The Development of Resistance to Reinoculation and of Circulating Cytotoxins in Response to Heterologous Ocular Tumor Transplantation in the Guinea Pig*

ALBERT C. SNELL, JR., AND BENEDICT V. FAVATA

(Divisions of Cancer Research and Ophthalmology, Department of Surgery, The University of Rochester School of Medicine and Dentistry, Rochester, N.Y.)

The superiority of the eye as a site for the transplantation of cancers and other tissues might be due to differences in the development of immunological events in this area as compared to other transplantation sites. Investigation of this hypothesis is stimulated by two considerations. First, there is increasing evidence that immunological factors determine the fate of many homografts and heterografts (5, 6, 7, 14). Secondly, there is evidence that immune reactions on the part of the eye are, in a few circumstances, peculiar in rate and character. For example, some of the peculiarities of the interstitial keratitis of congenital syphilis have been attributed to a relative failure of the cornea to participate in immunological developments occurring elsewhere in the infected individual (16). The fact that the eye contains two organ-specific substances, lens protein and uveal pigment (15), suggests the possibility of a compensatory local suppression or block in the mechanism of foreign protein reactions. In view of these considerations, it appeared worth while to attempt to determine if a delay or failure in the occurrence of immunological reactions could be responsible for the superiority of the eye as a transplantation site.

Immunity to grafts of homologous adult tissue transplanted in the eye, and subcutaneously, has been demonstrated by Woodruff and Woodruff (14). These authors studied the relative immunizing capacity of these two routes for homografts of thyroid tissue in thyroidectomized guinea pigs. They observed that both intraocular and subcutaneous grafts produced a state of relative resistance to the establishment of second grafts of the same tissue; immunizing grafts in either site produced a resistant state toward future grafts in both sites.

Medawar (8) observed immunity of the eye to homologous grafted skin only when skin grafts in the anterior chamber were vascularized; non-vascularized grafts of skin in the immune eye behaved no differently from those in normal control eyes.

Greene (4) investigated the possibility that tumor transplants in the anterior chamber might be segregated from tumor anti-substances in the circulation by the action of the blood-aqueous barrier. He studied homologous transplants of various rabbit tumors and observed that, following growth of the tumor in one eye, both the testicle and the anterior chamber of the opposite eye were resistant to reinoculation, indicating a free passage into the eye of the factors responsible for the immunity.

The participation of the eye in immunity against the Brown-Pearce carcinoma in rabbits was studied by several observers, with conflicting results. Besredka and Bardach (1) observed that the anterior chamber participated in the generalized resistance to reinoculation on the part of rabbits immunized against this tumor. Saphir, Appel, and Straus (9), on the other hand, successfully grafted the Brown-Pearce carcinoma into the eyes of tumor-immune rabbits. Cheever and Morgan (2) found a relative but not absolute immunity under the same circumstances. These observers found that a monocular transplant failed to confer immunity on the opposite eye.

Resistance to reinoculation after heterologous ocular tumor transplants was observed by Schilling and Snell (10) with the mouse carcinoma MTK8. The resistant state was found in both eyes after a monocular transplant.

In general, most authors agree that transplantation of alien tissue within the eye is followed by immune reactions, as is transplantation in other areas. The rate of development of immune reactions following ocular grafts, as compared with grafts elsewhere, has not been closely studied; it
is conceivable that this rate is of critical importance in the superiority of the eye as a transplantation site.

METHODS AND MATERIALS

The experiments reported herein were designed to test the development of immune reactions in response to transplantation within the eye, as compared to subcutaneous transplantation of tumors of alien species. Evidence for the occurrence of immune reactions following such transplantations was of two kinds: (a) the development of a refractory state, in which an initial transplantation was followed by failure of the same tumor to “take” when transplanted a second time to the same animal; (b) the development of circulating anti-tumor substances having a lytic effect on tumor cells growing in tissue culture.

Two different transplantable neoplasms were studied. One of these was a human breast carcinoma, which had been removed from an intracranial metastasis (19). This tumor had been transplanted to the anterior chambers of guinea pig eyes and maintained for 22 months by serial transplantations. The other tumor, more extensively studied, was the mouse carcinoma MT8 (10) carried in C3H mice. Guinea pigs were used throughout as the secondary or heterologous hosts.

The technics of the preparation of tumor samples and their transfer to the anterior chamber have been described (10, 11). Subcutaneous transplants were made with the same trocars used for the anterior chamber transplants, so that the amount of tumor introduced and the degree of maceration obtained were similar. Subcutaneous transplantations in the guinea pig were made principally with the mouse carcinoma. This tumor, when so transplanted, did not grow progressively. A severe inflammatory reaction developed about these transplants within 48 hours and subsided after 10-14 days.

The usual criteria (10) for the definition of growth of a tumor in the anterior chamber have been described. Subcutaneous transplants were made with the same trocars used for the anterior chamber transplants, so that the amount of tumor introduced and the degree of maceration obtained were similar. A severe inflammatory reaction developed about these transplants within 48 hours and subsided after 10-14 days.

The usual criteria (10) for the definition of growth of a tumor in the anterior chamber were applied: the transplanted fragment must vascularize and enlarge at least threefold, and histological identification must confirm the existence of growth of the tumor, at least in instances where the gross appearance of events in the eye left any doubt.

The tissues to be cultured in vitro were placed on cover slips where they were allowed to dry momentarily. This produced adhesion to the cover slip without the addition of other material. The cells were cultured at 37.5° C. in a mixture consisting of 4 parts of Fischer V-614 synthetic medium (3) and 1 part of Simms's ultrafiltrate of ox blood (12). A small particle of dried chick embryo extract was added. Each flask contained five to eight pieces of tissues and 1.0-1.5 cc. of medium. The aqueous humor or serum to be tested was added to this medium. About three-fourths of the explanted specimens grew actively; those flasks with poor tumor cell growth were discarded.

EXPERIMENTAL

The refractory state.—Previous work (10) had demonstrated that transplantation of MT8 to the guinea pig eye produced, after regression, a marked decrease in the survival of second ocular transplantations of portions of the same tumor. Transplantation into one eye conferred the refractory state on both eyes. The refractory state developed whether or not the tumor “took” and grew on the initial transplantation. It remained to be determined if subcutaneous transplantation also produced the refractory state to intraocular transplantation. Accordingly, seventeen guinea pigs (in four groups) received subcutaneous transplants of MT8. Seven to 12 days later new specimens of the same tumor freshly removed from other mice were transferred to both eyes. No growth of the tumor occurred in any of the 34 eyes. The expected incidence of growth of this tumor in the eyes of normal guinea pigs is about 50 per cent (10, 13).

It was desired to determine if transplantation via the ocular route produced the refractory state as quickly as was the case with the subcutaneous route. Four groups of guinea pigs, 38 animals in all, received monocular transplants of MT8, made in the right eye. Seven to 12 days later each animal save one received a new transplant of the same tumor in the opposite (left) eye. Growth of the transplants occurred in the right eyes in 24 animals, and in the secondly transplanted left eyes in four animals.

Previous work (13) had shown that it is occasionally possible to obtain growth of heterologous tumors transplanted within the stroma of the corneas of guinea pig eyes. The following experiment was done to determine whether the refractory state was exhibited by the cornea as well as by the anterior chamber. A series of fifteen guinea pigs was rendered “immune” by bilateral ocular transplantation of MT8 into a group of four animals, and by subcutaneous transplantation into eleven animals in two groups. After regression of the intraocular tumor and absorption of the subcutaneous tumor, new transplants of MT8 were made into 27 of the corneas of the above animals. Following this procedure, the corneas usually became vascularized, but the transplants usually did not. In 22 cases the transplant was absorbed without becoming vascularized. In the last group
of six animals, however, five of the twelve intracorneal transplants did become vascularized as in a successful “take,” but regression followed almost immediately, and the transplant failed to enlarge threefold. Four corneas in two normal animals received transplants of MT8 as simultaneous controls for this group, and typical growth with marked enlargement occurred in three. In an earlier series (13) the incidence of “takes” in the cornea was 69 per cent for this tumor. Evidently the cornea, as well as the anterior chamber, participates in the refractory state.

In summary, the above data (Table 1) indicate that subcutaneous and ocular heterotransplantation of the mouse tumor MT8 produce a state refractory to the establishment of the same tumor on ocular retransplantation; the refractory state develops within 7 days after either subcutaneous or ocular transplantation.

In the affected cells, large numbers of cytoplasmic droplets or granules appeared. The cell walls became crenated, and the characteristic short processes disappeared. The cells often became noticeably swollen and rounded. Many cells disintegrated entirely, leaving clumps of granules (Fig. 1). Some of the granules adhered to the cover slip, and even after repeated washings and reculturing they remained unchanged. Many cells were seen to float off the cover slip and were washed away. The main mass of the explant became smaller and broken. Finally, growth was re-established in some flasks after washing and the addition of fresh normal medium (Fig. 2).

The effects of the anti-substance on growing tumor cells were usually vigorous and dramatic and appeared within 8–24 hours. On the other hand, changes in pH or in temperature, or the presence of bacterial contamination or several months’ aging did not produce nearly so rapid or so extensive effects. The human carcinoma, the mouse tumor MT8, human fibroblasts, mouse fibroblasts, and chick embryo heart could be grown in tissue culture equally well when the usual media were replaced in whole or in part with the aqueous humor of normal horse, beef, or guinea pig eyes.

Five tissue culture flasks containing actively growing cells of the human breast carcinoma explanted from the guinea pig eye were treated by adding 1 or 2 drops of aqueous from guinea pig eyes in which other specimens of the same tumor had grown and had regressed 4–12 weeks previously. In every flask so treated the growing cells were injured in the manner described above.

Portions of the aqueous humor exhibiting the anti-tumor effect against the human mammary carcinoma did not alter cultures of human testis, chick embryo heart, MT8 tumor, and mouse and rat fibroblasts.

One flask in which the anti-tumor effect of “immune” aqueous humor had occurred was washed with standard media and further incubated. A few of the less affected cells appeared to recover, and multiplication was resumed. The anti-tumor effect occurred again when “immune” aqueous humor was now added a second time.

Similar procedures were carried out with the mouse tumor MT8. Ten flasks in which this tumor was growing in standard media were inoculated with 1 or 2 drops of aqueous humor from guinea pig eyes in which MT8 had been transplanted 4–12 weeks earlier and had grown and regressed. The tumor cells growing in tissue culture again were profoundly altered by such material. The cells became granular, shrank, and often disintegrated within 8–48 hours after the aqueous humor was added. In many of these flasks, multiplying tu-
mor cells were intermixed with actively growing fibroblasts, presumably mouse connective tissue explanted along with the tumor. These fibroblasts were not affected by the aqueous humor which destroyed the neighboring tumor cells. Aqueous humor exhibiting the in vitro effect against the mouse tumor did not affect cultures of the human breast carcinoma, embryonic lung epithelium of the mouse, or adult mouse or rat fibroblasts. MT8 explants were not altered by normal guinea pig aqueous humor or by aqueous humor exhibiting an effect against the human breast carcinoma. Aqueous humor obtained from either eye was effective, regardless of which eye had harbored the tumor.

In a second series, blood was withdrawn from the hearts of guinea pigs in which ocular growth and regression of MT8 had occurred. The blood was centrifuged, and 1 or 2 drops of the serum was added to each of five flasks of growing MT8 tumor cells. The tumor cells so treated were destroyed in each flask in the same manner as previously. Portions of the same serum did not affect mouse embryo lung epithelium in the one flask so tested, nor did it affect mouse fibroblasts in seven of the eight flasks tested. A sample of serum containing considerable hemolysed blood suppressed the growth of fibroblasts explanted from adult mouse connective tissue in one flask.

In a third experiment, three guinea pigs received subcutaneous transplants of MT8. Seven days later, aqueous humor was withdrawn from the eyes of these animals and added to six flasks in which MT8 tumor cells were actively growing in tissue culture. Again, the anti-tumor cell effect was observed in all flasks, most of the cells disintegrating within 48 hours.

In a fourth experiment, an additional eleven flasks of actively growing MT8 tumor cells received 1 or 2 drops of aqueous humor removed from guinea pigs which had received ocular transplants of MT8 7 days earlier. The anti-tumor effect in vitro of this aqueous humor was already present, as evidenced by the fact that most or all the cells in each flask were destroyed by the aqueous humor. In several of these eyes the tumor continued to grow. Two flasks were treated with aqueous humor from an eye with the anterior chamber half full of growing tumor. This material also exhibited the anti-tumor effect in vitro.

The data obtained by the tissue culture experiments are given in Table 2. In summary, a fairly specific in vitro anti-tumor cell effect was produced either by subcutaneous or by intraocular transplantation of the tumors investigated, and with the mouse tumor the effect was present at 7 days, regardless of which transplantation site was used.

### DISCUSSION

The results of the experiments described in this report agree with the majority of recorded observations on this topic; the eye does participate in immunological reactions to transplanted alien tissues, in the sense both that transplants within the eye excite systemic immunity, and that transplants within the eye are exposed to and may react with circulating immune bodies. The evidence presented here indicates that, in the case of the tumor studied, systemic immune reactions have occurred by the seventh day in response to ocular as well as to subcutaneous transplants. This makes it unlikely that the superiority of the eye as a transplantation site, at least for this tumor, is due to a relative slowness in the development of anti-substances following transplantation in this area.

The hypothesis of an ocular immunological weakness, even though it is not in accord with the available evidence, is by no means ruled out as a possible explanation for the unusual success of ocular transplants. While it is known that immune reactions do occur following ocular transplants, and that they occur essentially as quickly in the case of the eye as in other areas, it is not known if these reactions occur to the same degree or as strongly for the ocular as for other transplantation routes; quantitative titrations are needed.

---

**Table 2**

<table>
<thead>
<tr>
<th>Transplantation site</th>
<th>Interval between transplantation and removal of aqueous humor or of blood serum</th>
<th>No. of flasks with growing tumor tested for cytotoxic effect</th>
<th>No. of flasks with growing tumor in which cytotoxic effect occurred</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior chamber</td>
<td>4-12 weeks; aqueous humor</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Anterior chamber right eye</td>
<td>7 days; aqueous humor right or left eye</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Skin</td>
<td>7 days; aqueous humor</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Anterior chamber</td>
<td>4-12 weeks; blood serum</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

The CYTOTOXIC EFFECT in Vitro Exhibited by Aqueous Humor or by Blood Serum Following Transplantation of MT8 In the Anterior Chamber or Subcutaneously.
Moreover, only two immunological effects have been studied; it is not known if other kinds of immunological reactions occur following ocular tissue transplantation. Finally, it is not certain that the immunity which follows both ocular and subcutaneous transplantsations is the critical event in determining the survival or destruction of an intraocular tumor transplant.

There is even some evidence that the reactions studied are not actually those which determine the fate of the original intraocular transplants. For example, the tumor cytotoxin so quickly effective against the tumor in vitro appears at or before the time the tumor is growing in the eye; as the tumor vascularizes, the local supply of circulating anti-substances must improve, yet this is the time of most vigorous tumor growth. The anti-substance is not being absorbed by the tumor faster than it can be delivered, because, as described above, the aqueous humor from an anterior chamber half-full of growing tumor still exhibited the cytotoxic effect against the tumor in vitro. The same apparent conflict applies in the case of the state of resistance to retransplantation; this resistance appears just at a time when the original transplant has gained a precarious vascular supply and is beginning to grow most actively. Woodruff and Woodruff (14) observed prolonged survival of vascularized homologous thyroid grafts in the eyes of thyroidectomized guinea pigs even when a high degree of immunity to the same tissue was evidenced by the behavior of secondary grafts made subcutaneously or into the opposite eye. Resistance appeared to be directed against the establishment of a new graft, while a vascularized and already established graft appeared to be protected and survived.

It is interesting that Medawar’s observations (8) are directly at variance with those of Woodruff and Woodruff (14). Medawar, in studying the fate of homologous intraocular skin grafts in immunized rabbits, observed that those skin grafts that did not vascularize also escaped rapid destruction, and, conversely, that vascularized homologous skin grafts within the eyes of immunized rabbits were injured promptly and profoundly.

We have observed vascularization of the cornea in response to intraconal transplants of MT8 in immune as well as in normal animals. This suggests that immunity does not depend upon a suppression of a vaso-trophic effect. The occurrence of corneal vascularization under these circumstances makes it impossible to reach any conclusions about the presence of immune factors in the undisturbed cornea.

The observed specificity of the cytotoxicity for the particular tumor cells which provoked it is unusual. In the case of the mouse tumor, tumor cells were destroyed while fibroblasts from the mouse host, explanted along with the tumor, were not affected. There are two explanations for this considerable specificity. In the first place, this tumor excites a minimum of desmoplasia in the mouse and (apart from host vascular tissue) is almost a pure culture of tumor cells. The same is true of the human carcinomas growing in the guinea pig eye, in which no human vascular or connective tissue is represented. Secondly, the degree of specificity of tumor and other cytotoxins has been considered by Harris (5) to be a function of their concentration—specificity diminishing as the titer rises. In our experiments, the concentration of the cytotoxic agent must have been low, because frequently a few of the cells in a single flask were not destroyed by the toxin; presumably its concentration happened to be optimal for specificity.

SUMMARY

1. A state of resistance to the growth of an ocularly transplanted mouse tumor MT8 develops following either subcutaneous or intraocular transplantation of the same tumor in guinea pigs.

2. The resistant state to retransplantation in the eye is detectable at 7 days whether the original immunizing transplantation is made in the eye or subcutaneously.

3. Cytotoxins injurious to the cells of the same tumor growing in vitro are detectable at 7 days after transplantation either in the eye or subcutaneously; the cytotoxins are found in both eyes and in the blood plasma after a monocular or a subcutaneous tumor transplant.

4. It is concluded that the rate of development of these immunological reactions is not a critical factor in the superiority of the guinea pig eye as a transplantation site for these tumors.

REFERENCES


8. ———. Immunity to Homologous Grafted Skin. III. The Fate of Skin Homografts Transplanted to the Brain, to Subcutaneous Tissue, and to the Anterior Chamber of the Eye. Ibid., 29:55-69, 1948.


Fig. 1.—(A) Phase photomicrograph of MTS tumor cells as cultured in vitro and (B) the same field 12 hours after the addition of aqueous humor from the eye of a guinea pig which had received an ocular transplant of MTS 2 weeks previously.
FIG. 2.—Phase photomicrograph of MT8 tumor cells (A) before and (B) 48 hours after the addition of aqueous humor from the eye of an immune animal, and 24 hours after washing and substitution with normal media. The original cells have disappeared and new cells have grown out from the margin of the explant.
The Development of Resistance to Reinoculation and of Circulating Cytotoxins in Response to Heterologous Ocular Tumor Transplantation in the Guinea Pig

Albert C. Snell, Jr. and Benedict V. Favata


Updated version Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/11/5/335

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.