Lymphoma Allografts Abrogate Immune Privilege Within the Anterior Chamber of the Eye

Jerry Y. Niederkorn* and J. Wayne Streliein|

Immune privilege is extended to allogeneic tissues placed into the anterior chamber of the eye and results in part from the induction of anterior chamber-associated immune deviation (ACAID)—a condition in which the host is capable of making humoral antibodies and cytotoxic T cells specific for the antigens in question, but is selectively suppressed in its capacity to generate delayed-type hypersensitivity (DTH). Privilege is extended only transiently to tissue differing at the major histocompatibility complex; by contrast, privilege is complete for tissues offering multiple minor H incompatibilities, but no MHC disparity. During examination of host responses to tumor cell lines in this latter category, it was observed that privilege was extended to all histologic categories of tumors examined except T cell lymphomas. Moreover, the capacity of minor H incompatible tumors to maintain immune privilege and sustain prolonged survival within the anterior chamber could be abrogated if T cell lymphomas were placed into the anterior chamber of the contralateral eye. Thus, T cell lymphomas exert a systemic influence which robs the anterior chamber of its immune privilege. It is concluded that this systemic effect is intimately related to lymphokines produced by T cell lymphoma allografts. Since immune privilege seems to be a physiologic property of the anterior chamber, it is suspected that local features within this site conspire to insure that antigens placed therein interact with the immune system in a manner that bypasses lymphokine production, thus allowing for the induction of ACAID and selective suppression of DTH. Invest Ophthalmol Vis Sci 27:1235-1243, 1986

Many antigens, when inoculated into the anterior chamber (AC) of the eye, impact upon the immune system in a highly unusual way. In our laboratory, we have studied the immune response that mice make to allogeneic tumor cells placed in the anterior chamber. We have discovered that the alloantigens on these cells gain access to the systemic immune apparatus and elicit a deviant form of immune reactivity termed “anterior chamber-associated immune deviation” (ACAID). The unusual spectrum of immune effectors produced by anterior chamber inoculation comprises high titers of serum antibodies directed at alloantigens present in the inoculum, primed cytotoxic T cells present within lymph nodes and spleen, and suppressor T cells which selectively interfere with the development of DTH specific for the alloantigens in question. We have proposed that ACAID is the important mechanism responsible for immunologic privilege as it has been described in the anterior chamber of the eye.

The immunologic privilege extended to allogeneic tumors placed into the anterior chamber of the eye may be permanent or transient, depending upon the strength of the alloantigens in question. For example, tumors which differ from their recipients across the entire H-2 complex or express a single strong class I disparity enjoy only a transient period of growth within the eye and then elicit a vigorous immune response which causes their rejection. By contrast, tumors which are H-2 identical with their recipients, but offer multiple minor H incompatibilities, experience long-lasting privilege. Previous investigations have studied the P815 mastocytoma (DBA/2 origin) and the B16 melanoma (C57BL/6 origin) which were inoculated into H-2 compatible but minor H incompatible recipients. Routinely, these tumor cells, when placed into the anterior chamber, grow progressively, form increasingly massive tumors, and ultimately kill their host. By contrast, these tumor cells are rejected swiftly following subcutaneous inoculation into similar minor H incompatible hosts.

Recently, we inoculated the T cell lymphoma, EL-4 (C57BL/6 origin), into the anterior chamber of minor H incompatible LP/J recipients. To our surprise, these cells grew transiently, but then a brisk inflammatory
reaction signaled the intrusion of the host’s immune response which led to rejection of the tumor. We were intrigued by this finding, since this was the first example of a tumor that does not present one or more MHC disparities, yet robs the anterior chamber of its immunologic privilege, thereby inducing tumor allograft rejection. This preliminary result suggested that other factors, in addition to immunogenetic disparity, must be important in the establishment of ACAID. Accordingly, we used the EL-4 tumor system as a tool for analyzing these putative factors.

Materials and Methods

Mice

Adult female mice of the following strains were maintained in our breeding colony: B10.D2 (H-2b), B10.A (H-2a), and C3H/HeJ (H-2k). Female BALB/c (H-2d), LP/J (H-2k), C57BL/6 (H-2b), and DBA/2 (H-2a) mice were purchased from Jackson Laboratories (Bar Harbor, ME). All mice were used as experimental subjects when 6–10 weeks of age. The present investigations conform to the ARVO Resolution on the Use of Animals in Research. Animals were maintained according to the recommendations outlined in the “Guide for the Care and Use of Laboratory Animals” prepared by the Institute of Laboratory Animal Resources, National Research Council. All surgical procedures were performed using ketamine hydrochloride anesthesia.

Tumors

P815 mastocytoma cells (DBA/2) were grown in suspension cultures in Dulbecco’s modified Eagle’s minimal essential medium (MEM; Gibco, Grand Island, NY) supplemented with 10% heat-inactivated fetal calf serum (FCS; Gibco) and gentamicin (0.05 mg/ml; Schering Corp., Kenilworth, NJ). B16F10 melanoma cells (C57BL/6) were grown in monolayer cultures in MEM supplemented as described above, with the addition of a vitamin solution (Gibco). BW lymphoma (AKR) and YAC-1 lymphoma cells (A/Sn) were cultured in suspension cultures with RPMI 1640 medium containing the same supplements described above, with the exclusion of the vitamin solution (= complete RPMI 1640). WEHI-3 fibrosarcoma (BALB/c) cells were maintained as monolayer cultures in complete RPMI 1640 medium. Three lines of EL-4 lymphoma (C57BL/6) were cultured separately in complete RPMI 1640 medium. The first EL-4 line (designated “IL-2 Producer Line”; a gift from Dr. Michael Bennett, University of Texas Health Science Center at Dallas) is known to produce IL-2 following stimulation with phorbol esters. A second EL-4 line (designated “IL-2 Nonproducer Line”; a gift from Dr. M. Bennett) fails to secrete IL-2 when stimulated similarly. A third line of EL-4 (designated “Standard Line”) was a culture originally obtained from Mason Research Institute Tumor Bank (Worcester, MA) and has been maintained in our laboratory for the past several years and has been used in numerous previous experiments.

Anterior Chamber Inoculations

A quantitative technique for transplanting a definite number of tumor cells into the anterior chamber of the mouse eye has been described elsewhere. Tumor cells were suspended to a concentration of 1 × 10^5 cells/μl in sterile Hank’s balanced salt solution (HBSS) and inoculated intracameraly (IC) into the various experimental panels of mice. Eyes were examined three times per week with a dissecting microscope and the tumor growth scored according to the percent of the anterior chamber occupied by tumor.

Assay For DTH

Delayed-type hypersensitivity (DTH) against relevant minor H antigens was measured as footpad swelling as described previously. Briefly, mice received 2 × 10^2 EL-4 cells administered either intracameraly (IC) or subcutaneously (SC), and 14 days later their footpads were challenged and measured for DTH responses following footpad challenge. Both hind footpads of each mouse were measured with a Mitutayo engineer’s micrometer immediately before footpad challenge. An eliciting dose of 1 × 10^6 x-irradiated (3,000 R) C57BL/6 lymph node cells suspended in 25 μl of HBSS was injected into the subcutaneous tissue of the right hind footpad. The left hind footpad served as a negative control and received 25 μl of HBSS without lymph node cells. Both footpads were measured 24 hr later and the difference in footpad size was used as a measure of DTH. Results are expressed as specific footpad swelling = (24 hr measurement – 0 hr measurement) experimental foot – (24 hr measurement – 0 hr measurement) negative control foot × 10^-4 in.

Stimulation of IL-2 Production

EL-4 lymphoma cells can be induced to produce significant quantities of interleukin 2 (IL-2) following in vitro stimulation with concanavalin A (Con A) and phorbol myristic acetate (PMA). Accordingly, Con A and PMA were used to stimulate IL-2 synthesis by various murine tumor cell lines in the present study. Briefly, tumor cells were cultured in either complete MEM or complete RPMI 1640 at a concentration of 1 × 10^6 cells/ml in the presence of Con A (2 μg/ml; Sigma Chemical Co., St. Louis, MO) and PMA (20 ng/ml; Sigma Chemical Co.). Cells were cultured in Costar 24-well tissue culture cluster dishes (Costar, Cambridge,
MA) in a volume of 2 ml. The cultures were incubated for 48 hr at 37°C in humidified chambers containing 5% CO2. Standard Con A-induced rat spleen cell cultures were also prepared as previously described.8 Briefly, 107 spleen cells were cultured in 2.0 ml of complete RPMI 1640 containing 1% FCS and 2.0 μg/ml Con A. The spleen cell supernatants were harvested 48 hr later and used as positive controls for IL-2 assays. All supernatants were treated with 0.05 M alpha-methyl-D-mannoside to remove residual Con A. It has been previously demonstrated that alpha-methyl-D-mannoside does not affect the assay systems used in this study.9

### Biological Assay For IL-2

A conventional bioassay for measuring IL-2 was employed.8,10 According to Gillis et al,11 the IL-2 microassay remains the only unequivocal assay for detecting the presence of interleukin-2 activity in conditioned tissue culture medium. Accordingly, we employed this assay to measure IL-2 in the media from various tumor cell cultures. The two IL-2-dependent cells used for measuring IL-2 were HT-212 and CTLL.10 The CTLL cell line (a gift from R. Rembecki, University of Texas Health Science Center at Dallas) has been used by other investigators for titrating IL-2 activity in conditioned medium.10,13 IL-2 was measured by placing 1 x 104 IL-2-dependent cells into flat-bottomed microtiter wells containing 100 μl of the various test supernatants. After 20 hr of culture, 0.1 μCi of 3H-thymidine was added to each well and the radioisotope uptake assessed 4 hr later. Each assay was performed in quadruplicate and the results expressed as mean counts per min incorporated.

### Skin Grafting

Full-thickness C57BL/6 skin grafts were prepared as described elsewhere.14 Grafts were applied orthotopically and were wrapped in plaster of Paris bandages. Casts were removed 7 days later and the grafts were inspected for evidence of rejection. Destruction was judged complete when all remnants of surface epidermis were gone.

### Statistics

Student’s t-test was used to determine the statistical significance of the various data.

### Results

#### Capacity of EL-4 Lymphoma Cells to Induce ACAID

Our preliminary experiments indicated that anterior chamber presentation of EL-4 lymphoma cells into minor H incompatible LP/J hosts failed to induce ACAID. These tumor cells grew transiently within the anterior chamber, but were subsequently rejected by a vigorous immune response. By contrast, the B16 melanoma, which is syngeneic with EL-4 lymphoma, grows progressively in the eyes of LP/J mice. Moreover, B16 melanoma cells induce ACAID as expressed by the capacity of LP/J recipients bearing these intraocular tumors to accept indefinitely orthotopic skin grafts from C57BL/6 donors.15

Since EL-4 was unable to secure permanent residence within the anterior chamber, we decided to determine whether animals inoculated intracameraly with EL-4 would accept skin grafts from C57BL/6 donors. Accordingly, LP/J mice received intracameral inoculations of EL-4 lymphoma or B16 melanoma cells. Fourteen days later, each animal received an orthotopic skin graft from C57BL/6 donors. As can be seen in Table 1, animals bearing B16 tumors in their anterior chamber accepted C57BL/6 skin indefinitely. By contrast, animals inoculated intracameraly with EL-4 cells, did not accept C57BL/6 skin. In fact, the median survival times of these grafts was 8 days, indicative of a second-set rejection and thus a state of allograft immunity. Moreover, the rapidity with which these grafts were rejected reinforced our suspicion that these animals were specifically sensitized to the minor H antigens of the C57BL/6 mouse strain. Thus, rather than EL-4 inducing ACAID when inoculated intracameraly, it induces specific sensitization.

#### Capacity of EL-4 Lymphoma Cells to Influence ACAID in LP/J Hosts

The availability of two tumors of syngeneic origin, but having opposite effects on the immune system following their inoculation into the anterior chamber, gave us the opportunity to inquire what effect the EL-4 tumor might have on the host’s ability to develop ACAID induced normally by B16 melanoma cells. In the first set of experiments, panels of LP/J recipients received intracameral inoculations of: (a) B16 melanoma cells alone, (b) EL-4 “standard line” lymphoma cells alone, or (c) a mixture of equal numbers of EL-4

### Table 1. Ability of a standard EL-4 lymphoma cell line to induce allograft immunity against C57BL/6 minor histocompatibility alloantigens following intracameral transplantation in LP/J mice

<table>
<thead>
<tr>
<th>Primary exposure to C57BL/6 alloantigens</th>
<th>C57BL/6 Skin graft survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (first set)</td>
<td>MST = 11.7 ± 0.9 days</td>
</tr>
<tr>
<td>B16F10 Melanoma Injected IC</td>
<td>MST &gt; 30 days</td>
</tr>
<tr>
<td>EL-4 Lymphoma Injected IC*</td>
<td>MST = 8.1 ± 0.5 days</td>
</tr>
<tr>
<td>C57BL/6 Skin Graft (second set)</td>
<td>MST = 7.2 ± 0.3 days</td>
</tr>
</tbody>
</table>

* Standard EL-4 lymphoma cell line that produces T cell growth factor (TCGF) constitutively in vitro (see Materials and Methods).

Each panel contained 10 recipient mice.
Day After Tumor Transplantation

Fig. 1. Growth patterns of intraocular tumors of C57BL/6 origin placed into the anterior chambers of LP/J mice. Mice received either 1 × 10⁵ "standard line" EL-4 lymphoma cells (●) or 1 × 10⁵ B16F10 melanoma cells (○) inoculated intracamerally on day 0. A third panel of LP/J mice received an intracameral inoculation containing a mixture of 1 × 10⁵ EL-4 lymphoma cells and 1 × 10⁵ B16F10 melanoma cells (△). Each point represents the mean accumulation of tumor within the anterior chamber as assessed by visual inspection with a dissecting microscope; e.g., 25 = 25% of the anterior chamber is occupied by tumor. Extraocular extension of progressively growing tumors is indicated by an asterisk (*). There were 5–6 mice per experimental group. Each experiment was performed twice.

Day After Tumor Transplantation

Fig. 2. Systemic effect of intraocular EL-4 "standard line" lymphoma on B16F10 melanoma-induced ACAID in LP/J mice. One panel of LP/J mice (○) received 1 × 10⁵ EL-4 "standard line" lymphoma cells inoculated into the left eye on day 0 and 1 × 10⁵ B16F10 melanoma cells inoculated into the right eye on day 0. A second panel of LP/J mice (●) received 1 × 10⁵ P815 mastocytoma cells inoculated into the left eye on day 0 and 1 × 10⁵ B16F10 melanoma cells inoculated into the right eye on the same day. The data were prepared and plotted in the same fashion as described in Figure 1 and represent the growth patterns for B16F10 melanoma. There were five mice per experimental group.

and B16 tumor cells. The growth patterns of tumor in recipient eye is depicted in Figure 1. In Panel C, the pattern of tumor growth—transient growth followed by vigorous inflammatory response and rejection—is essentially identical to that of Panel B. Thus, when inoculated into the same anterior chamber, the EL-4 tumor robs B16 cells of their ability to induce ACAID, as measured by the destruction of both tumor cell lines within the same globe.

To test whether this was a strictly local effect of EL-4, in the next experiments, EL-4 cells were inoculated into the anterior chamber of the left eyes and B16 melanoma cells were simultaneously inoculated into the right anterior chamber. These data are represented in Figure 2. It can be seen that EL-4 inoculated into one eye exerted its activity against B16 cells placed into the contralateral eye and prevented the latter from inducing ACAID and from growing in its typical progressive fashion within the anterior chamber. Since EL-4 was able to prevent B16-induced ACAID, even when the two tumors reside in opposite eyes, we concluded that EL-4 must produce a factor which has a systemic effect on the host.

Factor Production by Cultured EL-4 Cells

At the time these observations were made, we were aware of studies reporting that some but not all lines of EL-4 tumor cells possessed the capacity to secrete lymphokines, either constitutively or following stimulation with phorbol esters. Specifically, interleukin 2 (IL-2) has been demonstrated as a secretory product of some EL-4 lines. We therefore examined the supernatant culture fluid from our EL-4 "standard line" to determine whether it possessed the capacity to sustain growth and proliferation of an IL-2-dependent T cell line, HT-2. The supernatant from cultured EL-4 "standard line" cells was compared with an unmodified culture medium and with a supernatant from rat spleen cells specifically stimulated with Con A. This last material is known to contain large amounts of IL-2. The results of these experiments are displayed in Table 2. It can be seen that over a wide dilution spectrum, IL-2-containing Con A supernatant sustains HT-2 proliferation as measured by tritiated thymidine uptake. More importantly, it is clear that the supernatant from the cultured EL-4 cells also contains factors that sustain HT-2 cell proliferation. Thus, the EL-4 tumor cell line that fails to grow progressively in the anterior chamber of LP/J mice also liberates, in a constitutive fashion, T cell growth factors.

Capacity of IL-2 Producer and Non-Producer EL-4 Tumor Cells to Induce ACAID

We next obtained two well-characterized lines of EL-4. Line 1 is known to secrete IL-2 when stimulated
with phorbol esters. Line 2 is known to be incapable of secreting IL-2, even if stimulated with phorbol esters. These two cell lines were used in experiments to determine whether either or both were capable of inducing ACAID in LP/J mice.

We have recently demonstrated that a sensitive indicator of ACAID is the footpad assay for delayed-type hypersensitivity. ACAID, induced by P815 mastocytoma cells in the eyes of BALB/c mice, as well as that induced by B16 melanoma in LP/J mice, is characterized by the failure of animals bearing these intraocular tumors to mount DTH responses against relevant alloantigens expressed on the respective tumor cell membrane. Thus, the IL-2 producer and nonproducer lines of EL-4 were inoculated intracamerally into panels of LP/J mice. As displayed in Figure 3, the IL-2 producer line grew transiently in the anterior chamber and then was destroyed. By contrast, the IL-2 nonproducer line grew progressively in the anterior chamber in a fashion indistinguishable from the growth of B16 melanoma cells in the eyes of LP/J recipients. Ten days after intracameral inoculation, the footpads of each panel of mice were challenged with irradiated C57BL/6 lymph node cells. The results depicted in Figure 4 show that the recipients of IL-2 producer EL-4 cells displayed intense DTH reactivity; however, animals bearing the nonproducer EL-4 line in the anterior chamber failed to develop significant DTH reactivity. Thus, EL-4 cells which are incapable of secreting lymphokines, such as IL-2, are able to induce ACAID whereas their IL-2 secreting counterparts are not. We conclude, therefore, that a second factor crucial to the induction of ACAID and thus important in the development of immunologic privilege is intimately associated with the capacity of the allograft in question to secrete or to have induced the secretion of lymphokines.

Survey of Minor H Incompatible Tumors and Their Capacity to Induce ACAID

The possibility that lymphokine secretion by tumor allografts was instrumental in the abrogation of ACAID was explored further. Various tumor cell lines representing diverse histogenetic sources were examined for their ability to induce ACAID. We were particularly interested in examining allogeneic tumors having the potential for lymphokine production (i.e., T cell lymphomas). The list included the P815 mastocytoma, B16 melanoma, three T cell lymphomas (EL-4, YAC-1, and BW) and WEHI-3 fibrosarcoma. Recipient mouse strains are listed in Table 3. Each was selected on the basis of the immunogenetic disparity between the recipient and the tumor inoculum. In each instance, the tumor and recipient share identical H-2 chromosomal regions, but the tumor expresses numerous minor histocompatibility antigens not found in the recipient. Panels of five to eight recipient animals were arranged. Each animal received an inoculum of $10^5$ tumor cells suspended in 5 μl placed into the anterior chamber. Observations concerning in situ growth and inflammatory reactivity were made at regular intervals (every 2–3 days), with the aid of a dissecting microscope. Extent of tumor growth was observed and scored according to the proportion of the anterior chamber occupied by the tumor.

The results listed in Table 3 indicate that some tumors grew progressively within the anterior chamber of the recipients, without any evidence of host inflammatory response. Other tumors, however, grew transiently and then were destroyed by a vigorous inflammatory host response. In these animals, the neoplasm

<table>
<thead>
<tr>
<th>Supernate†</th>
<th>1:2</th>
<th>1:4</th>
<th>1:8</th>
<th>1:16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat spleen cells</td>
<td>94,898</td>
<td>42,211</td>
<td>41,213</td>
<td>20,800</td>
</tr>
<tr>
<td>EL-4 &quot;standard line&quot; cells</td>
<td>10,925</td>
<td>5,466</td>
<td>1,097</td>
<td>688</td>
</tr>
<tr>
<td>Medium</td>
<td>508</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

* Incorporation of $^3$H-thymidine by $1 \times 10^4$ IL-2-dependent HT-2 cells cultured for 20 hr in the presence of culture supernates. Results are expressed as mean counts per min of specifically incorporate $^3$H-thymidine. Similar results were obtained using IL-2-dependent CTLL cells (data not shown).

† Rat spleen cells were stimulated with Con A and PMA as described in Materials and Methods section. Culture medium from EL-4 "Standard Line" cells that were not stimulated with Con A or PMA.

Fig. 3. Progressive intraocular growth of an EL-4 lymphoma cell line that fails to produce interleukin-2. Panels of LP/J mice received either $1 \times 10^5$ IL-2 producing EL-4 tumor cells (●) or $1 \times 10^5$ EL-4 tumor cells that fail to produce IL-2 (○). Data were prepared and plotted as described in Figure 1.
was eradicated and the animals survived indefinitely. It is remarkable that all of the T cell lymphomas (with the exception of the IL-2 nonproducing EL-4 line) were unable to grow progressively in the anterior chamber. By contrast, all other histologic types of tumors grew progressively in the anterior chamber but failed to grow if inoculated subcutaneously (2 x 10^5 cells) in other panels of normal hosts of the respective mouse strains (data not shown). It appeared, therefore, that under these immunogenetic conditions, neoplastic cells of the T cell lineage possess a property which precludes their long-term acceptance in the anterior chamber of recipient mice.

IL-2 Production by Allogeneic Lymphomas

The results from the previous experiments indicate that T cell lymphomas fail to induce ACAID and therefore permit the development of a normal immune rejection process in an otherwise privileged environment (i.e., the anterior chamber of the eye). The results from experiments comparing the IL-2 producing EL-

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**Table 3. Immunologic privilege in the anterior chamber of the eye**

<table>
<thead>
<tr>
<th>Tumor Origin</th>
<th>H-2 Haplosite</th>
<th>Recipient*</th>
<th>Intraocular growth pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>P815 mastocytoma</td>
<td>DBA/2</td>
<td>d</td>
<td>BALB/c</td>
</tr>
<tr>
<td>B16 melanoma</td>
<td>C57BL/6</td>
<td>b</td>
<td>B10.D2</td>
</tr>
<tr>
<td>EL-4 T Cell Lymphoma† (IL-2 Producer)</td>
<td>C57BL/6</td>
<td>b</td>
<td>LP/J</td>
</tr>
<tr>
<td>EL-4 T Cell Lymphoma‡ (IL-2 Nonproducer)</td>
<td>C57BL/6</td>
<td>b</td>
<td>LP/J</td>
</tr>
<tr>
<td>YAC-1 T Cell Lymphoma**</td>
<td>A/Sn</td>
<td>a</td>
<td>B10.A</td>
</tr>
<tr>
<td>BW T Cell Lymphoma</td>
<td>AKR</td>
<td>k</td>
<td>C3H/Hej</td>
</tr>
<tr>
<td>WEHI-3 Fibrosarcoma</td>
<td>BALB/c</td>
<td>d</td>
<td>DBA/2</td>
</tr>
</tbody>
</table>

* All recipients shared the same H-2 haplotype with the tumor but differed with the tumor donor at multiple minor H loci. Each experimental group represents the summation of two independent experiments with 5-8 mice per group.

† EL-4 lymphoma IL-2 producer cell line that produces IL-2 when stimulated with phorbol myristic acetate (PMA).

‡ EL-4 lymphoma cell line that fails to produce IL-2 after in vitro stimulation with PMA.
4 lymphoma cell line and its nonproducer counterpart strongly suggest a correlation between lymphokine secretion and the abrogation of immune privilege in the anterior chamber. If true, the various lymphomas which undergo immune rejection in the anterior chamber of allogeneic hosts (Table 3) would be expected to be capable of producing IL-2. This was examined in the following experiments. The various tumor cell lines listed in Table 3 were cultured in vitro and co-stimulated with Con A and PMA as described elsewhere. Con A-stimulated rat spleen cell culture supernatants served as positive controls. Negative controls consisted of supernatants from identical media incubated in the absence of tumor cells. The various supernatants were tested for their ability to sustain the growth and DNA synthesis of two IL-2-dependent cell lines (HT-2 and CTLL).

The results shown in Table 4 indicate that the EL-4 lymphoma “Producer Line” and our original EL-4 “Standard Line” liberated significant quantities of IL-2 that sustained the growth of both IL-2-dependent cell lines. Although the supernatant from the EL-4 “Nonproducer Line” had a modest effect on growth of the IL-2-dependent cell lines, the magnitude of this response was only 11–15% of that observed for the other EL-4 cell supernatants. By contrast, the remaining tumor cell lines, including the BW and YAC-1 lymphomas, failed to elaborate significant quantities of IL-2 even when co-stimulated with Con A and PMA. Repeated efforts to stimulate IL-2 production using various modifications of culture conditions, incubation times, tumor cell concentrations, lectin concentrations, and PMA concentrations failed to elicit the production of demonstrable IL-2 by BW and YAC-1 cell culture supernatants (data not shown).

It is possible that, although the various lymphomas fail to produce IL-2 in vitro, relevant amounts of this lymphokine are elaborated in vivo following transplantation into the anterior chamber. However, a more plausible explanation is that the nonprogressor lymphomas elaborate lymphokines, other than IL-2, that are involved in the abrogation of immune privilege.

**Discussion**

The results of these studies provide new insights into the cellular and molecular basis of immunologic privilege in the anterior chamber of the eye. We had previously documented that an important factor governing the extent of immune privilege was the strength and number of alloantigens on the tissues placed within the anterior chamber. The studies we report now suggest that a further determinant is the ability of the graft in question to elaborate lymphokines, such as IL-2, which have a profound effect upon the immune system. On the basis of these studies, we would propose that immune privilege is achieved in the anterior chamber of the eye under circumstances in which IL-2 (or comparable lymphokines) is not produced as a consequence of antigen placement within the anterior chamber. As a corollary, we suspect that lymphokine production, in a setting that would otherwise lead to immune privilege, now abrogates the phenomenon. Thus, lymphokines produced either by the intraocular lymphomas or their metastases seem to govern whether the host can mount an effective immune response against the alloantigenic cells within the anterior chamber and produce a rejection reaction that destroys the tumor.

The effects of lymphokine production by T cell lymphomas placed within the anterior chamber are not exclusively local. That is, the IL-2 producing EL-4 cell lines also interfered with the systemic induction of ACAID. Recipients of non-IL-2 producing EL-4 cells not only permitted the tumors to grow within the anterior chamber, but they failed to develop systemic delayed-type hypersensitivity. By contrast, recipients of the IL-2-producing tumor cell lines developed potent systemic delayed-type hypersensitivity. We cannot tell from the results of these experiments whether both the local and systemic consequences of anterior chamber inoculation of EL-4 cells producing IL-2 result from a strictly local action of the IL-2 and/or other lymphokines within the eye, or whether the lymphokines act systemically. It has been reported that normal mouse serum contains potent inhibitors of IL-2 which render its half-life exceedingly short. This would seem to mitigate against IL-2 being produced within the ante-

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**Table 4. Growth of IL-2-dependent cells in the presence of lymphoma culture supernatants**

<table>
<thead>
<tr>
<th>Supernatant</th>
<th>1:2</th>
<th>1:4</th>
<th>1:8</th>
<th>1:16</th>
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<tbody>
<tr>
<td>Final dilution of supernatant</td>
<td>1:2</td>
<td>1:4</td>
<td>1:8</td>
<td>1:16</td>
</tr>
<tr>
<td>Rat spleen</td>
<td>12,846</td>
<td>10,464</td>
<td>10,433</td>
<td>5,545</td>
</tr>
<tr>
<td>Medium</td>
<td>229</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>EL-4 producer</td>
<td>14,452</td>
<td>6,881</td>
<td>4,962</td>
<td>4,468</td>
</tr>
<tr>
<td>EL-4 nonproducer</td>
<td>1,640</td>
<td>1,344</td>
<td>442</td>
<td>145</td>
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<tr>
<td>EL-4 standard line</td>
<td>10,925</td>
<td>5,466</td>
<td>1,097</td>
<td>688</td>
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<td>YAC-1</td>
<td>111</td>
<td>91</td>
<td>104</td>
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<td>BW</td>
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<tr>
<td>B16F10</td>
<td>242</td>
<td>255</td>
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</table>

*3H-Thymidine incorporation by 1 X 10⁴ IL-2-dependent CTLL cells cultured for 20 hr in the presence of culture supernates. Results are expressed as counts per minute. Similar results were obtained using IL-2-dependent HT-2 indicator cells (data not shown).

* Cells were stimulated with Con A and PMA as described in Materials and Methods section. All supernatants were treated with 0.05 M alpha-methyl-D-mannoside to remove residual Con A prior to use in proliferation assay.
rior chamber and having a systemic effect. EL-4 cells, however, are able to metastasize from their anterior chamber inoculation site. Therefore, the possibility exists that the lymphokines produced from metastatic foci of EL-4 cells at distant sites, such as the spleen, result in the induction of delayed-type hypersensitivity. There seems little question that prompt and vigorous systemic immunity against the minor histocompatibility antigens of the C57BL/6 strain is generated in LP/J mice receiving intracameral inocula of IL-2-producing EL-4 cells. The most dramatic expression is the observation that simultaneous inoculation of EL-4 lymphoma plus B16 melanoma cells (either within the same anterior chamber or in opposite eyes) leads to failure of the B16 melanoma cells to grow progressively.

In this regard, Silverstein has suggested that, under certain circumstances, the eye can function as a lymph node. Critical events of lymphocyte recognition of antigens placed within the eye had been observed to take place within the globe itself. It has been our bias that antigens placed within the anterior chamber of the eye undergo at least some degree of processing in situ. We suspect that unique processing is responsible for the signal that leaves the eye and induces ACAID. It is, therefore, appealing to consider that local production of IL-2 and other lymphokines by EL-4 cells in the anterior chamber forces immunocompetent lymphocytes that enter the chamber into a differentiation pathway that leads to the induction of delayed-type hypersensitivity. Taken at face value, the observation that B16 melanoma cells, which normally grow progressively when placed in the anterior chamber of LP/J mice, are prevented from so doing if they are inoculated into the chamber simultaneously with EL-4 cells, is consistent with the hypothesis that immunocompetent lymphocytes are activated in situ. However, the fact that B16 is similarly prevented from growing from the anterior chamber of one eye when EL-4 is inoculated into the contralateral eye argues that lymphokines or other soluble factors produced by EL-4 have their locus of action at a systemic site.

We confess ignorance as to the mechanism by which lymphokine production might interfere with the induction of ACAID. However, it is worth noting that a similar form of immune deviation occurs when hapten-derivatized spleen cells are injected intravenously into mice. Such hosts fail to develop DTH responses against the relevant hapten; however, an additional injection of Con A converts the haptenated spleen cells from a tolerogenic stimulus to an immunogenic one. Moreover, the tolerance induced by intravenous injection of haptenated spleen cells can be circumvented by administration of purified exogenous IL-2. Malkovsky and Medawar have recently suggested that immunologic tolerance may be a consequence of IL-2 deficiency at the time of antigen recognition. In support of their hypothesis are the observations that the inability of neonatal mice to produce IL-2 coincides with their vulnerability to immunologic tolerance induction, and that the induction of neonatal tolerance can be abrogated by the administration of exogenous IL-2. Thus, they conclude that exposure to antigen in the absence of IL-2 favors the development of tolerance. It is possible, therefore, that the induction of ACAID involves the impairment of lymphokine synthesis and function during antigen processing. If true, then secretion of large quantities of appropriate lymphokines by intraocular lymphomas may override this immunological down-regulation in a fashion analogous to the situation in the intravenous hapten model of tolerance mentioned above or the model of neonatal tolerance described by Malkovsky and Medawar. However, it is prudent to recognize that EL-4 cells are able to elaborate other lymphokines besides IL-2. While we have documented that IL-2 production is a feature of our EL-4 producer cell line, this does not allow us to conclude that IL-2 is the active agent responsible for interfering with ACAID induction. Although the IL-2 producer line of EL-4 elaborates IL-2 in vitro after stimulation with Con A and PMA, it is not known if IL-2 is similarly secreted following transplantation into the anterior chamber of the eye. This uncertainty regarding the role of IL-2 in the abrogation of ACAID is further evident when one considers the findings with BW and YAC-1 lymphomas. Both of these tumors prevented ACAID induction and underwent immune rejection in the anterior chamber, yet neither of these tumor lines elaborated detectable quantities of IL-2 following in vitro stimulation with lectin and phorbol ester. It is possible that these lymphomas are stimulated by the host to produce IL-2 following intracameral transplantation; however, it is equally possible that lymphokines other than IL-2 are involved in the ablation of ACAID. Thus, the identity of the lymphoma factor(s) responsible for abrogation of ACAID remain to be identified.

We have pursued an analysis of ACAID in the belief that, under certain circumstances, induction of ACAID by antigenic substances within the eye (such as microorganisms, viruses, or tumors) may be deleterious to the host, robbing it of an effective immune response to rid the chamber of the offending agent. Identification of the lymphokine capable of interfering with ACAID induction may suggest potential therapeutic strategies in which exogenously administered IL-2, either into the anterior chamber or perhaps even systemically, would abrogate ACAID and permit the host now to mount an effective and curative immune response.
Key words: Intraocular tumors, immune privilege, interleukin-2, allografts, delayed-type hypersensitivity

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