Intraocular Transplantation of Malignant Lymphomas of the Mouse, Dog, and Man in Heterologous Species

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The transplantation of malignant human tumors in the eyes of guinea pigs and rabbits has been a helpful method for studying some neoplasms (4, 7, 10). Recently it has been advocated as a clinical-pathological technique to aid in the diagnosis of malignancy and degree of autonomy of tumors (11, 12). This method has, however, not been sufficiently well developed for use as a routine laboratory procedure. All types of tumors have not as yet been studied from this point of view, and the question is still unanswered why many neoplasms which are clinically highly malignant fail to establish a foothold in the anterior chamber and grow (5, 7, 14). That these tumors have not attained autonomy and therefore cannot grow after heterologous transplantation may indeed be an explanation for this phenomenon (12), but just what autonomy in this sense means to the welfare of the patient host and to a biological understanding of cancer is obscure. Several years ago experiments were begun in this laboratory to study malignant lymphomas by this method in the hope that an accurate earlier method of diagnosis of this difficult group of tumors might be developed, and that transplants which would be suitable for chemotherapeutic studies might be obtained. As yet no extensive study of malignant lymphomas has been reported although mention has been made in several instances that attempts at heterologous intraocular transplantation were unsuccessful (5, 12).

MATERIALS AND METHODS

Transplantation of tissue into the anterior ocular chamber was made by the method of Greene (6). As soon as possible after its removal from the donor, the tissue was inserted through a slit in the cornea. The pieces were trimmed aseptically to about 1 mm. or less in greatest dimension. Most tissues were introduced with 18 to 20 gauge needles; in a few instances firm rubbery tissues were inserted with a small forceps. The intraocular transplant was then moved into the inferior corneal angle by pressure from the outside with a glass rod. All instruments were sterilized before each operation in creosote solution and several washes in 70 per cent alcohol which was, in turn, permitted to evaporate.

The conjunctivae were anesthetized with cocaine solution. In mice, the corneal slit was made with a sharp knife and an emulsion of tumor was injected into the anterior chamber, using a syringe and a 26 gauge needle with a short bevel. Our successful growth of a canine melanoblastoma through 2 generations of guinea pigs in other experiments showed that the techniques used in these experiments were adequate.

These experiments employed 70 young guinea pigs, averaging 500 grams in weight; 29 rabbits, averaging 2.5 kilograms in weight; 6 pure bred cocker spaniels, 8 weeks old; 20 strain Ak mice; and 20 strain CF mice obtained from Carworth Farms. Pieces of tissue were usually implanted in both eyes of each guinea pig and rabbit. Transplants were made into only one eye of each animal in the first group of experiments and when dogs and mice were used. In all, transplants were studied in 111 eyes of guinea pigs, 56 eyes of rabbits, 2 eyes of dogs, and 20 eyes of mice. The eyes of the larger animals were examined on the average of 3 or 3 times a week for the first 4 to 8 weeks and then at irregular intervals for the next 6 months in guinea pigs and 4 months in rabbits and dogs. The eyes of the mice were examined every other day until all Ak mice were dead, and at increasingly long intervals after that for the next year and a half in the CF mice. At these observations schematic drawings were recorded of the findings in each eye.

Eleven separate groups of experiments were made. Seven human malignant lymphomas were studied; 3 leukemias, 2 lymphosarcomas, and 2 cases of Hodgkin's disease. One suspected human leukemia turned out to be an acquired hemolytic anemia but the study of this tissue is included in this paper for comparative purposes. Three malignant lymphomas of animals were studied; canine malignant lymphoma, and 2 mouse leukemias. In 4 cases (Nos. 1, 2, 4, and 8) the clinical diagnoses were only provisional at the time of transplantation. It was hoped that this procedure would aid in reaching the correct diagnosis. In all cases the final diagnosis was made clinically and confirmed by pathological study of either biopsy or necropsy material.

Half of the tissue from each donor was studied histologically. In experiment 1, the lymph node obtained by biopsy from a boy aged 9 years contained blastic cells of unknown nature which were beginning to obscure the architecture of the node. In experiment 2, the lymph node obtained by biopsy from a boy 8 years old contained many hemocytoblasts distributed focally throughout the node. In experiment 3, femoral marrow...
Human leukemias and lymphomas.—The results of the first 8 groups of experiments, using human tissues, are summarized in Table 1. In these experiments no "takes" were obtained. No transplant was seen to increase in size during the 4 to 6 months of observation; all resorbed promptly. Typically, the appearance of the piece of tissue and of the anterior chamber followed a well-defined course. Twenty-four hours after transplantation the aqueous humor was hazy and the color of the piece of tissue was fading. By 3 days the conjunctiva at the limbus was hyperemic and the transplant was dull gray or yellow. By 7 days the piece was about one-half of its original size; occasionally it could no longer be found. The mild ophthalmitis was usually resolved by this time. By 14 days the pieces of tissue were about one-third their original size and ivory or light gray. The pieces decreased very little in size during the next 5 weeks although an occasional piece disappeared entirely. At the end of this time the tissue was minute and almost pure white. During the following months no changes were noted in its appearance. An occasional eye developed suppurrative panophthalmitis. In most cases the transplants in the eyes of the guinea pigs resorbed faster than did those in the eyes of rabbits. In rabbits, during the first 48 hours, it was not unusual for a definite gray halo to develop around the transplant. Histologic study of such a halo (Fig. 6) showed that it was composed of fibrin. Most of the lymphocytes and granulocytes had died or disappeared from the transplant at this time (48 hours). The reaction and sequence of events appeared the same in the eyes regardless of whether one or both were implanted.

A histologic study of the fate of the transplanted tissue was made in experiment 9 in guinea pigs killed at 2, 4, 6, 8, and 14 days after the operation (Figs. 1 to 5). At 2 days the pieces were almost completely necrotic; they were eosinophilic and almost structureless. Most of the cells were dead. A few viable hemocytoblasts in the transplant were surviving in contact with the cornea in one eye (Fig. 1). In addition scattered endothelial and reticular cells were stainable. No viable cells were present in the transplant in the opposite eye; it was completely necrotic. At 4 days the transplants still contained minute foci of nuclear debris and they now contained many proliferating fibroblasts. These growing cells seemed to stem from the contiguous iris as no line of demarcation remained, and melanophores also were migrating into the transplants (Fig. 2). By 6 days, the fibroblastic tissue had produced collagenous fibers in the portion adjacent to the iris and was continuing to proliferate in the remaining part (Fig. 3). By 8 days, this connective tissue was almost completely differentiated; the transplant was composed of condensing collagenous connective tissue.
Lymphocytes and plasma cells were now present in the adjacent iris and in the margins of the transplant. They were probably of host origin since they were intermingled with pseudoeosinophilic leukocytes of the guinea pig (Fig. 4). After 14 days, the piece was a minute round body composed entirely of collagenous connective tissue (Fig. 5).

Table 1

<table>
<thead>
<tr>
<th>No. of experiment</th>
<th>No. of animals*</th>
<th>Material</th>
<th>No. of eyes</th>
<th>No. of Final diagnosis</th>
<th>Fate of donor</th>
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<td>66</td>
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<td>Myeloblastic leukemia</td>
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<td>12</td>
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<td>0</td>
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<tr>
<td>4</td>
<td>11</td>
<td>Sternal marrow</td>
<td>6</td>
<td>0</td>
<td>Reticulum cell sarcoma</td>
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<tr>
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<td>Lymphosarcoma</td>
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<td>0</td>
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<tr>
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<td>12</td>
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<td>0</td>
<td>Hodgkin's disease</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>Axillary lymph node</td>
<td>6</td>
<td>0</td>
<td>Acquired hemolytic anemia</td>
</tr>
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* GP = guinea pig; R = rabbit.
† Also transplanted into additional guinea pigs for serial sacrifice (see Figures 1 to 5).
‡ Tissue obtained at necropsy and transplanted within 1.5 and 3.5 hours after death.

Table 2

<table>
<thead>
<tr>
<th>No. of experiment</th>
<th>Source of Tumor</th>
<th>Type of Lymphoma</th>
<th>Fate of donor</th>
<th>No. of animals</th>
<th>No. of eyes</th>
<th>No. of Takes</th>
<th>No. of Regressions</th>
<th>No. of Transplants</th>
<th>No. of Regressions</th>
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<td>Malignant lymphoma</td>
<td>Death in 30 days</td>
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<td>7</td>
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<td>4 GP*</td>
<td>7</td>
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<tr>
<td>10</td>
<td>Strain Ak mice</td>
<td>Lymphatic leukemia</td>
<td>Death in 14 days</td>
<td>6 GP</td>
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<td>6 GP</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
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<td>Lymphatic leukemia</td>
<td>Death in 30 days</td>
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* GP = guinea pigs; R = rabbits; D = pure bred cocker spaniels; Ak = strain Ak mice; CF1 = strain CF1 mice.
† These mice died with generalized leukemia after regression of the intraocular tumor.

There was no evidence in these sections that any cells of the donor survived over 48 hours.

**Canine malignant lymphoma.**—The canine malignant lymphoma did not take in the eyes of guinea pigs or of rabbits in experiment 9 (Table 2). The visible sequence of events resembled that of the human tissues. A transient take was apparently obtained in the eye of the cocker spaniel puppy that had previously been irradiated. Since an emulsion of lymphoma (rather than a piece) was injected into this eye, no tumor was seen for the first 2 days. On the third day a minute round mass was visible on the iris. By the fourth day this mass was seen as a tannish-white projection 1 mm. long, protruding from the angle of the iris; it had 3 tiny polypoid rounded masses at its free end. Four days later this projection was whiter and its margins were no longer distinct. By 12 days it was a stringy white structure; later it gradually con-
FIGS. 1-5.—These illustrate the histologic changes between 2 and 14 days in transplants of a lymph node from a case of stem cell leukemia (Experiment 2) in the anterior ocular chambers of guinea pigs. These photomicrographs are reduced from a magnification of X180. The cornea is always to the left and the iris or lens is on the right. Some illustrations show artifacts.

Fig. 1.—At 2 days the transplant is partly necrotic.

Fig. 2.—At 4 days transplanted blast cells have disappeared and fibroblastic proliferation has begun.

Fig. 3.—At 6 days organization has proceeded and collagen is now visible near the iris.

Fig. 4.—At 8 days organization is almost complete.

Fig. 5.—At 14 days the organization is complete and the transplant is small.

Fig. 6.—Shows that the halo seen around the transplant in a rabbit was composed of fibrin. Unfortunately there is also present much staining artifact. Reduced from a magnification of X54.
other recipients are alive and well 2.5 years later.

Mouse leukemias.—In experiments 10 and 11, the highly malignant lymphatic leukemias of Ak mice did not grow in the eyes of the guinea pigs and rabbits. In fact, the transplants resorbed more rapidly than did human tissues in these animals. In experiment 11 the mouse leukemia grew well in the eyes of all Ak and CF₁ mice. These data are summarized in Table 2. All of the Ak mice injected intraocularly died with leukemia between the twenty-second and thirty-ninth days; the growth in the eyes of 2 of these mice had completely regressed by the twenty-fifth day. Only one CF₁ mouse with intraocular transplant died with leukemia (twenty-third day). The other 9 intraocular transplants regressed and all were completely gone by 60 days. The first regression in these animals was seen at 25 days. Histologically, the eyes of the Ak mice contained leukemic cells in all chambers and infiltrating all orbital structures. There was little necrosis of the tumor tissue; it contained a few blood vessels. The eye of one CF₁ mouse that died was similar but had more necrosis in the tumor, no vascularization and many degenerating and atypical leukemic cells.

All Ak mice, injected subcutaneously, died with leukemia; the average survival time was 19 days. One CF₁ mouse survived, along with those injected intraocularly, for an average of 18 months. None developed leukemia or hematopoietic disease. Nine died with pulmonary tumors and the other with nephritis or pneumonia.

COMMENTS

These observations demonstrate that malignant lymphomas did not grow following transplantation into the ocular anterior chamber of alien species. The invasiveness and metastasizing ability of the diseases used in these experiments were high as evidenced by the short course of the diseases in the donors and their generalized distribution at physical or necropsy examination. Growth of lymphatic leukemias was obtained in eyes of homologous species but regression proceeded rapidly unless the tumors were in eyes of the homologous strain. In so far as one can generalize from this limited number of experiments, these observations would seem to set this group of neoplastic diseases apart from the others which have been shown to grow in heterologous eyes after they have developed metastases in the host. Present behavior can, however, be explained on other bases than nonmalignancy and/or absence of autonomy, which are the explanations given by Greene (10, 11, 12) for failures to grow.

According to Greene (12), (a) normal adult tissues, (b) inflammatory lesions, and (c) benign tumors fail to survive while (d) embryonic tissues and (e) malignant neoplasms grow after intraocular transplantation in heterologous species. If this view is correct, failure of these malignant lymphomas to survive after transplantation means that they belong in one of the first three categories. Yet, in an homologous strain these diseases, in the two tests made, not only survived but infiltrated widely locally and metastasized to all parts of the body, killing the animals. In their biological behavior they conformed to the accepted criteria of malignant neoplasms. The deductions which can be made from these observations are that either there exist non-neoplastic diseases whose manifestations are indistinguishable from those of neoplasms, or that successful intraocular transplantation in alien species is inadequate as a test for malignancy, it being too severe. The host may die from the effects of a vigorously growing mass of atypical cells from which there has been dissemination and formation of secondary masses in other parts of the body, yet, according to the view of Greene, the disease is not necessarily an autonomous malignant neoplasm. At this point the laboratory is deserted in favor of the field of semantics.

It is accepted that there are in tumors different degrees of malignancy. No doubt there are also different degrees within the definition of autonomy. To set up a standard (such as ability to grow after transplantation in the anterior ocular chamber of an alien species) and state that all tumors which do not meet this standard are not malignant and autonomous for the homologous individual, or even species, is to change the previously accepted definition for this word.

Failure of takes with these malignant lymphomas might, however, be explained on several other bases: (a) lack of resistance of these cells to various types of trauma and adverse conditions; (b) short life span of these cells; or (c) the rapid development of immunity.

Cells of the hematopoietic system are notoriously susceptible to injury. Temperature changes, radiations, adrenocorticotrophic and adrenocortical hormones, nonspecific heterologous body fluids, various drugs and metabolites all are capable, together with other causes, of injuring these cells under certain conditions. Transplants are put into an environment which is hostile. Some cell death is to be expected. But the cells of a malignant lymphoma, as contrasted with the hardier cells of fibrosarcomas and certain other neoplasms, would be expected to die at a high rate due to their susceptibility to noxious influences of these non-
of hemopoietic cells; these normally have a life span which is among the shortest for any type of tissue (3). If the cells die because of their short life span before a new source of nutrition is established, the transplant will resorb. However, successful growth of a malignant lymphoma of mice in homologous and heterologous strains shows that short life span alone did not prevent takes. The failures of human lymphomas to grow must therefore be explained with the aid of additional factors.

Failure of takes with these malignant lymphomas might be explained on the basis of a rapid development of immunity. This factor is seemingly dependent on the previous two factors. The cells normally have a life span which is among the shortest exhibited by any type of tissue. They are subjected to adverse environmental influences. After transplantation early necrolysis of some of the transplanted cells from the above mentioned two causes might release antigen which could quickly arouse a humoral defense reaction (9). According to Greene and others (1, 9) the anterior chamber is not isolated but it exhibits antibodies formed elsewhere. The entrance of these antibodies into the eye might augment the nonspecific causes for cell death. The successful transplantation of a mouse leukemia into recipients of the same species, under which conditions antibody formation and cell death would be at a minimum, shows that the immune reaction might be the determining factor in failure of heterologous transplants. Furthermore, total body x-radiation, which inhibits antibody production (2, 13), apparently enabled a canine lymphoma to take in an irradiated dog whereas the same tumor transplanted to a non-irradiated littermate failed to grow. At the same time the failure of these two lymphomas to grow in alien species indicated, therefore, not that they lack high malignancy and autonomy, but merely that immunological and other factors were probably operating.

If short life span and high susceptibility to cell injury resulting in early cell death provide antigen for antibody production, and if antibodies can unfavorably influence intraocular transplants, then the success or failure of intraocular transplantation may be dependent less on the inherent autonomy and malignancy of cells, than on the other three factors mentioned. This might explain the successful takes of brain tumors (of low grade malignancy but long cell survival) (10), of fibrosarcomas (5, 8), of chondrosarcomas (11), carcinomas of the prostate (4), and others. Likewise it could explain the failures in the highly malignant tumors of childhood (which probably consist of comparatively short-lived, labile cells) (5), and the malignant lymphomas.

This technic was of no aid in differential diagnosis in these malignant lymphomas. A review of the reports in the literature give little support to this test as a practical diagnostic procedure. The largest series of tests reported is that of Eichwald (5) who found 1 positive case in 27 malignant tumors in children (1 guinea pig positive in 158 tested). Greene (7) obtained 4 positive cases in 11 tested. Masina reported negative results with 3 carcinomas of the prostate (14), but of three kidney tumors 2 were positive (15). Many papers report positive results but fail to state how many clinically malignant tumors tested during the same period of time were negative. A tabulation or statistical analysis of the reports in the literature up to the present time is, therefore, not justified as it would be unfairly weighted in favor of the positive results. Even so, the reported negative cases outnumber those that were positive. This test as it now stands does not appear to offer great promise as a practical diagnostic procedure. Nevertheless the procedure has merit as offering a means for studying the biology of certain cancers.

SUMMARY

Intraocular transplantsations in 7 cases of human malignant lymphomas (leukemia, lymphosarcoma, and Hodgkin's disease), 1 canine malignant lymphoma, and 2 leukemias of mice were unsuccessful in alien species (guinea pigs, rabbits, dogs, and mice). The mouse leukemias were transplantable in the eyes of homologous and heterologous strains of mice, but regression of the disease occurred rapidly in the latter strain. Histologic fate of transplants was rapid necrosis and partial resorption followed by organization of the debris.

The interpretation given to these experiments is that the failure of successful intraocular transplantation of malignant lymphomas in alien species was the result of the short life span of the cells, their high susceptibility to adverse conditions, and their antibody-engendering capacity, rather than to lack of a high degree of malignancy and autonomy.
This procedure was not of value in differential diagnosis.

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REFERENCES

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