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Intraocular Tumor Antigen Drains Specifically to Submandibular Lymph Nodes, Resulting in an Abortive Cytotoxic T Cell Reaction

Zita F. H. M. Boonman,* Geertje J. D. van Mierlo,† Marieke F. Fransen,‡ Kees L. M. C. Franken,† Rienk Offringa,† Cornelis J. M. Melief,† Martine J. Jager,* and René E. M. Toes†‡

Ocular immune privilege is considered essential in the protection against sight-threatening immune responses, as illustrated by the ability of the ocular environment to permit the growth of tumors that are rejected when implanted at other sites. Although several studies indicate that soluble Ag can drain directly into the spleen when injected into the anterior chamber, the primary site of intraocular tumor Ag presentation to tumor-specific CTLs has not been studied. To gain a better understanding of the mechanism involved in ocular immune privilege, we examined to which lymphoid organs anterior chamber tumor Ags primarily drain. Our data show that intraocular tumor Ag drains exclusively to the submandibular lymph nodes, resulting in activation of tumor-specific CTLs, whereas no Ag drainage was found in spleen. However, these tumor-specific CTLs do not distribute systemically and, as a consequence, intraocular tumor growth is unhampered. A similar lack of CTL efficacy has been observed in mice bearing s.c. tumors, which is converted to a systemic tumoricidal CTL response by administration of agonistic anti-CD40 mAb. In contrast, systemic anti-CD40 treatment of eye tumor-bearing mice did not result in mobilizing tumor-specific CTLs or tumor eradication. Together, these results show that intraocular tumor Ag drains to regional lymph nodes for activation of tumor-specific CTLs. However, the induced tumor-specific immunity is insufficient for tumor clearance, even combined with otherwise highly effective immune intervention protocols.


The anatomy and environment of the eye are part of a unique and complex system ensuring that sight is preserved when the integrity of the eye is challenged by invading infectious pathogens. This is crucial for the survival of the host, as loss of sight is often a life-threatening event. Therefore, the eye has evolved special immunological features to protect itself from immune-mediated pathology. This concept of immune privilege was suggested by the observation that transplanted allografts survive longer in the anterior chamber (AC) of the eye than in nonprivileged body sites, such as the skin (1).

Several factors are involved in maintaining the eye’s immune privilege, of which the exact mechanisms are not completely understood. Ocular immune privilege is best exemplified by a phenomenon called AC-acquired immune deviation (ACAID), describing that inoculation of Ag into the AC can generate systemic tolerance. Several lines of evidence indicate that ACAID is induced by eye-derived APCs, expressing macrophage-like markers on their cell surface. These cells can drain via the major outflow pathway of the eye directly into the blood to the spleen, where the intraocular Ag is presented to several immune cells (2–4). As the eye-derived APCs have been exposed to various immune modulatory cytokines present in the eye, such as TGF-β and IL-10, they are thought to induce regulatory (NK) T cell circuits in the spleen that suppress subsequent delayed-type hypersensitivity responses to s.c. injected Ag identical with the intraocular Ag (5).

A second factor involved in ocular immune privilege are the immunosuppressive cytokines found in the aqueous humor that inhibit effector immune responses in the eye (6–9). Third, the blood-aqueous barriers shield the eye mechanically from molecules, proteins, and Ag from within the body (10). Fourth, the intraocular tissues express Fas ligand, inducing apoptosis of Fas+ CTLs and other Fas+ inflammatory cells that may enter the eye (11, 12). Moreover, the inner eye seems to lack direct anatomical lymphatic drainage (13–16). Aqueous humor circulating in the AC can directly drain into the blood vasculature (17). Therefore, intraocular Ag can traffic directly into the venous circulation, bypassing the immune surveying cells in the draining lymph nodes. Nevertheless, inoculating soluble OVA into the posterior or anterior chamber of the eye has been shown to lead to an accumulation of OVA-specific TCR-transgenic CD4+ T cells mainly in the draining submandibular lymph nodes (18, 19). These findings point to a functional lymphatic drainage between the inner eye and the regional lymph nodes.

The environment in which naive tumor-specific T cells initially encounter Ag is likely to influence the outcome of the antitumor response. Consequently, it is of crucial importance to have detailed knowledge of the path through which intraocular tumor Ag primarily drains. Furthermore, it is essential to determine the site of first AC Ag encounter, because the fate and efficacy of Ag-specific CD8+ T cell immunity are highly dependent on initial Ag experience (20–24). Adoptive transfer of in vitro activated tumor-specific CTLs leads to the eradication of intraocular tumors (25).
Nonetheless, the fate and function of endogenous tumor-specific CTLs directed against tumors growing in the AC have not been well characterized, because important tools to study tumor-specific CTLs have only recently become available. We explored AC tumor-specific CTL immunity by analyzing kinetics and function of naïve tumor-specific CD8\(^+\) T cells adoptively transferred to mice bearing tumors in the AC. We used a tumor expressing an Ag derived from the human adenovirus type 5 early region 1A (Ad5E1A). This tumor grows progressively in the AC of C57BL/6 mice. Protective immunity against this tumor crucially depends on E1A-specific CTLs, as depletion of E1A-specific CTLs leads to the inability of mice to control s.c. growing tumors (26). Our results show that tumor-specific CTL activation predominantly takes place in the submandibular lymph node, rather than the spleen or other lymph nodes, indicating that intraocular tumor Ag is able to travel via a functional lymphatic drainage pathway from the AC of the eye to one local lymph node in the neck. However, these stimulated tumor-specific CTLs do not distribute systemically and CTL activity is insufficient to eradicate intraocular tumors even if systemic anti-CD40 triggering is applied.

Materials and Methods

**Mice**

Male C57BL/6 mice (H-2\(D^b\)) were obtained from The Jackson Laboratory (Bar Harbor, ME). C57BL/6 Kh (H-2\(D^b\)) mice were bred at TNO-PG (Leiden, The Netherlands). Thy-1.1 C57BL/6 (H-2\(D^b\)) were derived from the Jackson Laboratory. Strain 42 (Thy-1.2) mice, bred at TNO-PG, are TCR-transgenic mice expressing the TCR \(\alpha\) and \(\beta\)-chains derived from the H-2\(D^b\)-restricted, Ad5E1A234-243-specific CTL clone 5 (27).

**Murine tumor cell line**

The characterization of murine embryo cells transfected with the Ad5E1A plus E1-raf used in this study have been described previously (27, 28). Cell lines were maintained in IMDM (Life Technologies, Rockville, MD) supplemented with 8% FCS, 50 \(\mu\)M 2-ME, glutamine, and penicillin.

**E1A protein**

The E1A gene (13S) of the human adenovirus type 5 was amplified by PCR and cloned by Gateway Technology (Invitrogen, San Diego, CA) in a baculoviral expression vector containing an N-terminal histidine tag. The protein and cloned by Gateway Technology (Invitrogen, San Diego, CA) in a baculoviral expression vector containing an N-terminal histidine tag. The protein was overexpressed in *Escherichia coli* BL21(DE3) and purified, as described before (29). The E1A protein was dissolved in DMSO and diluted in PBS. For protein inoculation, mice were injected intracamerally with 2\(\mu\)g E1A protein diluted in 4 \(\mu\)l PBS and s.c. injections with 20 \(\mu\)g E1A-protein diluted in 200 \(\mu\)l PBS.

**Intracameral inoculations**

A previously described technique for deposition of a definite number of tumor cells into the AC of the mouse has been used (25). Mice were anesthetized with a mixture (ratio 1:1) of xylazine (Rompun 2%; Bayer, Leverkusen, Germany) and ketamine hydrochloride (Aescoken; Aesculap, Boxtel, The Netherlands) given i.p. The eye was viewed by low power (\(\times 8\)) under a dissecting microscope, and a sterile 30-gauge needle was used to puncture the cornea at the corneoscleral junction, parallel and anterior to the iris. A glass micropipette (80 \(\mu\)m in diameter) was fitted into a sterile 0.1-ml Hamilton syringe (Hamilton, Whittier, CA). The pipette, loaded with Ad5E1A-transformed tumor cells (0.5 \(\times 10^7\) cells/\(\mu\)l) or E1A protein suspension (20 \(\mu\)g/\(\mu\)l), was introduced through the puncture site of the cornea, and 4 \(\mu\)l of the tumor cell or protein suspension was delivered into the AC. The eyes were examined three times per week with a dissecting microscope to observe and document tumor growth. The draining lymph nodes were the submandibular, superficial cervical nodes and facial nodal ipsilateral to the injection. The nondraining lymph nodes were the neck lymph nodes contralateral to the eye with an intracameral inoculation.

**Subcutaneous inoculations**

E1A-expressing tumor cells (1 \(\times 10^7\) cells) or E1A protein (20 \(\mu\)g) suspended in 200 \(\mu\)l of PBS were inoculated s.c. into the right flank of 7- to 10-wk-old male C57BL/6 mice. The draining lymph node was the inguinal node at the site of injection. The nondraining lymph node was the contralateral inguinal node.

**Anti-CD40 treatment**

The FGK-45 hybridoma cells producing a stimulatory anti-CD40 Ab were provided by A. Rolink (Basel Institute for Immunology, Basel, Switzerland). Mice received 100 \(\mu\)g of the anti-CD40 mAb in 200 \(\mu\)l of PBS given i.v., at days 0, 1, and 2 of treatment. Control tumor-bearing mice were left untreated or received a control Ab, 100 \(\mu\)g of rat IgG specific for human CD40 in 200 \(\mu\)l of PBS.

**Histology**

Serial paraffin-embedded 4-\(\mu\)m sections of a murine tumor-bearing eye were stained with H&E staining.

**CFSE labeling and adoptive transfer of transgenic T cells**

CFSE labeling was performed, as previously described (30). Cells in peripheral lymph nodes and spleens from TCR-transgenic mice (strain 42) were resuspended in PBS at \(1 \times 10^8\) cells/ml and incubated with 0.5 \(\mu\)M CFSE (Molecular Probes, Eugene, OR) for 30 min at 37°C. FCS was added to a concentration of 5%, and the cells were washed in PBS. CFSE-labeled TCR-transgenic CD8\(^+\) T cells (3 \(\times 10^6\)) were injected in the tail veins of (tumor-bearing) mice in 200 \(\mu\)l of PBS.

**FACS analysis**

Cells from draining and nondraining lymph node and spleen were stained for two-color FACS analysis using the following reagents: allophycocyanin-conjugated monoclonal anti-CD8 (BD PharMingen, San Diego, CA) and PE-conjugated E1A\(_{234-243}\)-loaded H-2\(D^b\) tetramers, prepared as described (31), with the following modifications. During the refolding and subsequent purification steps, a mixture of protease inhibitors (Boehringer Mannheim, Mannheim, Germany) was added. BSA and glycerol were added to final concentrations of 0.5 and 16%, respectively. Tetramers were aliquoted, stored frozen, and used at a final concentration of 10 g/ml. For CFSE experiments, cells were stained with APC-labeled anti-CD8. Four-color FACS analysis has been performed on CFSE-labeled s42 T cells in lymphoid tissue using: PE-loaded mAb against various activation markers CD44, CD69, CD11a, and CD62L; PerCP-conjugated CD8; and APC-loaded Thy-1.2 H-2\(D^b\) tetramers. Data acquisition and analysis were performed on a BD Biosciences FACScan (BD Biosciences, San Jose, CA) using CellQuest software.

**IFN-\(\gamma\) assay**

The cells in draining and nondraining lymph nodes and spleen were assayed for IFN-\(\gamma\) secretion in mice with 35-day-old intraocular tumors. Lymph nodes of naïve C57BL/6 mice (tumor free) were also evaluated for IFN-\(\gamma\) production. Cells were incubated at 2.5 \(\times 10^7\) cells/well in 96-well plates containing irradiated Ad5E1A-expressing tumor cells (0.3–2 \(\times 10^7\) cells/well) in IMDM medium supplemented with 8% FCS, 50 \(\mu\)M 2-ME, glutamine, and penicillin in a humidified incubator with 7% CO\(_2\) at 37°C. Cultures were incubated for 3 days. Supernatant was analyzed for IFN-\(\gamma\) production by a standard sandwich ELISA.

**Results**

**Intraocular tumor Ags drain to local submandibular lymph nodes**

Based on the observations that AC Ag in the aqueous humor can directly drain into the blood to the spleen in combination with the absence of any obvious anatomical lymphatic drainage from the inner eye, it has been proposed that the spleen is the initial site in which intraocular Ag presentation occurs (32, 33). We studied this mechanism in more detail by injection of Ad5E1A-transformed tumor cells into the AC of the eye (Fig. 1) of C57BL/6 mice. Despite the presence of a highly immunogenic CTL epitope, this readily leads to the development of AC tumors. The developing intraocular tumor grows on the iris into the AC (Fig. 1) and progressively expands into the posterior segment of the eye, eventually filling up the whole eye without parting or damaging the barriers of ocular tissue or orbit (Fig. 2).

To examine where the E1A epitope derived from the intraocular Ag is initially presented to the immune system in vivo, we adoptively transferred CFSE-labeled E1A-specific TCR-transgenic T
cells into Ad5E1A intraocular tumor-bearing C57BL/6 mice. Normally, these T cells start proliferating within 3 days at the site draining the Ag, leaving this site at day 7, as determined after i.m. injection of human adenovirus 5 in the hind paw (unpublished data). Therefore, 3 days after adoptive transfer of the TCR-transgenic cells (day 19 after tumor injection), lymphoid organs of tumor-bearing recipients were removed and prepared for FACS analysis. Fig. 3A shows that proliferating CD8\(^+\) T cells were only observed within the ipsilateral submandibular lymph node draining the intraocular tumor. Very little proliferation of tumor-specific CD8\(^+\) T cells in the ipsilateral superficial cervical lymph node was seen, and no division was found in the facial and internal jugular lymph nodes (data not shown) or in the spleen. Fig. 4 gives the exact location of these cervical lymph nodes. Thus, proliferation of tumor-specific T cells could be detected as early as day 3 post-transfer only in regional draining lymph nodes, but not in nondraining lymph nodes or spleen. The selective draining of Ag from an AC tumor to the local submandibular lymph nodes might reflect the common physiological behavior of Ag present in the AC, but it could also be a unique consequence of AC tumor-derived Ag. We therefore analyzed the draining pattern of protein injected into the AC. CFSE-labeled E1A-specific T cells were isolated from the draining lymph nodes, nondraining lymph nodes, and spleen 3 days postprotein delivery in the AC. As shown in Fig. 3B, proliferating E1A-specific CTLs were only found in the local submandibular lymph nodes and not in other nodes or spleen. This was not a consequence of E1A protein leakage out of the AC into the conjunctival space, as E1A protein applied directly onto the conjunctiva did not lead to E1A-specific CTL expansion in the draining lymph node (Fig. 3C). Together, these findings indicate that a functional and dominant drainage pathway for Ag presentation to CTL is located between the AC and the local submandibular lymph node.

**Ocular Ag drainage leads to the induction of endogenous tumor-specific CTLs in draining lymph nodes**

The results described above show that naive transgenic E1A-specific TCR-transgenic CD8\(^+\) T cells encounter tumor Ag in the AC tumor-draining submandibular lymph nodes in vivo, indicating that tumorigenic events occurring in the inner eye probably elicit
a response from the immune system. Nonetheless, these intraocular tumors seem to grow unhampered in immunocompetent hosts. As this could be a consequence of the inability to mount an endogenous tumor-specific CTL reaction, we explored whether the recipients would generate tumor-specific endogenous CD8^+ T cells against AC growing E1A-expressing tumors. As the E1A Ag predominantly drains to the ipsilateral submandibular lymph nodes, we investigated these nodes in comparison with the non-draining nodes and spleen, for the presence of endogenous E1A-specific CTLs, using E1A/Db tetramers. As shown in Fig. 5, tumor-specific CD8^+ T cells were observed in the tumor-draining submandibular lymph nodes in ~75% of animals. There were no CTLs found in nondraining lymph nodes and spleens. Although these findings indicate that endogenous CTLs have been primed following Ag recognition in the draining local lymph nodes, it is not known whether these cells are functional. Therefore, the effector function of the primed endogenous tumor-specific T cells following intracameral tumor growth had been subsequently assessed by studying the IFN-γ production ex vivo. Cells of draining and nondraining lymph nodes were examined for their ability to secrete IFN-γ in response to irradiated Ad5E1A-transformed tumor cell exposure ex vivo. Lymph node cells were collected 35 days posttumor injection. The results in Fig. 6 show IFN-γ secretion from the draining submandibular lymph node cells in response to E1A-expressing tumor cell stimulation. The cells of the non-draining lymph nodes did not produce IFN-γ. Together, these findings indicate that AC tumor growth can lead to priming and expansion of functional tumor-specific T cells in the AC tumor-draining lymph nodes.
Activated and dividing E1A-specific T cells do not spread systemically

Despite the seemingly appropriate expansion and activation of tumor-specific CTLs, these CTLs were only found at tumor-draining lymph nodes, suggesting that they do not migrate systemically, leaving the AC growing tumor intact. Indeed, the intraocular tumors did not resolve in wild-type mice or even in mice that had received high numbers of E1A-specific TCR-transgenic T cells (3 × 10^6). To analyze in more detail whether tumor-specific CTLs activated in the local tumor-draining lymph nodes do not distribute to other sites, we followed activated E1A-specific CTLs from TCR-transgenic mice in time. As already outlined above, these CTLs commonly leave the Ag-draining lymph nodes ~7 days after exposure to adenovirus (our unpublished observations). We analyzed the CFSE-labeled E1A-specific CD8^{+} T cells in draining/nondraining lymph nodes and spleens up to 14 days after transfer. As shown in Fig. 7A, dividing tumor-specific CD8^{+} T cells were still only found in the tumor-draining lymph nodes 14 days posttransfer. This was not an exclusive property of Ag presentation from intraocular tumors, as similar results were obtained when mice were intracamerally inoculated with 20 μg of E1A protein following an i.v. injection of CFSE-labeled E1A-specific T cells (Fig. 7B), despite the fact that strong proliferation of E1A-specific CTLs was already observed 3 days posttransfer (Fig. 3).

Importantly, the inability to detect activated E1A-specific CTLs in nondraining lymph nodes and spleens was not due to dilution of the CFSE dye. CFSE-labeled E1A-specific CD8^{+} T cells from TCR-transgenic (Thy-1.2) mice were adoptively transferred into tumor-bearing Thy-1.1 C57BL/6 mice. Seven days posttransfer, the cells from tumor-draining lymph nodes, nondraining lymph nodes, and spleens were isolated and labeled with various activation markers, anti-CD8, and Thy-1.2 to detect all activated transgenic tumor-specific CD8^{+} T cells. CFSE-labeled E1A-specific (Thy-1.2) transgenic TCR cells in nondraining lymph nodes and spleen still displayed a naive phenotype, as determined by the expressed profile of four different activation markers (Fig. 8). Together these findings indicate that tumor-specific CD8^{+} T cells can recognize intraocular tumor Ag in the AC-draining lymph node, resulting in activated CTLs in vivo. However, this does not lead to systemic distribution of antitumor-specific CTLs.

Systemic CD40 activation does not lead to eradication of the AdE1A-expressing AC tumor

The observation that AC tumor growth can lead to the presentation of intraocular tumor-derived Ag, resulting in the activation of tumor-specific CTLs, is important, as it opens the possibility to enhance tumor-specific immunity against intraocular tumors. The inability of the CTLs to resolve the intraocular tumor is not due to failure in Ag presentation or Ag recognition, but apparently a failure to generate a systemic CTL response, as activated CTLs cannot be detected at other sites.

It has been shown that anti-CD40 treatment can be a very powerful method to improve tumor-specific CTL immunity in tumor-bearing mice, leading to the eradication of otherwise lethal tumors (26, 34). CD40 signaling has been shown to be a very powerful way to enhance tumor-specific immunity, most likely through the activation of DCs (35, 36) that cross-present tumor Ags to CTLs, as also exemplified by the up-regulation of activation markers and costimulatory molecules on CD11c^{+} cells after administration of anti-CD40 Abs (data not shown) (37). Therefore, we investigated whether systemic anti-CD40 treatment would lead to the elimination of AC tumors by the induction of an effective antitumor response similar to the observations made in the s.c. tumor system. Accordingly, we injected either intracamerally and/or s.c. E1A-expressing tumor cells into naive C57BL/6 mice. Twenty-one days after tumor inoculation, at a time when all mice had developed an AC, or s.c. tumor, or both tumors simultaneously, mice were treated systemically with agonistic anti-CD40 Ab. Although CD40 treatment was successful in the s.c. tumor settings, no significant effects were observed in mice with AC tumors (Fig. 9). Furthermore, systemic anti-CD40 treatment of mice bearing s.c. and AC tumors resulted in the eradication of the s.c. tumor, but not in the clearance of tumors growing in the AC, indicating also that local factors in the eye, rather than systemic effects, prevent AC tumor elimination. Together, these findings emphasize the notion that...
treatment of established intraocular tumors by modulating the immune system is more demanding than treatment of s.c. growing tumors, as immune-intervention protocols successful in clearing otherwise lethal s.c. tumors did not cure tumors growing in the AC of the eye.

**Discussion**

Our results provide for the first time, using a sensitive in vivo adoptive transfer model, evidence that intraocular tumor Ag drains directly into the regional lymph nodes, however not into the spleen, indicating a functional lymphatic drainage pathway from the eye involving primarily the submandibular lymph node. This observation is important, as it demonstrates that intraocular tumor Ag can be available to tumor-specific CD8+ T cells in the local draining submandibular lymph node and that these T cells are therefore not ignorant of the presence of intraocular tumor Ag.

A direct anatomical lymphatic flow from the AC to the cervical lymph nodes has never been found, and the actual exit of fluid from the eye into lymphatic spaces has not been demonstrated (13–16). It is well known that an important drainage pathway of the eye is into the venous circulation. The bulk of the aqueous humor drains via the canal of Schlemm, where a trabecular meshwork allows interaction with the venous plexus, directly into the vasculature (38). A much smaller portion of the intraocular compartment fluids is believed to drain via the uveoscleral pathway in which the flow is channeled via the ciliary muscle through the sclera and conjunctiva into the lymphatics and local draining lymph nodes (39, 40).

It has been shown that OVA injected into the AC or posterior chamber of the eye leads to the accumulation of OVA-specific T cells mainly in the hosts’ submandibular lymph nodes and spleen (18, 19, 24). The latter finding might reflect the fact that OVA is
The observation that the primed tumor-specific CTLs were not able to eradicate the intraocular tumor is, among others, explained by the fact that activated CTLs did not spread systemically and therefore were not capable of reaching the target organ, as also became evident by the absence of tumor-infiltrating CTLs in mice bearing AC growing tumors (data not shown). We consider it likely that the activated tumor-specific CD8+ T cells die a programmed cell death before they could depart the tumor-draining submandibular lymph node, as we were not able to detect these CTLs at other sites. CD8+ T cells start declining in a programmed mode as soon as Ag recognition and the 8–10 clonal cell divisions after initial Ag encounter have taken place (46), thereby contributing to maintenance of homeostatic integrity of the immune system.

An important factor in setting the balance between immunity and tolerance is the activation status of the APC. Ag presentation by immature DCs is thought to result in tolerance, whereas Ag presentation by activated DCs favors CTL immunity (47–49). Indeed, tumor-specific CTLs reacting against ocular tumors are most likely activated by professional APCs of the host, as we did not detect proliferation of CFSE-labeled tumor-specific CTLs when adoptively transferred into TAP-deficient mice (data not shown). The microenvironment of the eye is full of immunosuppressive cytokines such as TGF-β and IL-10. Ag-pulsed peritoneally derived macrophages exposed in vitro to TGF-β can generate systemic tolerance in mice (7). Likewise, IL-10-treated DCs can induce an alloantigen- or peptide-specific anergy in CD4+ and CD8+ T cell populations (50). Therefore, eye-derived APCs will most likely exhibit an immunosuppressive phenotype. More importantly, the presence of these immunosuppressive factors might even result in alternative activated DCs that have acquired a strong T cell-tolerizing capacity after exposure to maturation stimuli. This could also explain the discrepancy between CD40 treatment of mice bearing s.c. tumors and AC tumors (26, 34), as systemic anti-CD40 treatment in animals could perhaps not induce the desired maturation level of the eye-derived APC required for CTL induction, but rather an alternative activated DC endowed with the capacity to tolerate T cell responses. Because the unique microenvironment of the eye might prevent proper DC activation after CD40 ligation combined with the observation that AC tumor Ag can be presented to CTLs, it is tempting to speculate that neutralizing eye-derived APC-modulating cytokines will greatly improve immune intervention protocols. For example, proper DC activation might occur after anti-TGF-β treatment, followed by administration of DC-maturing stimuli, possibly allowing efficacious CTL induction.

Our findings provide evidence that tumor-draining submandibular lymph nodes ipsilateral to the intraocular tumor are potential sources of ongoing stimulation of tumor-specific T cells. However, additional factors are required to convert the proliferative T cell response into an effective tumor-destructive comeback. The lack of an effective immune response to intraocular tumors is not due to an inability to recognize intraocular tumor Ag, but rather to a failure of the immune response to develop and persist following Ag recognition. Attempts should be made to focus on factors that decisively drive this proliferative phase into a tumor-destructive response. Systemic anti-CD40 triggering helps CTLs eradicating conventional tumors, but does not have similar good results in AC tumors, although both tumor models show similar primary CTL activation. These findings indicate that treatment of established intraocular tumors by modulating the immune system is more demanding than treatment of s.c. growing tumors. Nevertheless, the finding that AC tumor-specific CD8+ T cells can be primed in
local draining lymph nodes offers hope for the development of more potent immune interventions against intraocular tumors.

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References