CHAPTER 3

Ocular Immune Responses

Highlights

- Unique regional immune responses influence ocular pathology.
- Several immunoregulatory mechanisms modulate the intraocular immune environment as part of the immune privilege of the eye.
- Anterior chamber–associated immune deviation is a recognized mechanism of ocular immune privilege.
- Increased understanding of the immune responses of the eye has been valuable to advances in corneal transplantation, retinal gene therapy, and various developing cell-based therapies.

Regional Immunity and Immunologic Microenvironments

Regional immunity is the concept that many organ and tissue sites demonstrate modifications to the classic immune response arc that may affect some or all 3 phases—afferent, processing, and effector. Tissue-specific or organ-specific variation in immune response is determined by differences in the immunologic microenvironments. There are multiple microenvironments within and around the eye (Table 3-1).

Immune privilege encompasses anatomical, physiologic, and immunologic adaptations that limit inflammatory damage to vital structures. Operationally, immune-privileged sites include the central nervous system, eye, testis, ovary, pregnant uterus, hair follicle, and adrenal cortex. *Ocular immune privilege* is a unique feature of the ocular microenvironment. First described in the 1940s, the concept was based on the observation that foreign antigens placed in the anterior chamber did not elicit an inflammatory response. Multiple mechanisms contribute to ocular immune privilege, including the following:

- physical barriers (partial blood–ocular and blood–retina barriers, lack of efferent lymphatics)
- inhibitory ocular microenvironment (cell-bound and soluble immunosuppressive factors)
- anterior chamber–associated immune deviation (ACAID; suppression of foreign antigen–specific inflammation in the eye)

Zhou R, Caspi RR. Ocular immune privilege. *F1000 Biol Rep*. 2010;2:3. doi:10.3410/B2-3

Table 3-1 Comparison of Immune Microenvironments in Various Normal Ocular Sites

	Conjunctiva	Cornea, Sclera	Anterior Chamber, Anterior Uvea, Vitreous	Retina, RPE, Choriocapillaris, Choroid
Anatomical features	Lymphatics, follicles	Lymphatics at limbus, none centrally Macromolecules diffuse through stroma	No well-defined lymphatics, antigen clearance through trabecular meshwork Partial blood–uveal barrier	No lymphatics Blood–retina barrier Permeable uveal circulation
Resident APCs	Dendritic and Langerhans cells, macrophages	Langerhans cells at limbus Rare APCs in central and paracentral cornea No APCs in sclera Epithelium/endothelium can be induced to express HLA class II molecules	Many dendritic cells and macrophages in iris and ciliary body Hyalocytes are macrophage derived	Microglia in the retina Dendritic cells and macrophages in choriocapillaris and choroid RPE expresses TLR and can be induced to express HLA class II molecules
Specialized immune compartments for localized immune processing	Possibly follicles	None	None	None
Resident effector cells	Mast cells, T lymphocytes, B lymphocytes, plasma cells, rare neutrophils	Central cornea—none Sclera—none	Rare to no T lymphocytes or B lymphocytes, rare mast cells	Retina—normally no lymphocytes Choroid—mast cells, some lymphocytes
Resident effector molecules	All antibody isotypes, especially IgE, IgG subclasses; IgA in tears Complement and kininogen precursors present	Peripherally—all immunoglobulins (minimal IgM) Centrally—minimal antibody, some complement present Sclera—low antibody concentration, minimal IgM	Kallikrein but not kininogen precursors Some complement present, but less than in blood Minimal immunoglobulins in iris, some IgG in ciliary body and aqueous humor	Retina—minimal to no immunoglobulins Choroid—IgG and IgM
Immunoregulatory systems	Conjunctival-associated lymphoid tissue	Immune privilege—apoptosis-inducing ligands (Fas ligand, TRAIL, PD-L1), avascularity, lack of central APCs	Immune privilege—ACAID, immunosuppressive factors in aqueous; Fas ligand, TRAIL	Immune privilege—RPE can secrete immunosuppressive molecules (TGF-β2) Complement regulator expression

ACAID = anterior chamber–associated immune deviation; APC = antigen-presenting cell; HLA = human leukocyte antigen; Ig = immunoglobulin; PD-L1 = programmed death ligand; RPE = retinal pigment epithelium; TGF = transforming growth factor; TLR = Toll-like receptors, TRAIL = tumor necrosis factor–related apoptosis-inducing ligand.

Immune Responses of the Conjunctiva

Features of the Immunologic Microenvironment

The conjunctiva shares many of the features that are typical of mucosal sites. It is well vascularized and has good lymphatic drainage to preauricular and submandibular nodes. The tissue contains numerous Langerhans cells, other dendritic cells, and macrophages that serve as potential antigen-presenting cells (APCs). Certain ocular surface infections or inflammation results in enlarged conjunctival follicles that contain T lymphocytes, B lymphocytes, and APCs. By analogy with similar sites, such as Peyer patches of the intestine, these follicles are likely sites for localized immune processing of antigens that permeate through the thin overlying epithelium. See Chapter 10, Figures 10-7 and 10-9 for examples of conjunctival nodules in sarcoidosis.

The conjunctiva, especially the substantia propria, is richly populated with potential effector cells, predominantly mast cells. All antibody isotypes are represented, with immunoglobulin (Ig) A as the most abundant type in the tears. Local antibody production presumably occurs as well as passive leakage. Soluble molecules of the innate immune system, especially complement, are also present. The conjunctiva appears to support most adaptive and innate immune effector responses, especially antibody-mediated and lymphocyte-mediated responses, although IgE-mediated mast cell degranulation is one of the most common and important. See also BCSC Section 8, *External Disease and Cornea,* for further information on conjunctival immune responses.

Immunoregulatory Systems

The conjunctival substantia propria contains *conjunctiva-associated lymphoid tissue (CALT)* and serves as the main T-lymphocyte inductive site of the *ocular mucosal immune system (OMIS),* which also includes the lacrimal gland, conjunctival and corneal epithelia, and draining lymph nodes. CALT and OMIS are part of the mucosal immune system, which is anatomically and functionally distinct from the systemic (primarily bloodborne) immune compartment. The mucosal immune system utilizes innate and adaptive immune responses to maintain the homeostasis of mucosal surfaces. The OMIS can be divided into mucosal inductive sites and mucosal effector sites. APCs take up and process antigens (pathogens and allergens) and then present them to T lymphocytes at mucosal inductive sites, usually in local (cervical, preauricular, and submandibular) lymph nodes. Antigen-specific T lymphocytes are induced in these lymph nodes and then return to ocular mucosal effector sites, where they carry out their immune functions.

Within the mucosal immune system, certain sites may elicit a more far-reaching distal mucosal immune response than others. Due to the nasolacrimal drainage system, there is direct anatomical communication between the OMIS and the *nasal-associated lymphoid tissue (NALT),* which are thought to be immunologically connected and interdependent. For example, intranasal immunization induces IgA within the nose and salivary glands as well as on the ocular surface and may elicit more tear IgA antibody responses than does direct ocular exposure. Increased understanding of the integrated nature of the OMIS and NALT

systems has influenced the development of intranasally administered immunotherapeutic agents against inflammatory dry eye disease.

Farid M, Agrawal A, Fremgen D, et al. Age-related defects in ocular and nasal mucosal immune system and the immunopathology of dry eye disease. *Ocul Immunol Inflamm.* 2016;24(3):327–347.

Immune Responses of the Cornea

Features of the Immunologic Microenvironment

The cornea is unique among ocular tissues because the peripheral and central portions of the tissue represent distinctly different immunologic microenvironments. In normal eyes, only the limbus is vascularized and richly invested with Langerhans cells. The avascular paracentral and central cornea are mostly devoid of APCs. Various stimuli, such as mild trauma, certain cytokines (eg, interleukin 1), or infection, can recruit APCs to the central cornea. Immune-mediated corneal changes such as marginal keratitis often occur adjacent to the vascularized limbus.

Plasma-derived proteins (eg, complement and IgG) are present in moderate concentrations in the periphery, but only low levels of IgM are present centrally. Corneal cells also synthesize various antimicrobial and immunoregulatory proteins. Localized immune processing probably does not occur in the cornea, and effector cells are scarce or absent; however, neutrophils, monocytes, and lymphocytes can readily migrate through the stroma if appropriate chemotactic stimuli are activated. These immune cells can also adhere to the endothelial surface during inflammation, giving rise to keratic precipitates or the classic Khodadoust line of endothelial rejection (Fig 3-1). See also BCSC Section 8, *External Disease and Cornea.*

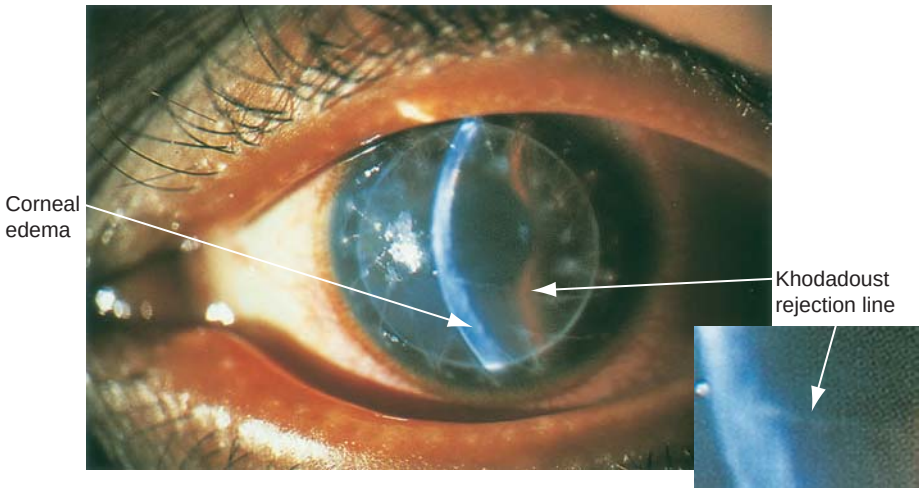

Figure 3-1 Endothelial graft rejection with stromal and epithelial edema on the trailing aspect of the migrating Khodadoust line *(inset).*

Immunoregulatory Systems

Immune privilege of the cornea is multifactorial. Normal limbal physiology is a major component, especially the maintenance of avascularity and the scarcity of APCs in the paracentral and central cornea. The lack of APCs and lymphatic channels partially inhibits the afferent response in the central cornea. The absence of postcapillary venules centrally can limit the efficiency of effector recruitment, although effector cells and molecules can infiltrate even avascular cornea. The corneal endothelium is exposed to anti-inflammatory and immunosuppressive molecules in the aqueous humor such as transforming growth factor β, α-melanocyte–stimulating hormone, macrophage migration inhibitory factor, and vasoactive intestinal peptide. In addition, corneal epithelial and endothelial cells have cell membrane–bound molecules such as Fas ligand (FasL) that resist immunologic attack by inducing apoptosis of infiltrating neutrophils and lymphocytes that express the FasL receptor. (See Clinical Example 3-1.)

CLINICAL EXAMPLE 3-1

Corneal Allograft Rejection

Penetrating keratoplasty, or the transplantation of corneal allografts, has an extremely high success rate (>90%) even in the absence of systemic immunomodulation.This rate is substantially superior to acceptance rates after transplantation of other donor tissues. Corneal allograft survival has been attributed to the mechanisms of immune privilege, including suppression of the immune response against allograft antigens, generation of regulatory T (Treg) lymphocytes that can suppress the destructive alloimmune reaction, and induction of apoptosis of inflammatory cells at the graft–host interface. Experimental models and clinical studies show that factors contributing to rejection include the following:

- stimuli for corneal vascularization that also induce ingrowth of lymphatic vessels and infiltration of dendritic cells
- induction of stromal human leukocyte antigen (HLA) molecule expression (normally low)
- contamination of the donor graft with donor-derived APCs prior to the transplant
- loss of immune privilege via maneuvers (eg, splenectomy) that prevent induction of ACAID

Changes in host immune status may influence graft rejection as well. Graft rejection has been reported in association with systemic checkpoint inhibitor treatment, SARS-CoV-2 infection, and recent vaccination; however, isolated case reports are not necessarily evidence of causality.

Niederkorn JY. The eye sees eye to eye with the immune system: the 2019 Proctor Lecture. *Invest Ophthalmol Vis Sci.* 2019;60(13): 4489–4495.

Yin J. Advances in corneal graft rejection. *Curr Opin Ophthalmol.* 2021; 32(4):331–337.

Immune Responses of the Anterior Chamber, Anterior Uvea, and Vitreous

Features of the Immunologic Microenvironment

The anterior chamber is a fluid-filled cavity; circulating aqueous humor provides a unique medium for intercellular communication among immune cells and resident tissue cells of the iris, ciliary body, and corneal endothelium. Although aqueous humor is relatively protein depleted compared with serum (containing about 0.1%–1.0% of the total serum protein concentration), even normal aqueous humor contains a complex mixture of biological factors, such as immunomodulatory cytokines, neuropeptides, and complement inhibitors, that influence immunologic events within the eye.

A partial blood–ocular barrier is present in the anterior chamber: fenestrated capillaries in the ciliary body allow a size-dependent concentration gradient of plasma macromolecules to permeate the interstitial tissue. Smaller plasma-derived molecules are present in higher concentration than larger molecules. The tight junctions between the pigmented and nonpigmented ciliary epithelium provide a more exclusive barrier, preventing interstitial macromolecules from permeating directly through the ciliary body into the aqueous humor. Nevertheless, a small number of plasma macromolecules bypass the nonpigmented epithelium barrier and enter the anterior chamber by diffusion directly through the anterior iris surface.

A few resident T lymphocytes and mast cells are present in the normal anterior uvea; B lymphocytes, eosinophils, and neutrophils are usually absent. Very low concentrations of IgG and complement components occur in normal aqueous humor. The iris and ciliary body contain significant numbers of macrophages and dendritic cells that serve as APCs and possible effector cells, but immune processing is unlikely to occur locally. Since the inner eye does not contain well-defined lymphatic channels, clearance of soluble substances depends on aqueous humor outflow channels. Nevertheless, antigen inoculation into the anterior chamber results in efficient communication with the systemic immune system. Particulates can be removed via endocytosis by trabecular meshwork endothelial cells or macrophages. Intact soluble antigens can enter the venous circulation that transports them to the spleen.

The vitreous has not been studied as extensively as the anterior chamber. Studies employing proteomics reveal the vitreous as a physiologically active, complex tissue containing diverse proteins originating from inside and outside the eye. An important source of these inflammatory mediators is the retina. The vitreous gel can electrostatically bind charged protein substances to serve as an antigen depot as well as a substrate for leukocyte cell adhesion. The vitreous may also serve as an autoantigen because of the presence of collagen type II, which has been implicated in the pathogenesis of rheumatoid arthritis. Hyalocytes, which are modified resident macrophages, are important in vitreal immunoregulation/modulation and frequently act as APCs. They also respond to different cytokines, playing a role in the immunopathogenesis of several disorders, including proliferative vitreoretinopathy.

Immunoregulatory Systems

Relatively mild inflammation that would be harmless in the skin can cause severe vision loss in the eye. Fortunately, a variety of immunoregulatory mechanisms modulate

intraocular immune responses, including in the anterior chamber, anterior uvea, and vitreous.

Ocular immune privilege has been observed with various antigens, including alloantigens (eg, transplant antigens), tumor antigens, haptens, soluble proteins, autoantigens, bacteria, and viruses. The best-studied mechanism of immune privilege in the eye is ACAID. Although subcutaneous inoculation of antigen elicits a strong, delayed hypersensitivity (DH) reaction, anterior chamber inoculation with the identical antigen results in a robust antibody response but a virtual absence of DH. In fact, preexisting DH can be suppressed by ACAID.

ACAID represents an attenuated effector arc in which cell-mediated immune responses such as DH and cytotoxic T-lymphocyte responses are suppressed by regulatory T (Treg) cells. ACAID blocks T helper-1 (Th1) immune responses and blunts Th2-mediated inflammatory disease. ACAID also shifts the antibody responses to preferential production of non-complement-fixing antibodies, thus reducing the likelihood of antibody-mediated ocular tissue injury from activation of the complement cascade.

Note that the immunoregulatory environment can still be overcome by sufficient immune stimulation.

Boneva SK, Wolf J, Rosmus DD, et al. Transcriptional profiling uncovers human hyalocytes as a unique innate immune cell population. *Front Immunol.* 2020;11:567274. doi:10.3389/fimmu.2020.567274

Niederkorn JY. The induction of anterior chamber–associated immune deviation. *Chem Immunol Allergy.* 2007;92:27–35.

Sen HN. Elements of the immune system and concepts of intraocular inflammatory disease pathogenesis. In: Whitcup SM, Sen HN, eds. *Whitcup and Nussenblatt's Uveitis.* 5th ed. Elsevier Health Sciences; 2022:1–28.

Skeie JM, Roybal CN, Mahajan VB. Proteomic insight into the molecular function of the vitreous. *PLoS One*. 2015;10(5):e0127567.

Immune Responses of the Retina, Retinal Pigment Epithelium, Choriocapillaris, and Choroid

Features of the Immunologic Microenvironment

The immunologic microenvironments of the retina, retinal pigment epithelium (RPE), choriocapillaris, and choroid have not been well characterized. An inner blood–ocular barrier is formed by tight junctions between the endothelial cells of the retinal vasculature, while tight junctions between the cells of the RPE provide an outer barrier between the choroid and the retina. The vessels of the choriocapillaris are highly permeable to macromolecules and allow transudation of most plasma macromolecules into the extravascular spaces of the choroid and choriocapillaris. Well-developed lymphatic channels are absent, although both the retina and the choroid have abundant potential APCs. In the retina, resident microglia (bone marrow–derived cells related to dendritic cells) are interspersed between the ganglion cell and outer plexiform layers and can undergo physical changes and migrate in response to various stimuli. The choriocapillaris and choroid contain an abundance of certain potential APCs, especially macrophages and dendritic cells.

The RPE can be induced to express human leukocyte antigen (HLA) class II molecules, suggesting that the RPE may interact with T lymphocytes in some circumstances. The presence of T lymphocytes or B lymphocytes within the normal posterior segment is not well characterized, but effector cells appear to be absent from the normal retina. Local immune processing does not seem to occur, either. Similar to macrophages, RPE cells also express Toll-like receptors. These special pattern recognition receptors are critical in the detection of pathogens and in the initiation of innate and adaptive immune responses, forming an initial line of defense against invading microorganisms.

A moderate density of mast cells is present in the choroid, especially around the arterioles, but lymphocytes are present only in very low numbers. Eosinophils and neutrophils appear to be absent. However, under various clinical or experimental conditions, a large number of T lymphocytes, B lymphocytes, macrophages, and neutrophils can infiltrate the choroid, choriocapillaris, and retina. The choroid can function as a repository for immunoreactive cells, manifesting clinically as lymphoid hyperplasia. It is hypothesized that the concentration of mast cells in the choroid may facilitate the spread of immunoreactive cells to other parts of the eye. The RPE and various cell types within the retina and choroid (eg, pericytes) can synthesize many different cytokines (eg, transforming growth factor β2) that may alter the subsequent immune response. See also BCSC Section 12, *Retina and Vitreous*.

McMenamin PG, Saban DR, Dando SJ. Immune cells in the retina and choroid: two different tissue environments that require different defenses and surveillance. *Prog Retin Eye Res.* 2019;70:85–98. doi:10.1016/j.preteyeres.2018.12.002

Taylor AW, Hsu S, Ng TF. The role of retinal pigment epithelial cells in regulation of macrophages/microglial cells in retinal immunobiology. *Front Immunol.* 2021;12:724601. doi:10.3389/fimmu.2021.724601

Immunoregulatory Systems

A mechanism of immune privilege, likely similar to ACAID, is present after subretinal injection of antigen. Iris, ciliary body, and RPE cells all contribute to immune homeostasis that is mediated by soluble or membrane-bound molecules. For example, soluble factors secreted by RPE cells can suppress APC and effector T-lymphocyte activation and induce Treg and myeloid-derived suppressor cell activity. These observations may be important because of growing interest in retinal transplantation, stem cell therapies, and gene therapy. (See Clinical Example 3-2.) The capacity of the choriocapillaris and choroid to

CLINICAL EXAMPLE 3-2

Retinal Gene Therapy

Retinal gene therapy is the transfection of neural retina cells or RPE with a replication-defective virus that has been genetically altered to carry a replacement gene. This gene becomes expressed in any cell infected by the virus. Immune clearance of the virus can cause loss of expression of the transferred gene in other body sites. In 2017, the US Food and Drug

Administration approved the first gene replacement therapy for biallelic *RPE65* mutation–associated retinal dystrophy (Leber congenital amaurosis). The agent, voretigene neparvovec-rzyl, uses an adeno-associated viral (AAV) vector to replace defective *RPE65*. It is administered via subretinal injection. Studies are currently under way to investigate gene therapy utilizing AAV for various inherited retinal dystrophies, such as achromatopsia, choroideremia, Leber hereditary optic neuropathy, X-linked retinoschisis, and X-linked retinitis pigmentosa. See also BCSC Section 12, *Retina and Vitreous*.

Despite the relative immune privilege of the eye, immunogenicity of viral vectors is an ongoing area of investigation. In animal models, intravitreal viral vector delivery has led to a humoral response, whereas subretinal delivery has not. Intraocular inflammation was observed in approximately 5% of patients receiving voretigene neparvovec-rzyl; therefore, systemic corticosteroids are started 3 days before injection of this agent.

Hassall MM, Barnard AR, MacLaren RE. Gene therapy for color blindness. *Yale J Biol Med.* 2017;90(4):543–551.

Moore NA, Morral N, Ciulla TA, Bracha P. Gene therapy for inherited retinal and optic nerve degenerations. *Expert Opin Biol Ther.* 2018;18(1):37–49.

function as unique environments for the afferent or effector phases of the immune response has not yet been evaluated.

Lee RW, Nicholson LB, Sen HN, et al. Autoimmune and autoinflammatory mechanisms in uveitis. *Semin Immunopathol.* 2014;36(5):581–594.

Mochizuki M, Sugita S, Kamoi K. Immunological homeostasis of the eye. *Prog Retin Eye Res.* 2013;33:10–27.

Vogt SD, Barnum SR, Curcio CA, Read RW. Distribution of complement anaphylatoxin receptors and membrane-bound regulators in normal human retina. *Exp Eye Res.* 2006;83(4):834–840.