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J Immunol 2005; 175:4247-4254; ;
doi: 10.4049/jimmunol.175.7.4247
http://www.jimmunol.org/content/175/7/4247

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Maintenance of Immune Tolerance Depends on Normal Tissue Homeostasis

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Ags expressed at immune privileged sites and other peripheral tissues are able to induce T cell tolerance. In this study, we analyzed whether tolerance toward an intraocular tumor expressing a highly immunogenic CTL epitope is maintained, broken, or reverted into immunity in the event the anatomical integrity of the eye is lost. Inoculation of tumor cells into the anterior chamber of the eye of naive B6 mice leads to progressive intraocular tumor growth, an abortive form of CTL activation in the tumor-draining submandibular lymph node, and systemic tolerance as evidenced by the inability of these mice to reject an otherwise benign tumor cell inoculum. Loss of anatomical integrity of the eye as a consequence of phthisis resulted in loss of systemic tolerance and the emergence of effective antitumor immunity against an otherwise lethal tumor challenge. Phthisis was accompanied by dendritic cell maturation and preceded the induction of systemic tumor-specific CTL immunity. Our data show that normal tissue homeostasis and anatomical integrity is required for the maintenance of ocular tolerance and prevention of CTL-mediated immunity. These data also indicate that tissue injury in the absence of viral or microbial infection can act as a switch for the induction of CTL immunity. * The Journal of Immunology, 2005, 175: 4247–4254.

Central tolerance, induced in the thymus, plays a pivotal role in the prevention of undesired immune attack against the own tissues of the body. However, next to central tolerance, tolerance induced in the periphery is also important in maintaining the homeostasis and integrity of the body (1). Recognition of Ags in a noninflammatory environment has been postulated to induce tolerance, whereas Ag recognition in a proinflammatory context will lead to immunity. For example, activation of Ag-presenting dendritic cells (DC), either through inflammatory signals delivered via the innate immune system, as occurs during microbial or viral infection, or through interaction with CD4+ T cells or anti-CD40 Abs results in the development of strong CD8+ CTL responses (2–5). In contrast, little information is available on the magnitude of the immune response against self-Ags or tumor Ags (6), particularly if alarm signals are derived from injured cells that have not been exposed to pathogens or toxins but instead are released during “spontaneous” tissue damage. For instance, it is not known whether tolerance against a defined Ag could be broken once the tissue (bearing this Ag) is damaged.

A classical example of an organ involved in preventing and dampening undesired immune attack is the eye. The eye being an “immune privileged site” is very efficient at preventing immunemediated elimination of cells present in the eye that express foreign Ag (7). Originally, an immune privileged site was an anatomic location where transplanted foreign tissue survives for an extended period of time in an immunocompetent recipient. Evolutionary, immune privilege of the eye is important because the few irregularities in the delicate anatomy of the eye can already severely affect vision and thus survival. Therefore, ocular immune privilege is likely to play an important role in the protection of the eye against hazardous immune responses.

Originally it was believed that immune privilege in the eye was due to lack of lymphatic drainage and the presence of blood-ocular barriers (8). Consequently, ocular Ag would be hidden from the immune system while immune cells could not enter the eye, thereby sparing the organ from the destructive effects of inflammatory cells (9). Currently, it is acknowledged that ocular immune privilege is much more multifaceted. It is an active process using numerous mechanisms to maintain organ function. These mechanisms include, among others, the production and activity of immunosuppressive cytokines and the expression of Fas ligand and TNF-related apoptosis-inducing ligand (10–12). Another aspect of ocular immune privilege is an experimental phenomenon called anterior chamber acquired immune deviation (ACAID). This feature describes how the inoculation of foreign Ag into the anterior chamber of the eye can generate systemic tolerance resulting in an inhibition of the delayed-type hypersensitivity (DTH) against the inoculated foreign Ag (13).

Although ocular immune privilege and ACAID apply to both MHC class II- and class I-restricted T cell responses, most studies on ocular immune privilege and ACAID have focused on MHC class II-restricted CD4+ T cell-mediated immunity (7, 14). These studies showed an important role for eye-derived APCs that activate NKT cells in the spleen that are required for the generation of CD8+ T regulatory cells capable of suppressing MHC class II-restricted DTH-responses (15). However, MHC class I-restricted CD8+ T cell responses are also affected by the presence of Ag in the eye. For example, CTL immunity toward OVA is inhibited when OVA is injected into the AC (16). The inhibition of CTL responses was the result of functional unresponsiveness rather than...
clonal deletion of Ag-specific CD8+ T cells. This result was determined by analyzing the response of Ag-specific T cells from TCR-transgenic mice. Likewise, it has been postulated that the emergence of T regulatory cells inhibits CTL immunity. ACAID can be induced in both naive mice and presensitized animals (17), and once induced, it is very long lasting and highly resistant to termination (7).

The disadvantage of ocular immune privilege is that intraocular tumors, although rare, can grow unhampered leading to severe mortality and morbidity. Typically, an intraocular tumor, such as a melanoma, grows quietly and insidiously without the patient noticing anything until vision becomes obscured by the enlarging tumor. At such time, distant metastases may have already occurred from which the patient will eventually die. In some cases, progressive intraocular tumors present as phthisis (i.e., disintegrate) in which the tumor-containing eye has shrunk (18, 19) because of extensive infiltration of tumor or inflammatory cells into the ciliary body (CB). The normal functioning CB produces aqueous humor to maintain the regular intraocular pressure of the eye. Once 90% or more of the CB is infiltrated or damaged by tumor or inflammatory cells, it cannot produce aqueous humor any longer resulting in hypotonia of the eye followed by phthisis, or literally shrinkage of the eye bulb.

Previously, we have shown that, although intraocular tumor Ags are constitutively presented to CD8+ T cells in the local draining lymph nodes (DLN), it did not lead to tumor eradication (20). Moreover, Ag presentation did not lead to proper systemic CTL immunity, as tumor-specific CTLs were not found outside the tumor DLN. We studied the consequences for ocular tolerance and immunity in case “spontaneous” phthisis occurs resulting from progressive tumor growth. Our data show that intraocular tumor growth leads to the inability of the AC tumor-bearing mice to control an otherwise unharmful tumor cell inoculum placed at a distant site in the body. Tissue damage in the absence of microbial or viral infection can break this established tolerance against tumor Ags, which suggests that normal tissue homeostasis is required for the maintenance of ocular tolerance.

Materials and Methods

Mice

C57BL/6 mice were purchased from Charles River Breeding Laboratories. TAP−/− mice and CD40−/− mice (both on C57BL/6 background) were purchased from The Jackson Laboratory. Strain 42 mice, bred at the Netherlands Organisatie voor Toegang Natuurwetenschappelijk Onderzoek-Preventie en Gezondheid (TNO-PI) are TCR transgenic mice expressing the TCR α and β chains derived from the H-2b-restricted early region 1A of human adenovirus type 5 (Ad5E1A)234–243-specific CTL clone 5 (21). Mice were kept at the Leiden University Medical Center Animal Facility (Leiden, the Netherlands) and used at 5–13 wk of age in accordance with national legislation and approval of the Animal Experimental Committee of the Leiden University.

Tumor cells

Mouse embryo cells transformed by the Ad5E1A plus EJ-ras were cultured in IMDM (Invitrogen Life Technologies) supplemented with 8% (v/v) FCS, 50 μM 2-ME, glutamine, and penicillin, as described (21, 22). Different kinds of Ad5E1A plus ras-transformed tumor cell lines were used. Some lines were progressively growing s.c. and therefore lethal in naive mice and others were nonlethal because they are rejected after a short period of growth following s.c. injection into naive mice (21, 23).

Tumor experiments

Intracameral inoculations. A previously described technique for deposition of a definite number of tumor cells into the AC of the mouse has been used (24). Mice were anesthetized with a mixture (ratio 1:1) of xylazine (2% Rompun; Bayer) and ketamine hydrochloride (Aescocoet; Aesculaap) given i.p. The eye was viewed by low power (×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 μm in diameter) was fitted into a sterile infant feeding tube, which was mounted onto a sterile 0.1-ml Hamilton syringe. The pipette, loaded with Ad5E1A-transformed tumor cells (0.75 × 106 cells/μl) was introduced through the puncture site of the cornea, and 4 μl of the tumor cell suspension was delivered into the AC. The eyes were examined three times a week with a dissecting microscope to observe and document tumor growth and the anatomical integrity of the eye.

Subcutaneous inoculations. EIA-expressing tumor cells (1 × 106) were injected s.c. into 7- to 10-wk-old male mice in 200 μl of PBS. Tumor size was measured twice weekly with calipers in three dimensions. Mice were sacrificed when tumor size exceeded 1 cm3 to avoid unnecessary suffering.

Histology

Intracameral inoculation

Single cell suspensions were generated from spleen and peripheral lymph nodes of strain 42 mice. Erythrocytes were depleted by ammonium chloride treatment (2 min on ice). Cells were washed once in cold medium and once in cold PBS, after which they were resuspended in PBS at 1 × 108 cells/ml and incubated with 0.5 μM CFSE (Molecular Probes) for 30 min at 37°C. FCS was added to a concentration of 5% FCS, and the cells were washed in PBS. TCR-transgenic CD8+ T cells (3 × 105) were injected into the tail vein of intracocular tumor-bearing mice in 200 μl of PBS.

Flow cytometry

Single cell suspensions of spleens, lymph nodes, and tumor-containing eyes were prepared by mechanical disruption. Blood samples and cell suspensions of spleens were depleted of erythrocytes by ammonium chloride treatment for 5 min at room temperature. Cells were stained with directly allophycocyanin-conjugated mAb against CD8 (clone 53-6-7; BD Pharmingen) combined with PE-conjugated EIAA244–255,444–455,444–465 tetramers (EIA-TCM) or PE-conjugated HPV16-loaded H-2Dd tetramers as a control. After CD11c enrichment, cells were stained with allophycocyanin-conjugated mAb against CD11c (clone HL3; BD Pharmingen) combined with stainings for CD80 (clone 16–10A1; BD Pharmingen), CD86 (clone GL1; BD Pharmingen), CD40 (clone 3/23; BD Pharmingen), I-A/I-E (clone M5/114.15.2; BD Pharmingen), or H-2Kb (clone AF6-88.5; BD Pharmingen). Data acquisition and analysis were done on a BD Biosciences FACScan with CellQuest software.

Separation of CD11c+ and CD11c− populations

Peripheral lymph nodes of tumor-bearing mice were treated with collage

nase (250 U/ml; Sigma-Aldrich) and DNase (50 μg/ml; Sigma-Aldrich) for 30 min at 37°C. CD11c+ cells were positively selected using magnetized Ab for CD11c (N418; Milteny Biotec). A purity of ~90% of the CD11c+ cell population was obtained as determined by FACS analysis.

Results

Collapse of the anatomical integrity of a tumor-containing eye leads to systemic distribution of endogenous tumor-specific CTL

Recently, we described that intraocular tumor Ags are presented to tumor-specific CTL in tumor DLN (20). The Ag is likely to be derived from the ocular growing tumor, as previous control experiments have shown that leakage of tumor cell suspension into the conjunctival sac during the inoculation into the AC does not lead to T cell priming in the DLN (20). However, no effective antitumor immune response could be found in the tumor-bearing animals because the endogenous tumor-specific CD8+ T cells do not distribute systemically. For these studies we used a tumor model of tumor cells transformed by Ad5E1A that do not metastasize to other parts of the body. These tumor cells harbor a highly immunogenic CTL epitope that is recognized by EIA-specific CTL. As no effective tumor-specific CTL responses are generated, EIA-transformed tumors are not cleared and grow progressively in the AC of the eye of syngeneic C57BL/6 mice. However, upon further experimentation it was found that in some cases, several weeks after tumor inoculation, systemic EIA-specific CTL responses were present. The presence or absence of EIA-specific CTLs in the
periphery appeared to be correlated with two distinct patterns of progressive intraocular tumor growth. The first pattern was characterized by a developing intraocular tumor that grows on the iris into the AC and progressively expands into the posterior segment of the eye. Eventually the whole eye is filled with tumor cells without damaging the barriers of ocular tissue or orbit, leaving the intraocular structures and surrounding eye bulb intact (Fig. 1A) vs phthisis (C). Both types show anterior and posterior tumor expansion. There is no tumor growth outside the eye bulb. Note the decrease in size of both intraocular tumor and eye in the phthisis intraocular tumor (C). Box (inset) in A–C are enlarged in D and E, which show the CB in detail of the naive eye (D), the nonphthisis (E), and phthisis intraocular tumor in which the CB has been completely destructed. C, cornea; L, lens; VB, vitreous body.

Phthisis precedes the emergence of tumor-specific CTL

To determine which of the two features, intraocular CTLs or phthisis occurred first, we set out to analyze the presence of endogenous E1A-specific CTLs in different stages of tumor growth: nonphthisis (B) vs phthisis (C). Both types show anterior and posterior tumor expansion. There is no tumor growth outside the eye bulb. The decrease in size of both intraocular tumor and eye in the phthisis intraocular tumor (C). Box (inset) in A–C are enlarged in D and E, which show the CB in detail of the naive eye (D), the nonphthisis (E), and phthisis intraocular tumor in which the CB has been completely destructed. C, cornea; L, lens; VB, vitreous body.

FIGURE 1. Two types of progressive intraocular Ad5E1A-expressing tumor growth in C57BL/6 mice. Sagittal view of a naive (tumor-free) eye (A) and two types of progressive intraocular tumor growth: nonphthisis (B) vs phthisis (C). Both types show anterior and posterior tumor expansion. There is no tumor growth outside the eye bulb. Note the decrease in size of both intraocular tumor and eye in the phthisis intraocular tumor (C). Box (inset) in A–C are enlarged in D and E, which show the CB in detail of the naive eye (D), the nonphthisis (E), and phthisis intraocular tumor in which the CB has been completely destructed. C, cornea; L, lens; VB, vitreous body.

FIGURE 2. Collapse of the anatomical integrity of a tumor-containing eye results in systemic distribution of endogenous tumor-specific CTLs. Endogenous E1A-specific CTLs are readily detectable systemically in mice undergoing complete phthisis of the eye; however not in mice with nonphthisis. In the latter case, E1A-specific CTLs were only found in the tumor DLN. Although, a relatively small number of E1A-specific CTLs were detected in some mice with prephthisis (20%), probably as a consequence of the ongoing phthisis process, no E1A-specific CTLs were detected systemically in the majority of mice (80%) with prephthisis intraocular tumors. Together these findings indicate that the occurrence of phthisis

FIGURE 2. Collapse of the anatomical integrity of a tumor-containing eye results in systemic distribution of endogenous tumor-specific CTLs. Endogenous E1A-specific CTLs are only observed in the tumor DLN of mice with nonphthisis intraocular tumor growth and have distributed systemically in mice with phthisis intraocular tumor growth. Mice bearing an Ad5E1A-expressing tumor were sacrificed 63 days after tumor inoculation. FACS analysis was performed in DLN, NDLN, spleen, and intraocular tumors using D\textsuperscript{D\textsuperscript{b}}E1A tetramers. One representative experiment of 10 is shown.
Intraocular tumor Ags are cross-presented by CD11c+ cells to naive CTL in local DLN

The results we describe illustrate systemic distribution of tumor-specific CD8+ T cells after collapse of the anatomical integrity of the tumor-containing eye (phthisis). As no such systemic distribution was observed in tumor-bearing mice with intact intraocular structures, these findings indicate that signals are released during phthisis development, which is beneficial to the induction of CTL response. These signals could relate to Ag presentation, as well as to the context in which Ag presentation occurs. Activation of CTL is generally accepted to result from cross-presentation of Ag by DCs that have acquired Ag from tumor cells. However in some instances, direct presentation of Ag by tumor cells have also been implicated as the predominant mechanism by which CTLs are activated. To study whether E1A-specific CTL are primed directly by tumor cells in tumor DLN or by host APC, we analyzed whether intraocular tumor cells can be found in the secondary lymphoid organs of tumor-bearing animals. Twenty-one days after intracameral injection of E1A-expressing tumor cells, secondary lymphoid organs were examined. Selective in vitro outgrowth of lymph node and spleen cultures followed by PCR showed the presence of tumor cells in tumor DLN, but not in NDLN or spleen (data not shown). Subsequently we investigated whether these tumor cells, which are able to reach the lymphoid organs, were capable of presenting tumor Ag in vivo. Therefore, we injected E1A-expressing tumor cells into wild-type and TAP−/− mice. TAP−/− mice cannot cross-present the E1A-Ag because presentation of this epitope is strictly TAP-dependent (25, 26). In case the E1A epitope is presented directly by tumor cells to E1A-specific CTL, it is expected that the E1A-epitope will also be presented in mice deficient from TAP. Nineteen days after tumor inoculation, at a time intraocular tumors had developed, CFSE-labeled E1A-specific CD8+ T cells derived from TCR-transgenic mice were injected. Three days later, division of tumor-specific T cells in different lymphatic organs was analyzed. As shown in Fig. 4, only division of CFSE-labeled cells was observed in tumor-bearing wild-type mice but not in TAP−/− mice, indicating that host-derived APC presented the ocular tumor Ag in a TAP-dependent fashion.

FIGURE 3. Phthisis precedes the emergence of tumor-specific CTLs. Endogenous E1A-specific CTLs are never observed in a nonphthisis intraocular tumor (A) or in the very early stages of prephthisis when the tumor-bearing eye starts to show some signs of collapse (B). Tumor-specific CTLs are found in small numbers in prephthisis tumors (C) and are found in large amounts in phthisis intraocular tumors (D). In the prephthisis intraocular tumor (B and C), the eye has started to deteriorate, increasingly showing reduced eye bulb size, opacified and thick cornea, and increasing collapse of the AC and vitreous body (VB). Mice bearing an Ad5E1A-expressing tumor were sacrificed at several time points after tumor inoculation either for histology of the intraocular tumors or for FACS analysis performed in tumor-bearing eyes using D9/E1A tetramers. One representative experiment of three is shown.

FIGURE 4. Host-derived APCs are required for presentation of tumor Ags. CFSE-labeled E1A-specific CD8+ T cells were adoptively transferred into wild-type mice or TAP−/− mice bearing 19-day-old tumors in the AC of the eye. Three days posttransfer proliferation of adoptively transferred E1A-specific T cells in DLN, NDLN, and spleen was analyzed by FACS. One representative experiment of three is shown.

precedes the emergence of systemically distributed tumor-specific CTL, suggesting that during phthisis signals are released that trigger proper CTL responses.
Because CD11c<sup>+</sup> cells are most likely the host-derived APCs responsible for cross-presentation of tumor Ags to CTL (27), and because we have shown recently that the E1A-Ag is presented by CD11c<sup>+</sup> cells (but not by CD11c<sup>-</sup> cells) from (s.c.) growing tumors, we subsequently studied whether phthisis would lead to maturation of these cells as APC activation is considered crucial for induction of CTL immunity (2, 28). For this purpose, CD11c<sup>+</sup> cells in tumor DLN of mice with phthisis eye tumors and nonphthisis intraocular tumors were analyzed. In Fig. 5 it is shown that CD11c<sup>+</sup> cells isolated out of the DLN from mice with phthisis intraocular tumor growth had up-regulated several surface markers such as CD80, CD86, CD40, and MHC class I and class II in comparison with the CD11c<sup>+</sup> cells isolated from nonphthisis eye tumor-bearing mice. CD11c<sup>+</sup> cells isolated out of the phthisis intraocular tumors also displayed an up-regulation of these activation markers contrary to the CD11c<sup>+</sup> cells out of the nonphthisis eye tumors (data not shown). Together these findings indicate that collapse of the anatomical integrity of a tumor-containing eye led to an activated phenotype of the CD11c<sup>+</sup> cells present in tumor DLN and the phthisis intraocular tumor itself. As presentation of the E1A epitope depends on host-derived APCs, our results indicate that the emergence of systemic CTL immunity results from the presentation of the E1A epitope by DCs that are endowed with the capacity to activate CTLs allowing their systemic distribution as a consequence of phthisis.

**Collapse or preservation of intraocular tumor-containing structures determines the outcome of sequential immune responses: immunity vs tolerance**

As outlined, an intraocular tumor in a deteriorating eye leads to systemic spread of tumor-specific CTLs. To analyze whether these systemic CTLs are effective in vivo, mice with primary intraocular tumors with or without phthisis were challenged s.c. with Ad5E1A plus ras-transformed tumor cell lines. For these experiments, we took advantage of two tumor cell lines that have different s.c. growth patterns in naive mice. One tumor cell line grows progressively and is lethal in all naive C57BL/6 mice (22). The other tumor cell line is rapidly eradicated after s.c. injection in naive C57BL/6 mice (21). In a group of 30 mice with intraocular tumors, 17 mice developed phthisis 8 wk post tumor inoculation. The remaining 13 tumor-bearing mice maintained “normal” intraocular integrity. Injecting the lethal progressive E1A-expressing tumor s.c. in both groups resulted in a 100% tumor-take and death in mice without any phthisis signs in contrast to 0% tumor outgrowth in mice with phthisis (Fig. 6A). All naive mice developed lethal s.c. tumors (Fig. 6A). As rejection of these E1A-expressing tumors crucially depends on E1A-specific CD8<sup>+</sup> CTL immunity (29), these results indicate that phthisis leads to the induction of strong tumor-specific CTL responses that protect against a secondary tumor challenge.

In another group of 30 mice with intraocular tumors, 20 mice developed phthisis compared with 10 mice with nonphthisis intraocular tumor growth. Inoculation (s.c.) of the regressor E1A-expressing tumor cell clone led, as expected, to complete tumor rejection in naive mice and in mice with phthisis intraocular tumors (Fig. 6B). However, secondary s.c. tumors developed in all mice with nonphthisis intraocular tumor growth (Fig. 6B). Thus, because the ability of the mouse to control a challenge of this otherwise nonlethal (spontaneous regressing) tumor is lost in the case of intraocular tumor growth, these results show that intraocular tumor growth results in systemic tolerance that allows the outgrowth of an otherwise nonlethal tumor. More importantly, they also indicate that this tolerance is broken if anatomical integrity of the eye is lost. Together, these findings indicate that collapse or preservation of intraocular structures in a tumor-containing eye determines the systemic immunological immune responsiveness, respectively resulting in immunity or tolerance against secondary tumor challenges.

**Discussion**

Our results show that ocular immune privilege is broken when the anatomical integrity of the eye is disrupted. Mice with established tumors growing in the AC are incapable of rejecting an otherwise nonlethal tumor inoculated s.c., but are able to resist an aggressive, otherwise lethal tumor, when phthisis occurs spontaneously. These findings are strongly correlated with the systemic emergence of tumor-specific CTLs in case of phthisis, explaining the rejection of a s.c. tumor, as rejection of this tumor crucially depends on CTL-mediated immunity (21). In case no phthisis occurred, although

**FIGURE 5.** Collapse of the anatomical integrity of the tumor-containing eye leads to activated CD11c<sup>+</sup> cells. C57BL/6 mice were injected intracamerally with 0.75 × 10<sup>6</sup> E1A-expressing tumor cells. When phthisis (broken histogram) or nonphthisis (shaded histogram) intraocular tumor growth had developed, mice were sacrificed and CD11c<sup>+</sup> cells were isolated out of the lymph nodes and intraocular tumors for analysis of CD80, CD86, CD40, MHC class I and class II expression. Naive tumor-free mice were included in the experiment (thick histogram). One representative experiment of three is shown.
tumor-specific CTL are present in tumor DLN, the animal could not mount a tumor-protective CTL response any longer, as reflected by the inability to reject a challenge with benign tumor cells. Because such CTL responses are crucial for the eradication of these s.c. tumors (21, 23, 30), these results indicate that ocular tumor growth in intact eyes leads to an “abortive” or “poised” CTL reaction.

Apparently, the tumor growing in the AC recruits effective suppressor mechanisms that do not only exert local, but also systemic effects that allow the s.c. growth of an otherwise nontumorigenic E1A-transformed tumor cell clone. The induction of such systemic effects appears to be specific of AC tumor growth because we did not observe these effects when the same tumor was injected s.c. (data not shown). The observation that the tolerance and suppressive mechanisms installed by the AC growing tumor are broken (data not shown). The observation that the tolerance and suppressive mechanisms installed by the AC growing tumor are broken (data not shown).

FIGURE 6. Collapse or preservation of intraocular tumor-containing structures determine the outcome of sequential immune responses: immunity vs tolerance. Secondary “lethal” (A) or “nonlethal” (B) syngeneic s.c. tumors were inoculated (10^6 E1A-expressing tumor cells) in mice with progressive nonphthisis (●) or phthisis (▲) intraocular tumor growth and in naive mice (primary s.c. tumor (■)). Secondary “lethal” tumors (A) develop s.c. in naive mice (■) and in mice with nonphthisis intraocular tumor growth (●). Secondary s.c. tumor growth was inhibited in mice with phthisis intraocular tumor growth (▲). Secondary “nonlethal” tumors (B) only develop in mice with nonphthisis intraocular tumor growth (●) and was inhibited in mice with phthisis intraocular tumor growth (▲) and in naive mice (■).

As DCs are the predominant, if not the only, cells capable of generating CTL immunity (27, 33, 34), it is likely as outlined that the outcome of immune reactivity is a consequence of DC activation.

The data presented indicate that CD11c^+ cells display a relatively “resting” phenotype in mice with nonphthisis intraocular tumor growth, allowing Ag presentation in a nonimmunogenic or even tolerogenic fashion (34, 35). It is conceivable that CTLs recognizing Ag on such quiescent DCs go through an abortive/posed form of activation that ultimately leads to deletion as is also observed in other systems (32, 36, 37). This would explain our observation that we do not observe a systemic dispersal of tumor-specific CTL that have been activated in the local DLN. However, the inability of the animals to control the growth of otherwise nonlethal E1A-expressing tumors implanted s.c. could also result from other mechanisms, such as the emergence of tumor-directed regulatory T cell responses. Stimulation of the immune system under tolerogenic conditions as operative in the ocular environment might lead to the systemic emergence of such regulatory T cells that inhibit the induction of tumor-specific CD8^+ T cells at other sides. Thus, two levels of ocular tolerance might occur, one is leading locally to an abortive form of T cell activation, whereas the other results systemically in active inhibition of tumor-specific immunity.

Either way, our results indicate that phthisis can bypass ocular tolerance leading to the induction of proper systemic antitumor immunity, conceivably as a result of local APC activation (34). Although we cannot rule out the possibility that eyes undergoing phthisis are susceptible to some kind of low-grade infection that provides danger signals, the acquisition of the immunostimulatory DC phenotype is acknowledged. The theory is in line with the concept that stressed cells release signals such as uric acid that locally activate APC so that they present captured Ag along with


