Ocular Immune Privilege and the Faustian Dilemma

The Proctor Lecture

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The eye and the immune system represent discrete organ systems in vertebrates. Each possesses a unique and vital function that ensures the viability of the host, and loss of either or both brings disease and death. For many years, it has been known that the eye and the immune system are joined in a curious phenomenon called "immune privilege." This phenomenon was first described experimentally more than 100 years ago, and for much of that time, immune privilege has been regarded chiefly as a laboratory curiosity. However, recent evidence strongly supports the view that immune privilege is an important physiologic adaptation that contributes in a significant way to the success of vertebrates. The interaction between the immune system and the eye that leads to immune privilege is best approached by considering the functions and challenges of these two very different organ systems.

FUNCTIONS OF IMMUNE SYSTEM

The primary function of the immune system is to protect the body against exogenous and endogenous pathogens—by eliminating or inactivating them. In this manner, host viability is sustained. If the immune system fails, the host is overcome by pathogens, and death is the inevitable consequence.

To achieve immune protection against pathogenic agents, the immune system must address four distinct challenges:

1. It must create a library of cells (lymphocytes) with recognition structures (T-cell receptors for antigens, and antibodies) sufficiently diverse to detect all biologically important molecules (antigens) in our universe.
2. Having achieved this goal, the system must then eliminate or inactivate those lymphocytes with antigen receptors that have the capability to recognize the body's own molecules (autoantigens). Elimination of these autoreactive lymphocytes avoids the threat of autoimmunity.
3. In addition to antigen-recognizing T-cell receptors and antibodies, the immune system must generate an array of effector mechanisms (immune cells and molecules; see Table 1) that are sufficiently diverse in functional properties to meet the enormously diverse pathogenic strategies devised by the numerous and diverse pathogenic agents in our environment.
4. With such a wide array of functionally distinct effector modalities, the immune system must then fashion particular immune responses in such a manner that protection is provided for individual organs and tissues without compromising the physiologic functions of these vital tissues.

FUNCTIONS OF THE EYE

The primary functions of the eye and visual system are to receive, at the level of the retina, accurate light images from the world, to initiate their processing in situ, and to transmit these processed images faithfully to the brain. For sighted organisms, maintenance of vision is vital to survival, and loss of vision leads inevitably to death.

To provide accurate sight, the eye and visual system must address four distinct challenges:

1. From neural crest and ectoderm, a vision apparatus must be developed, an apparatus that is composed of diverse cells (neuronal, epithelial, endothelial, supporting) that express a large array of unique molecules that are only displayed in the eye.
2. Once the eye's delicate microanatomic structure is created during differentiation, this structure must be preserved intact for the remainder of the organism's life. In part, this is accomplished by reducing and/or eliminating the ability of ocular parenchymal cells to replicate.
3. Given its anatomic location, the eye must be

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TABLE 1. Immune Effector Cells and Molecules

Effector	*Type*	*Function*	*Proinflammatory?*
CD4+	T cell	Delayed hypersensitivity	Yes (macrophages)
CD8+	T cell	Cytotoxic	No
IgM	Antibody	Intravascular	Yes (complement, PMNs)
IgG1	Antibody	Extravascular	No
IgG2a	Antibody	Extravascular	Yes (complement, PMNs)
IgA	Antibody	Mucosa	No
IgE	Antibody	Extravascular	Yes (Mast cells)

Ig = immunoglobulin; PMNs = polymorphonuclear leukocytes.

linked to the body (for sustenance and for immune protection) and to the brain (for information transfer).

4. To maintain accurate vision, the eye must develop strategies that limit its vulnerability to the blinding consequences of trauma, inflammation, neovascularization, regeneration, and autoimmunity.

It is at the intersection of the challenges respectively facing the eye and the immune system that the phenomenon of immune privilege arises. On the one hand, the eye, as do other organs, requires immune protection against pathogens, yet it must avoid the blinding consequences of autoimmune disease. On the other hand, the immune system can recognize unique molecules within the eye as "foreign," and can deploy "protective" effector mechanisms that are sight threatening.

THE FAUSTIAN DILEMMA

This intersection confronts the eye and the immune system with a dilemma that requires a novel solution to avert disability and disease, and that novel solution proves to have its own ambiguity for the integrity of the eye and the immune system. In certain ways, the dilemmas facing the eye and the immune system at this functional intersection resemble the dilemma faced by the legendary medieval character, Georg (or Johann) Faust. This unusual man was purported to be both an alchemist and a sorcerer. He was believed to have consorted with the devil to make gold from ignoble metals and to obtain material gains beyond his talents. Johann Wolfgang Goethe, the supreme literary figure in German culture, took this legend and recast Faust as an aging, but gifted and accomplished, professor who believed that his life was passing him by—without his having experienced that life to its fullest. In Goethe's drama,[1] Faust encounters a black poodle who is, in reality, the devil, Mephistopheles. Selected readings from Goethe's drama describe this encounter.

FAUST
I have become too overblown . . .
The threads of thought are torn to pieces,
and learning has become repugnant.

Faust sought to experience life to its fullest, to experience the greatest ecstasy and the deepest agony. Goethe's word for this supreme moment was (appropriately) *augenblicke,* which translates as "in the blink of an eye."

FAUST
Let in the throes of raging senses
Seething passions quench my thirst! . . .
Let me plunge into the rush of passing time,
into the rolling tide of circumstance!

MEPHISTOPHELES
I hope your pleasures may agree with you.

To reach his goals, that is, to achieve augenblicke, Faust proceeded to bargain with the devil.

FAUST
I told you I am not concerned with pleasure.
I crave corrosive joy and dissipation . . .
My breast no longer thirsts for knowledge . . .
I want to seize the highest and the lowest . . .
and thus expand my single self titanic ally
and in the end, go down with all the rest.
. . . I suppose a pact might be concluded with you, gentleman.

MEPHISTOPHELES
The promises we make you shall enjoy in full,
we will not skimp or haggle . . .
My friend, in this one hour you will gain
far more for all your senses
than in a year's indifferent course.
You will be bathed in ecstasy . . .

FAUST
And in return, what do you ask of me?

MEPHISTOPHELES
I pledge to serve you here and now.
. . . and if beyond we meet again,
you shall do the same for me.

FAUST

Werd' ich zum Augenblicke sagen:	*If ever I should tell the moment:*
Verweile doch! du bist so schoen!	*Oh, stay! You are so beautiful!*
Dann magst du mich in Fesseln schlagen.	*Then you may cast me into chains.*

Goethe's play then proceeds to describe Faust's life experiences after the compromise he makes with Mephistopheles. The dramatic episode that came to epitomize the augenblicke occurs when Faust sees and desires to make love to a beautiful maiden, Margaret.

It is our thesis that the eye also makes a compromise to achieve its augenblicke—unperturbed and accurate vision. This compromise is made with the immune system such that some, but not all, immune effector mechanisms are selected, and the chosen effectors provide protection that carries little or no risk of sight-threatening inflammation. Ocular immune privilege is the manifestation of this compromise.

RECONSIDERATION OF IMMUNE PRIVILEGE IN THE EYE

To set the stage for the studies conducted in our laboratory, it is important to define and describe the phenomenon of immune privilege, which exists at specific sites and for specific tissues. In the case of the former, foreign tissues placed in privileged sites experience extended (often indefinite) survival, whereas similar tissues placed in conventional (nonprivileged) sites are rejected promptly. In the latter case, privileged tissues grafted into conventional (nonprivileged) sites experience extended (often indefinite) survival, whereas nonprivileged tissues placed in conventional sites are rejected promptly.

In 1970, the established view of immune privilege had been formulated originally by Sir Peter Medawar in 1948.[2] Medawar based his synthesis on the fact that the eye lacks lymphatic drainage. To the transplant immunologists of that era, a lymphatic drainage pathway was essential for sensitization against the antigens expressed on solid tissue allografts. Because of the absence of lymphatic drainage, Medawar proposed that immune privilege existed because of "immunologic ignorance," that is, that antigenic material placed in the eye simply never escaped and, therefore, was never detected by the immune system.

In 1971, a postdoctoral fellow in my laboratory in Dallas, Texas, Henry J. Kaplan, MD, began a series of studies designed to reexplore the mechanisms of ocular immune privilege. During the course of those studies, we discovered an unusual form of systemic immunity that appeared after alloantigenic lymphoid cells were injected into the anterior chamber of rat eyes (F_1 lymphocyte-induced immune deviation).[3] After implantation of allogeneic lymphocytes in the anterior chamber, alloantibodies were detected in recipient sera, indicating that *antigens placed in the anterior chamber (AC) can escape and activate the immune response systemically.* Moreover, skin grafts from the original donor survived longer than expected when placed orthotopically on AC-treated rats, indicating that *immune responses activated by the AC are deficient in the immune effector mechanisms responsible for acute skin graft rejection.* Based on these findings, we concluded that immune privilege is not a passive process of immune ignorance of intraocular antigens, as originally proposed by Medawar. Instead, we proposed that immune privilege is an active process in which the immune response to ocular antigens is regulated in a deviant, stereotypic way.

This active process was explored in much greater detail by my second major collaborator, Jerry Y. Niederkorn, PhD, also at Dallas. Niederkorn developed a model system that enabled immune privilege to be studied in eyes of laboratory mice, and, as a consequence, the phenomenon of anterior chamber-associated immune deviation (ACAID) was described.[4,5] When weakly antigenic tumor cells from DBA/2 mice were injected into the AC of BALB/c mice, it was observed that tumors formed in the AC and grew progressively, confirming that *immune privilege is extended to foreign tumor cells in the AC.* Mice bearing ocular tumors of this type accepted orthotopic DBA/2 skin grafts, even though the mice acquired donor-specific cytotoxic T cells. Moreover, these mice failed to display DBA/2-specific delayed hypersensitivity (DH). Curiously, mice with tumors in the AC rejected DBA/2 tumor cells injected subcutaneously, indicating that *concomitant immunity also is elicited by AC injection of antigen.* Finally, it was observed that spleen cells from mice with AC tumors prevented DH from developing in syngeneic recipient mice immunized subsequently with the same tumor cells. Thus, *AC-injected antigens induce T cells that suppress DH.*

The constellation of features that characterize ACAID can be summarized as follows[5–7]: Antigens placed in the AC evoke a deviant systemic immune response that (1) lacks the T-cell mediators of DH, (2) includes a unique group of T lymphocytes that suppresses immunogenic inflammation, and yet (3) retains the immune mediators of concomitant immunity. ACAID is now regarded as a critical component of the active mechanism that creates and maintains ocular immune privilege. In separate experiments, Niederkorn and Kaplan both made the remarkable observations that led us to postulate the existence of a camero-splenic axis. When antigen is injected into the AC, ACAID fails to develop if either the injected eye or the spleen is excised within 5 days. This was interpreted to mean that *local factors within the eye, along*

with systemic factors within the spleen, contribute together to the successful development of ACAID and to ocular immune privilege.

OCULAR FACTORS IN IMMUNE PRIVILEGE

In 1984, our laboratory moved to the Department of Microbiology and Immunology at the University of Miami School of Medicine, where my next major collaborator was Bruce R. Ksander, PhD. Ksander directed his experimental approach toward the observations that mice with ocular tumors possess tumor-specific T cells in their lymph nodes and spleens but that these potential effector cells do not cause rejection of eye tumors. He isolated lymphocytes from tumor-bearing eyes to assay them for cytotoxic activity and to examine their functional responses to helper factors secreted by other T cells. Ksander found[8,9] that tumor-specific precursor cytotoxic T cells (pTc) accumulated in ocular tumors but that the cells failed to kill tumor cells, revealing that *precursor cytotoxic T cells can enter the eye.* Moreover, he determined that pTc obtained from tumor-containing eyes can become "killers" if they are stimulated with an appropriate source and type of T cell-derived "help." Thus, *potential killer cells enter the eye but fail to acquire their lethal ability within this microenvironment.* This important conclusion drove us to ask the question, "Does the ocular microenvironment interfere with local expression of T-cell immunity?"

The most accessible form of the ocular microenvironment is aqueous humor, and it was with this fluid that we began an exploration of the eye's immunomodulatory properties. Charles Kaiser and Bruce Ksander took the lead in these experiments, working with aqueous humor harvested from murine, rabbit, and human eyes.[10] Irrespective of species of origin, aqueous humor displayed profound immunosuppressive features in vitro. When added to cultures of T lymphocytes stimulated with specific antigen or mitogens, aqueous humor inhibited T-cell activation, as measured by the cells' ability to proliferate or to secrete lymphokines, such as interleukin-2 and interferon-gamma. In addition, aqueous humor prevented pTc from differentiating in vitro into fully cytotoxic cells—as though confirming that the intraocular microenvironment can prevent pTc that enter this environment from developing into tumor cell killers in situ. However, aqueous humor did not act as a universal T-cell toxin because fully functional cytotoxic T cells were able to lyse appropriate target cells in the presence of aqueous humor. More recently, Andrew W. Taylor, PhD, has demonstrated[11] that aqueous humor also suppresses lymphokine-induced (interferon-gamma) activation of macrophages, preventing them from acquiring lytic effector functions, such as generation of nitric oxide and reactive oxygen intermediates. The range of inhibitory properties of *aqueous humor* implies that this fluid selectively interferes with the T cells and macrophages that are responsible for immunogenic inflammation.

Along with these experiments on aqueous humor, Dr. J. S. P. Williamson developed a method to culture cells from murine iris and ciliary body,[12,13] and she demonstrated that these cells secreted immunosuppressive factors into the supernatant, conferring on the fluid properties very similar to aqueous humor. Because aqueous humor is secreted by ciliary body epithelial cells, these findings support the view that parenchymal cells of the eye dictate the functional features of the intraocular microenvironment, and, in this context, *parenchymal cells create a profoundly immunosuppressive microenvironment that contributes to ocular immune privilege.*

Scott W. Cousins, MD, a young research-minded ophthalmologist, then proceeded to demonstrate formally that *the eye prevents intraocular expression of cell mediated immunity.*[14] Antigen injected into the eye of previously sensitized mice failed to elicit intraocular delayed hypersensitivity, even though similar injections intracutaneously evoked intense delayed reactions. Moreover, antigen mixed with aqueous humor and injected intracutaneously into specifically sensitized recipients failed to incite delayed hypersensitivity reactions, confirming that the intraocular microenvironment itself (as represented by aqueous humor) possessed immunoinhibitory activity.

In dramatic affirmation of the ability of the eye to suppress locally the expression of preexisting immunity, Ksander and Chen (unpublished observations, 1996) have discovered recently that weakly antigenic tumor cells grow progressively in the anterior chamber of eyes of preimmune mice, whereas tumor cells injected subcutaneously are rejected by similarly immune mice. In aggregate, these results indicate that in preimmune animals, the expression of T cell-dependent immunity (the type that generates immune inflammation) is curtailed sharply in the eye. Therefore, ocular immune privilege depends in part on *local inhibition of expression of systemic immunity.*

CONCERNING THE COMPOSITION OF AQUEOUS HUMOR

With so much evidence pointing to the presence of immunosuppressive factors in aqueous humor and the ocular microenvironment, we undertook studies to identify which factors might be important. This work was accomplished first by Scott W. Cousins,[15] and, more recently, by his student Andrew W. Taylor [16,17] A major inhibitory factor in aqueous humor has been

identified as transforming growth factor-β_2, which is present primarily in its latent form. In addition, aqueous humor contains alpha-melanocyte-stimulating hormone, vasoactive intestinal peptide, calcitonin gene-related peptide, and free cortisol (because there is virtually no cortisol-binding globulin present).[18] Each of these factors has its own unique type of immunosuppressive activity, and the sum of these factors largely explains the inhibitory activities of this ocular fluid. In addition to factors that limit the expression of T cell-dependent inflammation, aqueous humor contains inhibitors and inactivators of key components of the complement cascade. This leads to the prediction that the ocular microenvironment also acts to inhibit antibody-dependent, as well as -independent, complement-mediated inflammation, a prediction we are currently examining in detail in our laboratory.

OCULAR FACTORS IN ACAID

The evidence recounted above indicates that the eye displays immune privilege, in part because the ocular microenvironment can inhibit those aspects of immune effector function that elicit immunogenic inflammation. There is also compelling evidence that the same ocular microenvironment plays a crucial role in the induction of ACAID. This line of experiments was initiated in Miami by Garth A. Wilbanks, MD, and was subsequently pursued by Yoshiyuki Hara, MD, and Shigeki Okamoto, MD.[15-23] Reasoning that a camerosplenic axis requires that antigenic information travel through the blood from the eye to the spleen, Wilbanks obtained blood from mice that received AC injections of a soluble antigen (ovalbumin [OVA]) 48 hours earlier. To maximize the likelihood of success, these mice had their spleens extirpated 7 days before the AC injection. The blood was injected intravenously into naive, syngeneic mice that were then subjected to an immunizing dose of OVA in complete Freunds' adjuvant. The ear pinnae of these mice were challenged with OVA 7 days later to determine whether delayed hypersensitivity was present. The results of this series of experiments were as follows:

After an AC antigen injection, blood contains an ACAID-inducing signal, i.e., recipients of blood from AC-injected donors acquired ACAID.

The ACAID-inducing signal proved to be a blood-derived cell (probably a dendritic cell or a monocyte that differentially expressed the marker molecule F4/80).

Conventional dendritic cells–macrophages harvested from the peritoneal cavity (PEC) acquire ACAID-inducing properties when injected into the AC of the eye.

Conventional dendritic cells/monocytes that are pulsed with antigen in vitro in the presence of aqueous humor acquire ACAID-inducing properties. The active ingredient in aqueous humor turned out to be TGFb-2.

PEC exposed to aqueous humor in vitro migrated preferentially through the blood to the spleen when injected intravenously into normal mice.

Eye-derived, antigen-bearing cells, as well as PEC endowed with ACAID-inducing properties in vitro, migrate to the spleen and function as the proximate antigen-presenting cells responsible for ACAID.

Recent evidence supports the view that eye-derived antigens are presented preferentially on class I, rather than class II, molecules (Takeuchi M, unpublished observations, 1996).

The responding T cells are primarily $CD8^+$, rather than $CD4^+$.

We have formulated the following scenario as the mechanism of ACAID induction. TGF-β_2, which is constitutively present in the eye, confers ACAID-inducing properties on blood-borne dendritic cells–monocytes that continuously enter the eye, becoming transient residents of the stroma of the iris , ciliary body, and trabecular meshwork. These cells of hematopoietic origin capture ocular antigens, migrate to the spleen, process the antigen into peptides that are loaded preferentially onto class I major histocompatibility complex (MHC) molecules, and then present this immunogenic complex to resident splenic T cells (primarily CD8+). The activated T cells possess the unique property of suppressing immunogenic inflammation when they next encounter the relevant antigen—in the eye or elsewhere.

CHARACTERISTICS OF ACAID

Many laboratories have now contributed to our aggregate knowledge of ACAID,[5-7] and some general characteristics have emerged:

ACAID can be induced by virtually any type of antigen. Weaker (less immunogenic) antigens generate long-lasting ACAID, whereas highly immunogenic antigens (such as MHC class I molecules) generate transient ACAID.

ACAID has been induced by the injection of antigenic materials into the anterior chambers of mice, rats, rabbits, and even primates.

ACAID can be induced in individuals previously sensitized to the antigen injected into the AC, implying that the immune regulation characteristic of ACAID is dominant and powerful.[24]

ACAID cannot be induced in eyes that are inflamed, have neovascularized corneas, or contain Langerhans cells in the central cornea.[25]

ACAID is induced whenever antigen is injected into the anterior chamber, the vitreous cavity, or the subretinal space.[26]

Thus, *ACAID is a stereotypic, systemic immune response to intraocular antigens, a response that provides the eye with incomplete immune protection, but protection that is not, in and of itself, injurious to vision.*

THE FAUSTIAN DILEMMA

The Rewards

There are clearly advantages to the eye in having struck this compromise with the immune system. Similarly, there were rewards for Faust when he struck his deal with Mephistopheles.[1] The devil arranged for Faust to meet the beautiful maiden, Margaret. Within a short period of time, she fell deeply in love with him, and their passion drove them to consummate their love without the benefit of marriage. Faust was elated, for he interpreted this success with Margaret as his promised augenblicke—that transcendental moment when time stops, and mortal man becomes god! Mephistopheles had delivered on his promise, and Faust was pleased.

The compromise between the eye and the immune system affords the eye its own augenblicke—the maintenance of perfect vision despite constant threats from trauma, infection, and inflammation. A few examples follow.

Orthotopic corneal allografts are highly successful compared to other types of tissue allografts. This experimental and clinical fact has been ascribed largely to the existence of immune privilege. In eyes that clinicians define as not at high risk and in normal eyes of experimental animals, immune privilege is clearly responsible for the high frequency of long-term graft acceptance. However, in human eyes that are defined clinically as high risk and in experimental animals with eyes containing neovascularized corneas, Langerhans cells, or both in the central epithelium, privilege is lost, and graft rejection is as brisk and universal as orthotopic skin allografts.[27-29] But even in this setting, preemptive induction of ACAID to histocompatibility antigens of the cornea graft donor can mitigate graft rejection in high-risk mouse eyes, an outcome that suggests an approach to securing long-term survival of grafts placed in high-risk human eyes requiring keratoplasty.

The eye avoids autoimmune disease. The immunopathogenesis of ocular autoimmune disease has received considerable study, but it remains enigmatic. Experimental models, such as experimental autoimmune uveoretinitis and experimental acute anterior uveitis, can be evoked in animals immunized with retinal and melanin-related antigens, respectively. Experiments have been conducted in which eye-derived autoantigens have been injected into the AC of normal mice, and the recipients acquired antigen-specific ACAID. More important, preemptive exposure of mice and rats to ocular autoantigens in this manner rendered them significantly less vulnerable to autoimmune eye disease induced by typical uveitogenic regimens.[30] Thus, ACAID can be used to prevent autoimmune uveitis. Perhaps of greatest interest in this regard is the observation that an ACAID-inducing signal can be created in vitro using ocular autoantigens to pulse antigen-presenting cells in the presence of TGF-β. Intravenous injection of this ACAID-inducing signal into mice, before the induction of experimental uveitis or even after experimental uveitis has created intraocular inflammation, prevented or significantly ameliorated the intensity of uveitis. Once again, the potential to use ACAID to treat autoimmune uveitis exists.

It is of equal interest to understand whether ocular autoantigens are continually released from the eye in a manner that constitutively induces eye-specific ACAID. No important information exists on this possibility, but it has been proposed that this process may offer a primary physiologic reason for ACAID and immune privilege.

The eye avoids herpes stromal keratitis. In the setting of corneal infection with herpes simplex virus, stromal keratitis represents an immunopathogenic disorder in which immune effectors, rather than virus toxicity, account for injury to the cornea. Virus-specific T cells, especially those that mediate delayed hypersensitivity, appear to be the culprits. Several years ago, our laboratory tested the hypothesis that stromal keratitis occurs when ACAID, with respect to viral antigens, has failed to develop during ocular infection with the virus.[31] By using a model system of zosteriform spread of herpes simplex virus (HSV) from a snout infection through the trigeminal ganglion into the anterior ocular segment, it was determined that a high incidence and severity of stromal keratitis occurred only in corneas that already contained high numbers of centrally placed Langerhans cells. Previously, it had been shown that eyes with Langerhans cells in the central cornea failed to support ACAID induction when antigens were injected into the AC, and, in our study, a similar result was observed. Mice with HSV-infected, but clear, corneas displayed transient HSV-specific ACAID, whereas mice with stromal keratitis rapidly acquired virus-specific delayed hypersensitivity, coincident with the presence of virus in the cornea. We have reasoned from these findings that, in normal eyes, infection with HSV evokes a transient episode of ACAID, and

this temporary inability to mount delayed hypersensitivity corresponds to the interval when infectious virus is in the cornea. As the virus is cleared, virus-specific DH then emerges, but now that virus has been cleared from the cornea, the tissue is spared the ravages of immunogenic inflammation.

CELLULAR AND MOLECULAR BASIS FOR ACAID AND OCULAR IMMUNE PRIVILEGE

Within the last few years, our laboratory has relocated to Boston at the Schepens Eye Research Institute, and this shift has enabled us to develop some new initiatives in the search to probe ACAID at its cellular and molecular levels. Only a few of the current projects warrant comment because this is largely work-in-progress. The following statements are meant both to summarize novel findings and to give an indication of the direction our research is taking:

Masaru Takeuchi, MD, has examined the effects of active TGF-β_2 on the antigen-processing and -presenting functions of PEC, which when pulsed with antigen, can induce ACAID in naive mice. He has discovered[32] that TGF-β treatment of PEC in vitro impairs the cell's ability to process exogenous antigen (OVA) in a manner that enables immunogenic peptides to be loaded onto MHC class II molecules. As a consequence, OVA-specific, class II-restricted T cells are not activated. In addition, he has determined that TGF-β-treated PEC secrete immunosuppressive factors that inhibit the activation of $CD4^+$ T cells, and one of those factors is TGF-β itself. Thus, antigen-presenting cells treated with TGF-β acquire a defect in presentation of MHC class II-restricted peptides and begin to secrete TGF-β on their own, creating a local immunosuppressive microenvironment.

In separate in vitro model systems both Drs. Takeuchi and Taylor[33] have studied T cells activated by antigen in the presence of exogenous TGF-β. The results of these studies indicate that activation of T cells (by the T-cell receptor for antigen) under the cover of TGF-β produces T cells with a very unusual spectrum of capabilities. If the responding T cells are of either the T helper 1 (Th1) or the Th2 phenotype, secretion of IFN-g and IL-4, respectively, largely are curtailed. At the same time, these T cells upregulate their TGF-β genes and begin to secrete TGF-β in an autocrine fashion. Thus, in a manner similar to that for TGF-β-exposed antigen-presenting cells, T cells exposed to TGF-β, though stimulated through their cognate antigen receptors, change their functional programs, enabling them to create a TGF-β-containing immunosuppressive microenvironment.

Experiments such as those described above must be treated with circumspection because not all such findings translate directly, or at all, to in vivo physiologic situations. Experiments conducted by Michele Kosiewicz, PhD, however, have produced a set of results suggesting that the findings of Drs. Taylor and Takeuchi are relevant and important. Working with spleen cells from animals in which ACAID had been induced, Kosiewicz[34] has found that AC injection of OVA, followed by an immunizing regimen of this antigen, generates splenic T cells that secrete interleukin-4 and interleukin-10 preferentially (compared to interferon-γ), implying that conventional immunization imposed on a system pretreated with antigen through the AC veers toward Th2-like responses. More important, she has determined that antigen-specific T cells are detectable in spleens of mice that receive only AC injections of antigen. The responding cells are of neither the Th1 nor the Th2 phenotype; instead, they secrete TGF-β in response to antigen stimulation. Thus, even a single exposure to an antigen through the AC results in primed splenic T cells, and the functional program of the responding cells equips them to create in situ an immunosuppressive microenvironment.

SPECULATIONS ON THE RELATIONSHIP OF ACAID TO OCULAR IMMUNE PRIVILEGE

Our current working model of ocular immune privilege emphasizes intraocular immunosuppressive factors, especially TGF-β_2, which confer ACAID-inducing properties on intraocular antigen-presenting cells. Under the influence of TGF-β_2, these cells can capture ocular antigens and migrate to the spleen, where they present peptides from eye-derived antigen on class I molecules while simultaneously secreting TGF-β locally. In this TGF-β-rich microenvironment, antigen-specific T cells (especially $CD8^+$) are activated. These cells, in turn, begin to secrete TGF-β. Thus, antigen-presenting cells migrating from the eye carry with them information concerning intraocular antigen, and they carry a message that dictates the properties of the splenic microenvironment in which T cells will recognize the eye-derived antigen. In a very real sense, the microenvironment of the ocular privileged site has "metastasized" to the spleen. And this metastatic process does not stop in the spleen, for when ACAID T cells encounter antigen in other tissues (including the eye), TGF-β is once again produced. Consequently, the local tissue microenvironments are altered, and second-order *metastatic* immune-privileged sites are created.

Taken together, ocular immune privilege depends on inhibition of the *expression* of systemic immunity by immunosuppressive factors within the eye's microenvironment, and modification of *induction* of systemic immunity by ocular factors that coerce mobile local antigen-presenting cells to activate unique T cells that in turn, suppress immunogenic inflammation. These conclusions imply that immune-privileged sites not only create microenvironments that suppress immunogenic inflammation locally, but they can endow mobile immune cells passing through with the ability to create de novo privileged sites if and where the cells encounter ocular antigens again. If these cells meet antigen again in the eye, privilege is reinforced, and vision is further protected from immunopathogenic injury. However, if the immune privilege-carrying cells meet antigen at extraocular sites, where de novo privilege may be inappropriate, the host's very life may be threatened because of an ineffectual immune effector response.

THE FAUSTIAN DILEMMA

The Price

Which brings us to the issue of the price that the eye's compromise with the immune system demands. In Goethe's drama,[1] Faust's bargain with Mephistopheles enables him to meet Margaret and to consummate their passion. However, after this augenblicke of love without the benefit of marriage, Margaret bears a child. This breach of moral conduct horrifies her mother, infuriates her brother, and scandalizes her village. The consequences for Margaret and Faust are profound and disastrous. She is arrested and thrown into jail. Faust inadvertently kills Margaret's brother in a dual presided over by Mephistopheles. The devil then whisks Faust from town before he can be captured. Subsequently, during the Walpurgis-night festivities, Faust has a vision of Margaret's impending execution and convinces Mephistopheles to take him to her prison cell and to arrange for her escape. When Faust finds Margaret in her cell, the interchange is not what he expected:

MARGARET
Why is it that you don't recoil from me?
Do you know, my friend, whom you set free?
. . . I killed my mother,
drowned my child;
was it not a gift for you and me?

Margaret has been sentenced to death for the horrible crimes she committed in the name of love, yet she has accepted her fate as a just one.

FAUST
Come! Follow me! Beloved, be strong!
I'll love you with thousand-fold passion;
only come with me! This is all I beg of you!

MARGARET
No, no! You must remain among the living.
I will describe the graves to you:
Give the best place to my mother,
and lay my brother next to her;
place me a little to one side—
but not so very far away!
And place my baby by my breast.

FAUST
Trust me. Come with me!

MARGARET
Leave me! No, I will not be forced!
The final day is breaking.
. . . The blade quivers over every neck,
as it quivers over mine.
The world is silent as a grave.

FAUST
Oh, if only I had not been born!

MEPHISTOPHELES
Useless conversation! Dally and prate!
. . . Come now, or I will desert you both.

MARGARET
. . . Save me, Father! I am thine!
Angels! Sacred hosts!

MEPHISTOPHELES
She is condemned!
(to Faust) You come with me!
(Mephistopheles vanishes with Faust)

THE FAUSTIAN DILEMMA

The Price

As it turns out, by receiving immune privilege, the eye is rendered vulnerable to those pathogens that can be eliminated *only* by protective mechanisms that are selectively impaired in ACAID. A few examples make the point.

Ocular tumors grow without restraint and, if left unchecked, lead inevitably to blindness. Both Niederkorn and Ksander have demonstrated experimentally that tumor cells injected into the anterior chamber of eyes of normal mice grow progressively, in large measure because antigens on the tumors induce ACAID, and the selective immune deficiency robs the host of the ability to contain the tumor intraocularly. In addition to Ksander and Chen's recent demonstration that immune privilege in the eye is extended to tumor cells bearing antigens to which the recipient is already immune, Niederkorn and Apte have discovered[32] that immune privilege is extended even to tumors that would normally be rejected by natural killer

TABLE 2. Features of Immune-Privileged Sites and Tissues

Blood–tissue bariers
Deficient efferent lymphatics
Tissues fluids drain into blood vasculature
Reduced–impaired expression of major histocompatibility complex class I and II
Unconventional or deficient antigen-presenting cells
Constitutive expression of Fas ligand
Immunosuppressive microenvironment

cells, implying that immune privilege applies to both adaptive and innate immunity.

There is good evidence to suggest that ACAID and immune privilege play a central role in rendering the person with an ocular tumor vulnerable to aggressive systemic metastases, at least for experimental tumors in mice. For ocular melanomas in humans, the major threat is death from liver metastases, and it is likely that immune privilege also sets the stage for this outcome.

Acute retinal necrosis (ARN) occurs during ocular infection with herpes virus, and this syndrome has been demonstrated experimentally to be correlated with ACAID induced by antigens encoded by herpes virus. Judith Whittum–Hudson, PhD,[33] and Sally Atherton, PhD,[34] have demonstrated independently that injection of HSV-1 into the AC of one eye of BALB/c mice results within 7 to 10 days in acute necrosis of the retina of the contralateral eye. Atherton and her collaborators have shown that a tight correlation exists between the induction of HSV-specific ACAID and the development of ARN. Although the precise pathogenesis of ARN remains elusive, the deficiency of virus-specific delayed hypersensitivity—an effector modality known to be important in eliminating herpes virus from tissues—sets the stage for the virus to spread through the brain to the contralateral eye. Retinitis, leading to blindness, and encephalitis are the inevitable outcomes. Whether a similar mechanism operates in ARN in humans remains to be determined.

Thus, the eye is vulnerable to pathogens that can be eliminated *only* by protective mechanisms that have been *excluded* by the compromise between the eye and the immune system. *The consequence may be blindness.* For ocular pathogens of this type, the vulnerability extends beyond the eye to the rest of the body, and, in this situation, the *consequence may be death.*

MECHANISMS IMPLICATED IN OCULAR IMMUNE PRIVILEGE

During the last 20 years, a great deal has been learned about immune privilege in the eye. During this same interval, the phenomenon also has been studied at other similar sites and tissues, and from this effort has emerged a growing list of features that are often present and that are thought to be important in creating the privileged state. This list is presented in Table 2. It has been known for considerably more than 20 years that many privileged sites, including the eye, reside behind a blood–tissue barrier that limits the entry of blood-borne cells and molecules of the immune system into the site. In addition, many, but not all, such sites lack a demonstrable lymphatic drainage system, and, in the case of the eye and the brain, the specialized tissue fluids (aqueous humor, cerebrospinal fluid) drain directly into the bloodstream. For immunogenic signals leaving these organs, the spleen is the first lymphoid organ that is impacted. Thus, both afferent and efferent routes by which the immune system can communicate with privileged sites or tissues are unusual.

Expression of class I and class II molecules encoded by genes within the MHC is reduced, and often absent, on parenchymal cells within privileged sites or tissues, including the eye. And there is indirect evidence that cells of the eye resemble the trophoblast of the placenta in expressing constitutively atypical, or class Ib, molecules. It is speculated that class Ib molecules shift immune responses toward tolerance rather than sensitivity. Recently, cells within the eye and the testes[35,36] have been discovered to express the ligand for Fas (CD95) constitutively, and circumstantial evidence has been presented to suggest that local expression of Fas ligand induces apoptosis (and, therefore, clonal deletion) of Fas^+ T cells that enter these immune-privileged environments. In a similar manner, cells lining the anterior chamber have been found to express constitutively membrane-bound inhibitors of complement activation (such as CD59). Because aqueous humor itself possesses anti-complementary activity, the ocular microenvironment mitigates against complement activation by both classical and alternative pathways.

Perhaps of most importance, privileged sites and tissues create a local microenvironment that is profoundly immunosuppressive. Suppression is achieved by a variety of locally produced factors, such as TGF-β, alpha-melanocyte stimulating hormone vasoactive intestinal peptide, and so on, which influence the functional properties of migratory cells of the immune system that enter these compartments. As a conse-

quence, induction and expression of immunity to antigens placed in privileged sites, such as the eye, are modified in a direction that limits the local expression of immunogenic inflammation.

OCULAR IMMUNE PRIVILEGE

Resolving the Dilemma

Although much has been learned about immune privilege in the eye, a great deal more remains to understand. At present, there is considerable activity and enthusiasm for working this complex mechanism out. If we can understand eventually the cellular and molecular bases for immune privilege, we have reason to expect that we can create ACAID at will to prevent ocular autoimmunity and immunopathogenic disease, we will be able to restore immune privilege to damaged and diseases eyes in which sight is imperiled by immunogenic inflammation, and we can expect to terminate privilege in the eye when life is threatened (for example by an aggressively growing tumor), accepting blindness as the price for this intervention. Finally, we should be able some day to confer immune privilege on other solid tissues and thereby provide grafts that use the strategies of immune privilege to resist immune rejection.

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