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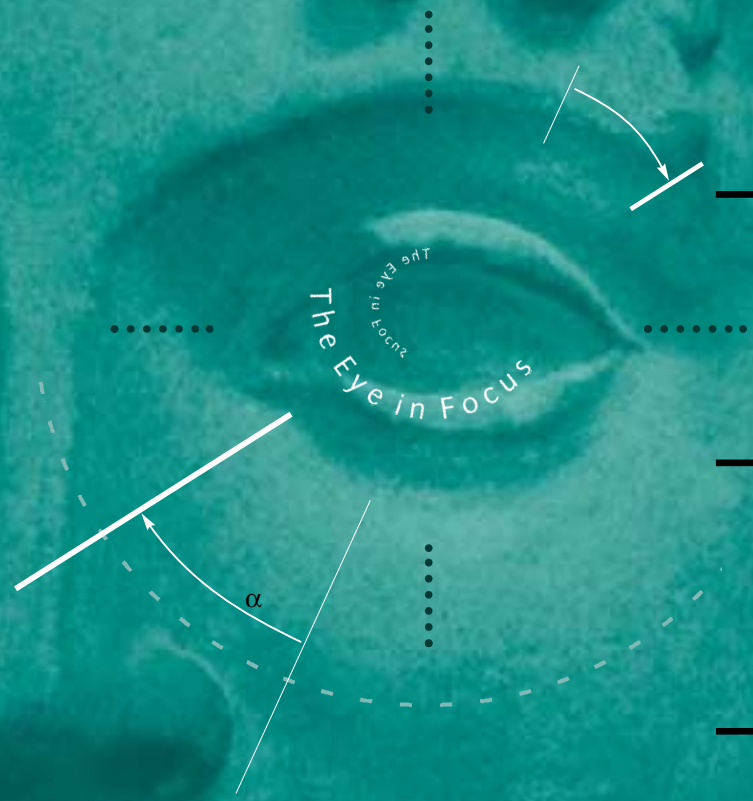
The Eye in Focus



The Evolution of Eyes

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Light is the ultimate source of earth's energy and serves as the premier source of information for many species. Indeed, since the beginning of biological evolution over 5 billion years ago, sunlight has fueled all organic life and defined biological time on earth. Light and the light/dark cycle have probably been the most important selective forces ever to act on biological organisms. One of the most remarkable consequences of light on earth has been the evolution of eyes that has made vision possible. At present, we do not know whether eyes arose once or many times, and, in fact, many features of eye evolution are still puzzling.

How did eyes evolve? Darwin, the great English naturalist who first brought the systematic explanatory power of evolution to bear on the bewildering biological complexity of our planet, felt that eyes offered a special challenge to evolutionary thinking because they are such '...organs of extreme perfection and complication...' (1859). He was quite explicit on this point, saying '...that the eye....could have been formed by natural selection seems, I freely confess, absurd in the highest possible degree'. More than a century later, with new insights that reach from molecular to macroscopic levels of analysis, new mysteries reinforce Darwin's prescient writing. We still have much to learn from the evolution of eyes, both about the existing eyes as well as the processes of evolution that produced them.

Current interest and excitement about eye evolution comes from discoveries at both ends of the full spectrum of biological investigation. Molecular biologists who seek fundamental similarities among organisms have found some genes implicated in eye development that are conserved in eyes from animals across a large phylogenetic distance. Evolutionary biologists interested in understanding why organisms and their parts are so different have found new types of eyes, both in the fossil record and in living animals. What do these different approaches to the evolution of eyes tell us? Together they offer complementary views of eye evolution and possibly the beginnings of a clear story. This article will examine features of eyes for clues about their origins.

Why Do We See What We See?

All eyes are sensitive to a common, rather narrow range of wavelengths within the broad spectrum of energy produced by the sun. Why is this? Why can't we see more of this spectrum? The most likely explanation is that eyes first evolved in animals living in water, and, water, due to its fundamental nature, filters out all but two quite narrow ranges of electromagnetic (EM) radiation [1, 2]. As shown in figure 1, the range of EM radiation 'visible' for most organisms is a narrow, sharply defined band, ranging from the very short wavelengths we think of as having a blue color to longer wavelengths we identify as red. It is particularly narrow when compared with the full range of EM radiation produced by the sun. In our language, we divide this narrow range of perceived wavelengths into seven names (red, orange, yellow, green, blue, indigo, violet), also called spectral colors. As is clear from the figure, in this very narrow band, EM radiation penetrates water better than the adjacent wavelengths by about 6 orders of magnitude. So, since our ultimate ancestors existed in a watery slime, the only radiation to penetrate water must have been the primary selective force. As we see now, this early selection for the narrow spectrum ultimately drove the evolution of biochemical mechanisms sensitive to these colors of light. This is true both for perception of light by animals and for photosynthesis by plants. Now, five billion years later, though many animal species have moved onto land where the sun's full spectrum is available, eyes remain sensitive only to this narrow region. That limit comes now, not from the filtering properties of water but rather from the biochemical mechanisms that evolved in response to the limited wavelengths penetrating the original slime. Once selection started organisms down that path, mechanisms that evolved limited future options.

It is true that many insect species as well as some species of fish and birds can 'see' in the ultraviolet, or very short wavelength end of the visible spectrum. However, they do so with slight modifications of the same biochemical system that the rest of us use to see, not with new mechanisms. This particular exploitation is remarkable because the energy in photons at the short wavelengths is very high.

As seen in figure 1, EM radiation penetrates water quite well at the very low frequency end of the spectrum (<10^3 Hz) explaining why it is dangerous to put power wires into water, among other things. This range of wavelengths is actually used by some organisms to gather sensory information. For example, weakly electric fish evolved independently in both Africa and South America, and species from both groups use low frequencies to signal conspecifics about reproduction and other important things in murky water where normal vision is not much use.

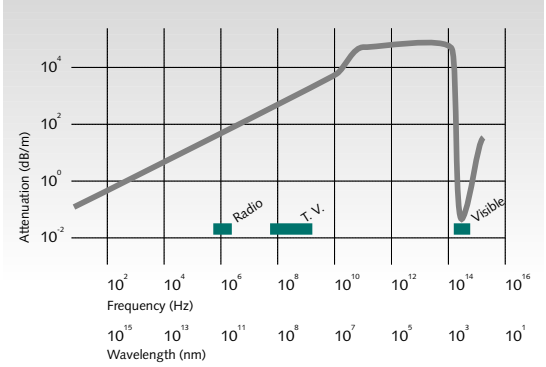
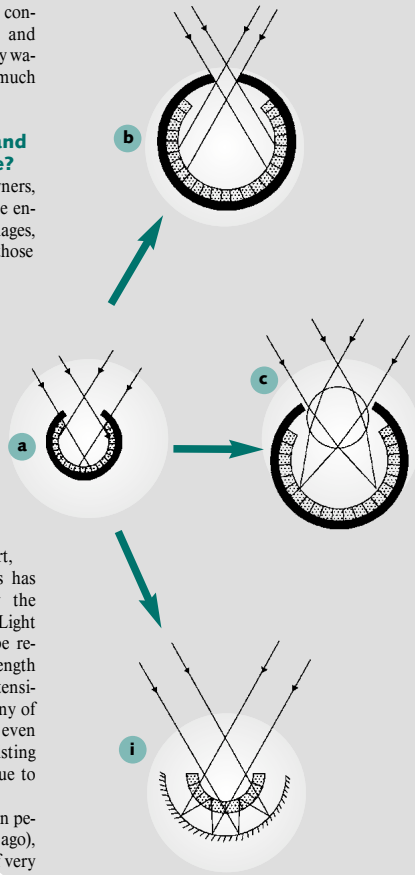


Fig. 1. Attenuation (dB/m) of electromagnetic (EM) radiation in sea water plotted as a function of frequency (Hz) and wavelength (nm) of that radiation. The narrow band of electromagnetic energy which corresponds to visible light is shown, as are the bands used for radio and television transmission. The band of EM radiation we now consider visible light is transmitted through water with an attenuation 6 orders of magnitude less than that of adjacent wavelengths. Redrawn from Fernald [1].

How Do Eyes Work and How Did They Evolve?

To be useful to their owners, eyes must collect light from the environment, resolve it into images, and then capture and forward those images to the brain. Despite decades of research, we still have only limited understanding of how vision actually works. It remains a deep puzzle how a seamless representation of the world is knitted together by the brain from visual snapshots. The functioning of the eye itself, however, is fairly well understood. This is, in part, because the evolution of eyes has been strictly constrained by the physical properties of light. Light travels in straight lines, can be reflected, and varies in wavelength (subjective hue or color) and intensity (subjective brightness). Many of the structural principles and even apparent flaws we find in existing eyes result from constraints due to the physical properties of light.

By the time of the Cambrian period (570 – 500 million years ago), eyes were present in the form of very simple eyecups, useful for detecting light but not for processing directional information. Although the causes are unknown, explosive speciation, or the 'Big Bang' of animal evolution happened during the Cambrian [3]. Existing eye types improved radically, coincident with the appearance of carnivory and predation. The evolution of ocular structures has proceeded in two stages (fig. 2) [4]. First was the production of simple eye spots which are found in nearly all the major animal groups and contain a small number of receptors in an open cup of screening pigment [4]. Such detectors cannot play a role in recognizing patterns but are useful for distinguishing light from dark. The second stage in eye evolution is the addition of an optical system that can produce an image. Image-forming eyes occur in 96% of known species distributed among 6 phyla [4].



Among the known eye types are at least eleven distinct optical methods of producing images, the most recently described is a telephoto lens, identified in the chameleon in 1995. Indeed, six of the optical mechanisms have only been discovered in the past 25 years.

Since camera-type eyes are demonstrably superior in several respects [5], why don't all animals have them? Certainly, camera-type eyes require big heads and bodies to hold them which may have restricted the number of animals that have followed this evolutionary path. Also, it is likely that having evolved one eye type, conversion to another type requires intermediate stages that are much worse or useless com-

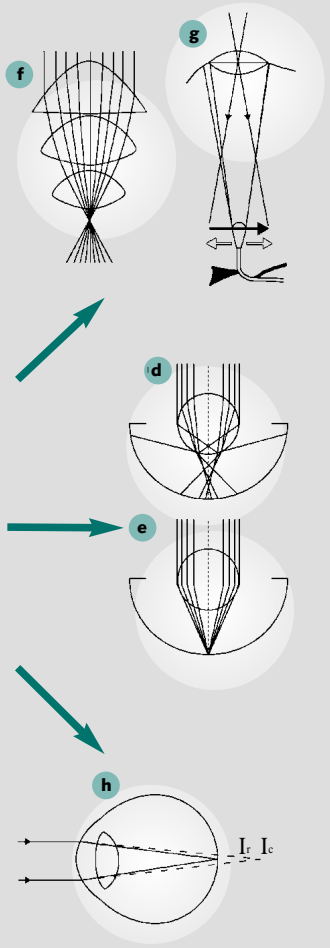


Fig. 2. The likely evolution of single-chambered eyes. Arrows indicate functional developments, not specific evolutionary pathways.
From Land and Fernald [4].

- **a** Pit eye, common throughout the lower phyla.
- **b** Pinhole of *Haliotis* (abalone) or *Nautilus*.
- **c** Eye with a lens.
- **d** Eye with homogeneous lens, showing failure to focus.
- **e** Eye with lens having a gradient of refractive index.
- **f** Multiple lens eye of male *Pontella*.
- **g** Two-lens eye of the copepod crustacean *Copilia*. Solid arrow shows image position and open arrow the movement of the second lens.
- **h** Terrestrial eye of *Homo sapiens* with cornea and lens; Ic= image formed by cornea alone; Ir= final image on the retina.
- **i** Mirror eye of the scallop *Pecten*.

pared with the existing design. This would make a switch essentially lethal to animals that depend on sight. Although this argument makes sense intuitively, some existing cases of novel optical combinations suggest this is probably not the whole story.

Textbooks tend to group animal eyes into two groups, the camera-type or 'simple' eyes and the compound eyes. Although this dichotomy reflects a real and fundamental difference in optical mechanisms, it conceals a remarkable diversity of optical systems subsumed under each heading [4, 5].

In 1994, Nilsson and Modlin described a mysid shrimp *(Dioptromysis paucispinous)* that has a combined simple and compound eye: partly compound with multiple facets exactly like the eye of an insect, and partly simple with a single lens focusing an image on a sheet of receptors like that of a human. The shrimp are about 5 mm long with nearly spherical eyes at the ends of stalks. In addition to the facets (ca. 800–900) there is a single giant facet facing the shrimp's tail, which the shrimp frequently rotates forward probably to get a better look at something since it has ca. 5 times the acuity (but much lower sensitivity) than the rest of the eye. It is as if the shrimp is carrying a pair of binoculars for the occasional detailed look at something ahead of it. The discovery that simple and compound eye types can be found in a single animal raises the question of how a developmental program could produce this outcome.

How Do Eyes Capture Photons?

Visual information from the environment is detected by specialized cells called photoreceptors located in a sheet covering the back of the eye. These cells are part of the retina, a thin (ca. 100 µm) layer of cells that is responsible for getting visual information to the brain. Photoreceptors contain two molecules that act together to collect photons. One, opsin, is a protein that sits in a membrane in close association with the other, a visual pigment or chromophore (11-cis-retinal), which is surrounded and held by opsin (fig. 3). When a photon is absorbed by the chromophore, it lengthens by 5 Å by rotating around a double bond. Through this slight transformation, the chromophore makes opsin enzymatically active, ultimately causing, via an amplifying cascade, a decrease in current flow across the outer segment membrane. The main result of this interaction is that the photon energy is transduced into electrical energy which can be interpreted by the nervous system.

The opsins have a family history that precedes eyes as evidenced by comparisons of their DNA. They consist of seven transmembrane helices with short loops on both sides of the membrane. The chromophore, retinal, is attached covalently to opsin at a site in the seventh transmembrane domain. These features are common to all metazoan opsins and, based on comparison of the DNA sequences, they must share a common ancestry. In particular, several regions of the molecule show close similarity among opsins from vertebrates, insects and *Octopus*, whose ancestries diverged in the Cambrian [4]. This homology suggests that the molecule responsible for the initial absorption of photons has been exquisitely tuned over evolutionary time. In addition, the high level of conservation has allowed relatively easy recovery of the cDNAs that encode opsin from the eyes of many different species, giving us a remarkable amount of information on its evolutionary history.

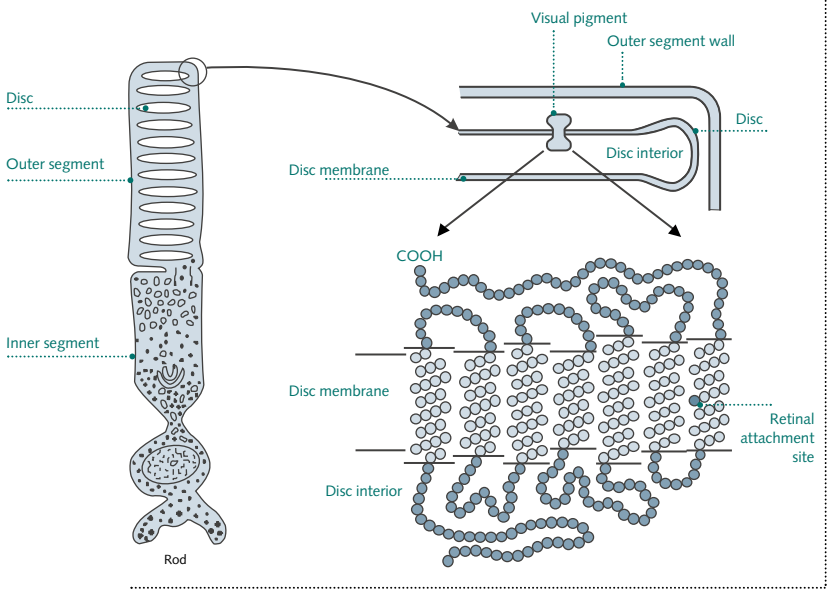
One source of evolutionary information has been the evolution of color vision. There are many selective advantages for animals having color vision including improved detection of food, mates and enemies. To see colors, animals must have photoreceptors sensitive to different wavelengths of light. This is possible through the evolution of slight variants in the opsin molecule through which subtle differences in the amino acids at particular sites 'tune' the chromophore to a particular peak absorbance wavelength. The discovery and clarification of a direct causal link between a molecular structure and its importance for a perceptual process is remarkable in its own right, but also because these features are common to all metazoan opsins. These evolutionary experiments have allowed detailed phylogenetic comparisons, suggesting that vertebrate visual pigments have evolved along at least five lines and diverged from an ancestral type before teleost fish diverged from other vertebrates.

Although metazoan opsins appear to have evolved along several separate lines from a common ancient ancestor, what happened earlier is not clear. Bacteriorhodopsin, from *Halobacterium* does not show significant amino acid similarity with cattle rhodopsin. Moreover, it is the double bond 13 of the chromophore, rather than 11 that is altered by light. Nonetheless, like metazoan opsins, bacteriorhodopsin belongs to a large superfamily of proteins, all of which have seven transmembrane helices and operate by activating second-messenger cascades. This family of proteins includes neurotransmitter and peptide receptors as well as the family of odorant receptor molecules. Whether similarities within the superfamily result from a very ancient common ancestry or a more recent recruitment is not yet known.

Where Do Lenses Come From?

The vertebrate eye develops from a diverse collection of embryonic sources through a complex set of inductive events [6]. Whereas the neural retina is derived from the diencephalon and is a part of the brain, the lens comes from surface ectoderm and the iris and ciliary body arise primarily from the neural crest. Mapping the genes known to play a role in mouse eye development, for example, shows that some of these genes are present on every chromosome [6]. The apparent patchwork assembly of the eye makes it all the more surprising that

Fig. 3. Schematic illustration showing the location of the visual pigment (opsin plus retinal) at several levels of magnification. A rod photoreceptor is shown on the left. Light enters the rod from below and strikes visual pigment molecules contained in the disc membranes as seen in the top right illustration. The lower right figure shows the opsin protein amino acid structure with the site of retinal attachment within the disc membrane on the 7th transmembrane domain.



common developmental programs seem to produce comparable outcomes across a broad phylogenetic divide [7]. Could we use the composition of lenses to gain insight into eye evolution?

Vertebrate lenses are formed from modified epithelial cells that contain high concentrations of soluble proteins known as crystallins because they are packed in a highly organized fashion. It is the change in relative concentration of these proteins from the periphery to the center of vertebrate lenses that produces the refractive index gradient necessary for a lens to be useful to the animal. In fact, the identity of the proteins seems not to be important since the crystallin proteins are not more transparent than others. Instead, the distribution of protein concentration as a function of radius is the key to a successful lens. Thus, the challenge in understanding lens evolution lies in discovering how the distribution of proteins within a lens is established and maintained.

Of the eleven lens crystallins now known, only three, α-, β- and γ-crystallins, are common to all vertebrates. In fact, until recently, all crystallins were thought to be unique to lens tissue and to have evolved for this special function. However, despite their apparently specialist role, most of the crystallins are neither structural proteins nor lens specific. There are two major groups of lens crystallins, those present in all vertebrates and those specific to a particular taxon. For example, in crocodiles and some bird species, the glycolytic enzyme lactate dehydrogenase B is a major protein in the lens. Indeed, 4 of the 8 taxon-specific crystallins are identical to metabolic enzymes and products of the same genes, suggesting these products share a gene.

Why might enzymes be recruited to make vertebrate lenses? Perhaps the robust regulation of enzyme production is advantageous for producing sufficient protein for a lens, but there is not much beyond speculation to support this notion. There may be some deeper reason, however, because this molecular opportunism seemed such a good idea, that certain invertebrates, e.g. mollusks, independently evolved the same strategy [8]. Squids have lenses whose protein content is nearly entirely the enzyme glutathione S-transferase. The common strategy of constructing lenses from different proteins seems to be a convergent evolutionary solution. This convergence of molecular strategy suggests that enzymes as lenses may have a functional meaning, or that it is easy to get lens cells to make a lot of enzyme, or there may be other as yet not understood reasons.

Eyes: Convergence or Homology?

Have the structural similarities among eyes resulted from evolutionary convergence due to similar selective pressures (analogous) or from descent from a common ancestor (homologous)? This distinction is particularly hard to draw when comparing eyes because the physical laws governing light greatly restrict the construction of eyes. Similar eye structures may have arisen in unrelated animals simply because of constraints imposed by light.

The most commonly cited example of evolutionary convergence are the eyes of squids and fish. Both of these are 'camera-type' eyes, in which an image is formed on the photosensitive retinal layer at the back. Moreover, both have evolved a spherical lens with an exquisitely constructed gradient of refractive index that allows good focus despite their spherical shape. In addition, both types of eyes use the same light-sensitive molecule, opsin, to convert photons into neural energy. However, the fish retina is inverted, meaning the light-sensing cells are at the very back of the eye (inverse) while those in squid are at the front of the retina (everse). Moreover, the parts of the eyes of fish and squid arise from very different embryological sources during development, suggesting different origins for these eye types.

Paired eyes in the three major phyla, vertebrates, arthropods and mollusks (fig. 4), have long been considered to be classic examples of evolutionary convergence. At the macroscopic level, this must be true since they arise from different tissues and have evolved radically different solutions to the common problem of collecting and focusing light. However, as discussed above, opsin has a significant DNA sequence homology across all phyla. Remarkably, recent work by Gehring and Ikeo [9] has shown that features of ocular development in different phyla can be coordinated by a homologous 'master' gene, *Pax-6*. That a single gene could trigger construction of an animal's eye in diverse species led to their proposal that eyes are monophyletic, i.e. evolved only once. This is an interesting hypothesis that goes against all the previous suggestions of multiple (i.e. polyphyletic) origins for eyes. There are several reasons why this hypothesis seems difficult to support. It is well known that *Pax-6* organizes other structures besides eyes and is even necessary for the onset of various actions outside the nervous system. Also, other genes can cause development of eyes [reviewed in 10]. Whether eyes are monophyletic or not, the work of Gehring and his colleagues has stimulated a great deal of new work on eye evolution, which is a good thing in itself.

Clearly, eyes have common molecular constituents whether they be opsins, *Pax-6*, or others. Yet, homology at the molecular level of organization does not predict homology at the organ or organismic level. Molecules are not eyes.

Conclusions

Eyes exist in a variety of shapes, sizes, optical designs and locations on the body, but they all provide similar information about wavelength and intensity of light to their owners. Different tissues have been recruited to build lenses and retinas across the phyla. In contrast, all eyes share the same mechanism of absorbing photons, i.e. the opsin-chromophore combination has been conserved across phylogeny. Despite new findings yielded by powerful molecular techniques, all evidence still suggests that eyes have a polyphyletic origin, with the caveat that they contain homologous molecules responsible for many structural, functional and even developmental features (fig. 5). Given a growing list of homologous gene sequences amongst molecules in the eye across vast phylogenetic distances, the challenge is now to discover what makes the eyes of *Drosophila*, squid and mouse so different. Since strictly homologous developmental processes must produce homologous structures, key elements responsible for the development of nonhomologous eyes remain missing. Understanding what makes eyes different may be a bigger challenge than finding what they have in common.

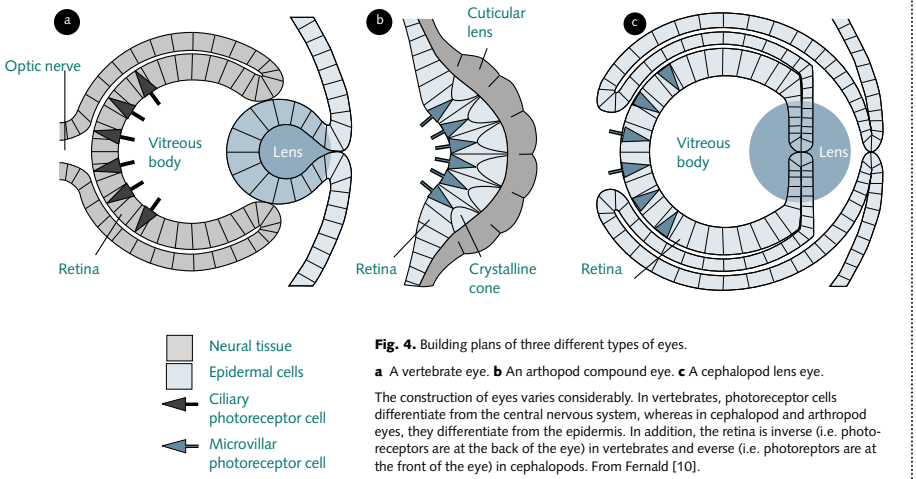


Fig. 4. Building plans of three different types of eyes.

a A vertebrate eye. **b** An arthopod compound eye. **c** A cephalopod lens eye.

The construction of eyes varies considerably. In vertebrates, photoreceptor cells differentiate from the central nervous system, whereas in cephalopod and arthropod eyes, they differentiate from the epidermis. In addition, the retina is inverse (i.e. photoreceptors are at the back of the eye) in vertebrates and everse (i.e. photoreptors are at the front of the eye) in cephalopods. From Fernald [10].

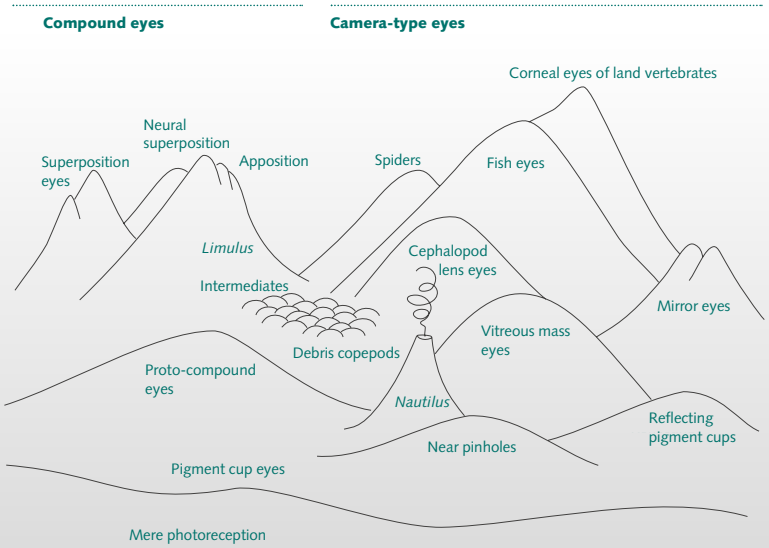


Fig. 5. A possible landscape of eye evolution created by Mike Land. Height represents optical quality and the ground plane evolutionary distance. Land writes that 'Climbing the hills is straightforward but going from one hilltop to another is near impossible'. From Dawkins R: Climbing Mount Improbable. New York, Norton, 1996.

Russell D. Fernald is Benjamin Scott Crocker Professor of Human Biology and professor of psychology at Stanford University, California. His research is focused on understanding how the vertebrate visual system develops and how vision influences the behavior of animals. Dr. Fernald is an editorial board member of the Karger journal *Brain Behavior and Evolution*.

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Ocular Immune Privilege –

Protection That Preserves Sight!

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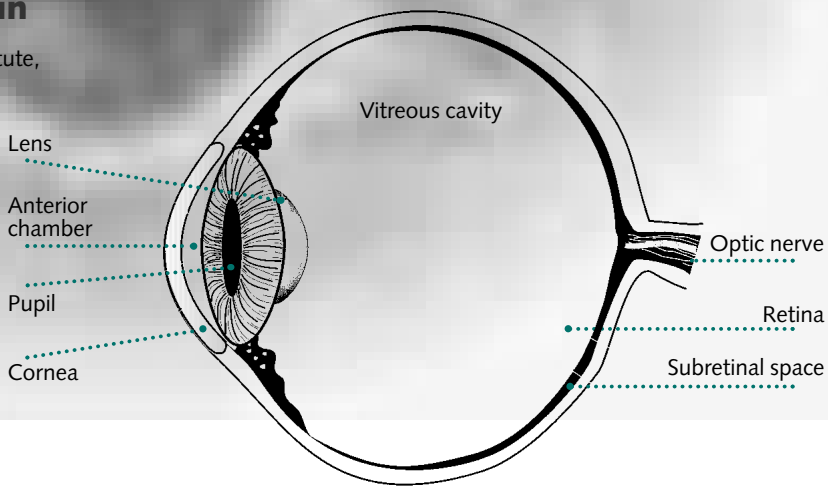


Fig. 1. Visual axis of the normal human eye. Unimpeded light passing through the ocular surface, anterior chamber, pupil, vitreous body, and inner retina falls, as a focused image, on the outer segments of the photoreceptor cells of the retina.

The Eye's Dilemma

The eye is an essential organ for survival – for humans, and for the vast majority of other vertebrates. Like other vital organs and tissues, it is vulnerable to a variety of external and internal pathogens that can abolish its critical function and threaten the host's survival. To avoid this catastrophe, evolution has provided vertebrates with a wide spectrum of general and local defense mechanisms designed to neutralize the virulence of pathogens. The fact that the spectrum of defense mechanisms is wide reflects, on the one hand, the extreme diversity of pathogens, each with a unique 'spin' on virulence strategies, and, on the other, the unique vulnerability of different organs and tissues to the distinct virulence strategies of different organisms. Thus, the protection against injury from pathogens that is conferred at any particular site in the body is both distinctive and appropriate for the range of potential pathogens and for the physiologic functions of that site. The term 'regional immunity' has been used to identify this dimension of immune protection against patho-gens. The eye is a good example of an organ that possesses 'regional immunity', and immune privilege in the eye is the experimental and clinical expression of this concept.

Especially for humans, precise vision of detailed images is vital for survival. This property depends upon absolute integrity of the so-called visual axis: the structures of the eye that permit light to enter the organ, and encourage and focus light images onto the neuronal retina (fig. 1). These structures include the ocular surface (tear film, cornea), the anterior chamber (aqueous humor) and pupil of the iris, the lens, the vitreous body, and the layers of retina immediately anterior to the photoreceptor cells. Each of these structures is uniquely endowed with the property of transmitting light with a minimum of distortion and diffraction. The precise anatomic relationships of these structures are critical to achieve a focused image at the level of the retina. Even very minor deviations (millimeters or less) in the anatomic integrity of the visual axis can result in impaired vision.

Not surprisingly, inflammation, if it occurs within the eye, is a profound threat to vision. In an inflamed eye, light transmission through the visual axis can be impeded and diffracted by leukocytes and plasma proteins, and the visual axis itself can be distorted, causing the focused light image to fall away from the photoreceptor outer segments. Thus, the dilemma! Inflammation is one of the most important pathways by which immune mechanisms protect a tissue against pathogens. It is this dilemma – the need for immune protection, and the vulnerability to the consequences of inflammation – that lies at the heart of immune privilege in the eye. Through adaptation, evolution has devised a special form of immune protection (we call it immune privilege) that enables the eye to resist the vast majority of pathogens by using processes largely devoid of inflammation, thereby avoiding loss of vision. We should remember that adaptations of this type represent biologic compromises, and in the case of ocular immune privilege, the compromise renders the eye vulnerable to those organisms whose pathogenicity and virulence can only be eliminated with the aid of overt inflammation.

Immune Privilege

Even before the modern concept of immunity had formed, scientists had discovered that certain tumors would grow progressively when transplanted into the anterior chamber of the eye, but not when transplanted elsewhere. With Medawar's discovery of the principles of transplantation immunology in the 1940s, it became possible to explain the biologic reasons for the surprising growth of tumors in the anterior chamber. Medawar demonstrated that foreign tissue grafts expressed transplantation antigens that were recognized by the recipient's immune system. In mounting a response against these antigens, immunity caused graft rejection. Medawar further demonstrated that this rule of transplantation immunology, which applied to most organs of the body, was relaxed in the eye. He found that foreign tissue grafts placed in the anterior chamber of the eye (as well as in the brain) often displayed prolonged survival without evidence of rejection, and coined the term 'immune privilege' to refer to this special property of the anterior chamber (and the brain).

By now, many other investigators have performed similar experiments by placing foreign tissue grafts at numerous special sites in the body. There is a long, but probably still incomplete, list of immune-privileged sites: anterior chamber, vitreous cavity and subretinal space of the eye, brain, pregnant uterus, ovary, testis, adrenal cortex and certain tumors. As researchers have probed the mechanisms responsible for immune privilege, it has become clear that similar, but not identical, processes are involved in conferring immune privilege on these various body sites.

Anterior Chamber of the Eye as an Immune-Privileged Site

Our understanding of the anterior chamber of the eye as an immune-privileged site is based on solid experimental evidence and is supported by considerable clinical experience. Allografts prepared from a variety of different tissues (skin, thyroid, islets of Langerhans, cornea, retina) experience prolonged, even indefinite survival when placed in the anterior chamber. Two types of experimental grafts make this point dramatically: (1) Allogeneic tumor cells that are summarily rejected when placed subcutaneously form progressively growing tumors when injected into the anterior chamber of eyes of immunologically normal mice and rats. (2) Corneal tissue obtained from the eye of one individual enjoys extended survival when grafted orthotopically to the anterior surface of the eye of a normal individual, even though corneal tissue grafted heterotopically to the skin surface is rejected promptly. The experience of corneal surgeons makes the same point. The most common solid tissue transplantion performed in humans is corneal transplantation, and these transplants enjoy the highest rate of success compared to all other types of solid tissue grafts. This is true even though the immunosuppression used to control rejection is applied topically, rather than systemically.

Over the past 30 years, investigators have learned much about the physiologic processes responsible for immune privilege in the eye: special architectural features of the anterior chamber, and unique immunomodulatory molecules present in the ocular fluids and expressed on ocular parenchymal cells. Together they govern and modify the manner in which antigenic material placed in the anterior chamber is recognized by cells and molecules of the systemic immune apparatus. In addition, these processes alter the ways in which immune effector molecules and cells respond to foreign and antigenic material that is present within the eye. The net effect of these forces is to limit the intraocular development of inflammation.

Inflammation in Relation to Innate and Adaptive Immune Responses

Immunity is a complex response made by the body in an effort to avoid invasion by pathogens and to nullify their virulence strategies. Two complementary forms of immunity conspire in this effort: the innate immune system and the adaptive immune system (table 1). In the effort to eradicate invading pathogens, inflammation is usually the final common pathway employed, and both innate and adaptive immune responses are known to trigger inflammation toward this end. Not surprisingly, immune privilege in the anterior chamber of the eye acts to thwart the triggering of inflammation by both types of immune response.

Innate immunity is activated by sets of rather stereotypic molecules that are expressed on microbial pathogens; these molecules are called pathogen-associated molecular patterns (PAMPs). Bacterial lipopolysaccharide (LPS) and (α-lipotechoic acid, expressed by gram-negative and gram-positive organisms, respectively, are good examples. Although PAMPs vary a lot among different pathogens, there is nonetheless considerable sharing, and therefore any given PAMP is not 'specific' to any particular organism in a molecular sense. PAMPs are recognized by cells of the innate immune system

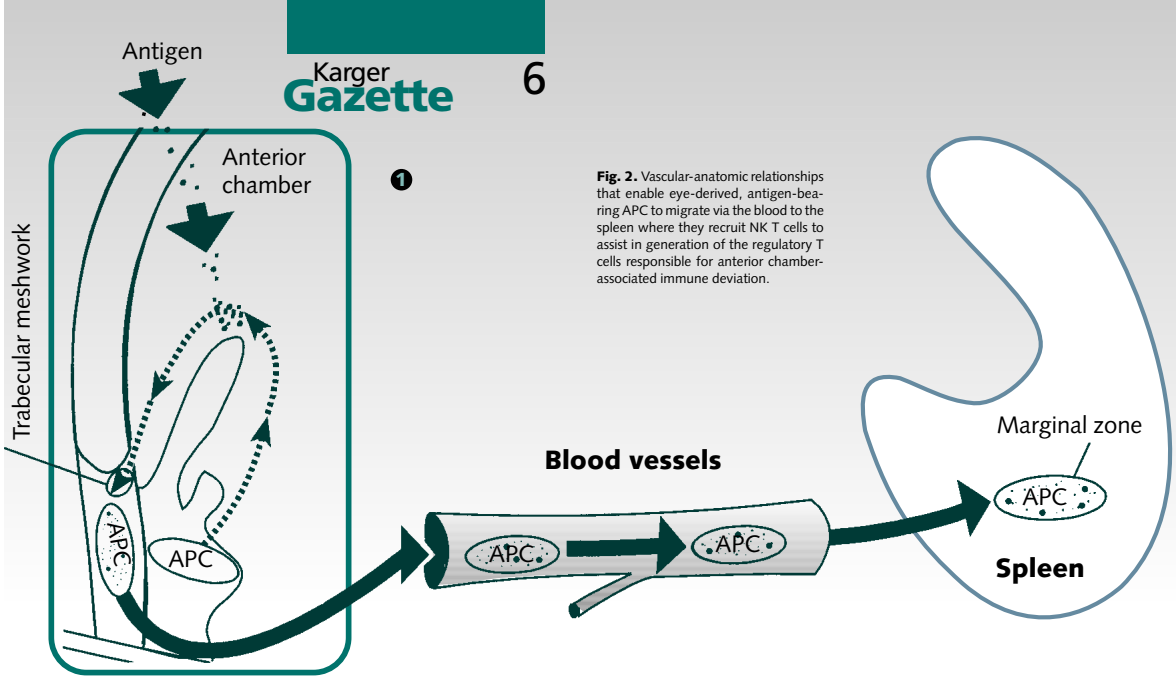


Fig. 2. Vascular-anatomic relationships that enable eye-derived, antigen-bearing APC to migrate via the blood to the spleen where they recruit NK T cells to assist in generation of the regulatory T cells responsible for anterior chamber-associated immune deviation.

that express receptors that recognize these patterns *directly* (called pattern recognition receptors, PRR). Examples of PRRs include certain complement components, C-reactive protein, mannose receptors, LPS-binding protein, CD14, and KIRs. PRRs are expressed on virtually all cells of the innate immune system: macrophages, dendritic cells, neutrophils, natural killer (NK) cells, mast cells, platelets, γ/δ T cells, and B-1 B lymphocytes. When PRRs bind PAMPs they trigger an immediate cellular or molecular response (immediate ≤ 3 h), and no prior exposure to the same PAMP is required for this immediate response. Mediators (cytokines, chemokines, prostaglandins, etc.) that are released during this response modify the local microvasculature and recruit additional inflammatory cells and molecules to the site. While cellular proliferation is not an important component of the ensuing response, an intense and potentially destructive inflammation *is*. Once the offending pathogen is eliminated, innate immunity subsides, along with its attendant inflammation. Very often an inflammatory episode, if especially intense, may produce tissue damage and leave a scar. However, no imprint of the encounter is left upon the innate immune system, and therefore 'memory' of the offending pathogen does not exist.

Adaptive immunity uses rather different mechanisms to detect and respond to pathogens, although the principle of using specialized receptors to recognize foreign molecules remains the same. Adaptive immune cells and molecules respond to unique 'fingerprints' on molecules expressed by pathogens (often very small peptides and carbohydrates derived from complex macromolecules). These molecules are called 'antigens', there are estimated to be more than 10^9 such molecules in our universe. More than one antigen may be expressed by any single pathogen, and each pathogen displays antigens which are uniquely its own. In an effort to devise a receptor system sufficiently diverse to detect this enormous diversity of antigens, receptors are generated somatically in the cells of the adaptive immune system: T and B lymphocytes. The recognition structures on B lymphocytes are antibodies (immunoglobulins) that can bind directly to their specific antigen. When B cells are activated by exposure to antigen, they secrete soluble versions of their surface receptors. Secreted antibodies are the effector modalities generated by B lymphocytes. Antigens are also recognized by specialized receptors on T lymphocytes (T-cell receptor – TCR – for antigen). In this case, what is recognized are small peptides derived from complex macromolecules that have been loaded onto special recognition structures (class I and II molecules encoded within the major histocompatibility complex, MHC) that are displayed by specialized antigen-presenting cells (APC). Thus, the conditions for recognition of antigen by T cells is that the antigen be processed and presented to the T cell by an APC. Unlike the cells of the innate immune system, T and B lymphocytes are not activated simply by having their receptors engage the relevant ligand. Instead, a second signal is needed for T-cell activation, and this is also prepared by the APC. This 'second signal' arises when APC is activated by an innate immune signal. Thus, activation of the adaptive immune system is dependent upon prior or simultaneous activation of the innate immune system.

Clonal expansion and differentiation of T and B lymphocytes are important consequences of antigen-specific activation. Not only are the numbers of antigen-reactive T cells increased because of clonal proliferation, but the emergent progeny display the capacity to carry out effector functions – delayed hypersensitivity, cytotoxicity, complement fixation. Moreover, many of these progeny become long-lived, helping to account for the acquisition of antigen-specific immunologic memory. Both clonal expansion and acquisition of antigen-specific memory are unique attributes of adaptive immunity, compared to innate immunity. Adaptive immune effectors are able to traffic via the blood vasculature to sites containing the offending, antigen-bearing pathogen. Upon local recognition of the antigen, these effectors trigger an inflammatory response that eliminates the pathogen. This is the second important point when the adaptive and innate immune systems cooperate in conferring protection. CD4+ T cells can only trigger a full-scale delayed hypersensitivity response if they are assisted by innate immune cells such as macrophages. Similarly, antibodies can only trigger phagocytosis of bacteria with the assistance of complement components. By contrast, in the case of CD8+ cytotoxic T cells, elimination of pathogens may occur *without* significant inflammation, and non-complement-fixing antibodies that neutralize viruses may similarly terminate an infection *without* inflammation. Thus, only a subset of adaptive immune effectors trigger destructive immunity when attacking their pathogenic targets, and it is to these effectors that immune privilege in the eye is directed.

Ocular Factors That Promote Immune Tolerance of Eye-Derived Antigens

Injection of antigen into the anterior chamber of the eye leads to the development of a systemic immune response that is deviant and unexpected. This response, termed anterior chamber-associated immune deviation (ACAID), represents a selective, antigen-specific deficit of T cells that mediate delayed hypersensitivity and B cells that secrete complement-fixing antibodies, i.e. immune effectors that trigger immunogenic inflammation. The selective deficiency of ACAID is due to the generation of antigen-specific regulatory T cells (CD4+ and CD8+) that inhibit both induction and expression of pro-inflammatory effector modalities. At the same time, these regulators have no effect on other effector modalities, leaving cytotoxic T-cell responses and non-complement-fixing antibodies intact.

Factors within the eye play a key role in the generation of ACAID. APC indigenous to the eye (stroma of iris and ciliary body) express CD1 and are heavily influenced by soluble molecules in the ocular microenvironment, especially transforming growth factor-β_2 (TGF-β_2). When these cells capture local antigen, they display the capacity to process and present peptides on class I and II MHC molecules, and they even express certain costimulatory molecules (B7-1). However, they fail to secrete IL-12 and they are impaired in their ability to upregulate expression of CD40 – costimulatory molecules that are central to the induction of T cells that mediate delayed hypersensitivity. In addition, eye-derived APC secrete active TGF-β. This is the distinct functional phenotype of antigen-bearing APC that leave the anterior chamber by migrating across the meshwork directly into the venous circulation (fig. 2). Traversing the bloodstream, eye-derived APC migrate preferentially to the marginal zone of the spleen where they secrete MIP-2, a chemokine that attracts NK T cells. The latter cells bear a special T-cell receptor that recognizes CD1. When APC-NK T-cell conjugates form, the NK T cells secrete additional chemokines which bring to the site antigen-specific T cells. From this multicellular aggregate emerge the regulatory T cells of ACAID.

The importance of ACAID to ocular immune privilege is that it pre-empts the systemic immune response to eye-derived antigens, molding all subsequent responses to the same antigens such that inflammation doesn't develop when these antigens are encountered within the eye (or anywhere else for that matter). The ocular factors that conspire to produce this form of tolerance are: (a) TGF-β_2, which endows ocular APC with special costimulatory properties, and (b) an outflow path for aqueous humor (AqH) that directs mobile antigen-bearing APC directly to the blood (rather than into the lymphatics) and therefore to the spleen. It is the spleen that presides over the inductive events that lead to ACAID. In animals devoid of a spleen, ACAID fails to develop.

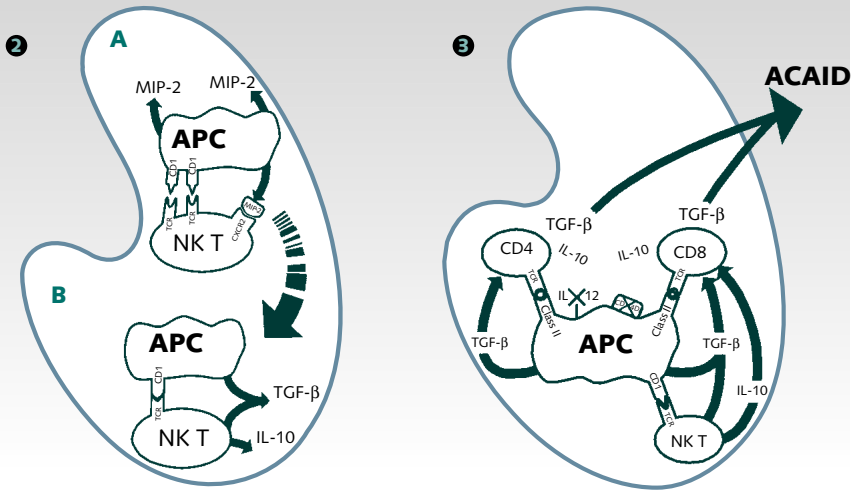
Factors That Modify Expression of Ocular Adaptive Immunity

Under normal circumstances, the anterior chamber is located behind a blood:tissue barrier. The content of plasma proteins within AqH is extremely low (<0.01%), and only T and B lymphocytes are ever detected within the tissues surrounding the anterior chamber. This anatomical barrier represents an important factor in preventing expression of adaptive immunity within the eye. In fact, only when this blood:tissue barrier is breached does the possibility of immune expression in the eye exist. This is because adaptive immune responses are necessarily triggered by antigen-specific T cells and antibodies, both of which are carried in the bloodstream and can only enter the eye from this source.

There are also soluble factors within the anterior chamber that act to modify adaptive immune effectors that gain access to this compartment. It has been known for more than a decade that AqH inhibits activation of T lymphocytes in vitro. Since this initial observa-

Table 1. Comparison of innate and adaptive immune responses

Property	Innate immunity	Adaptive immunity
Triggering molecules	repetitive molecular units	antigen + association structures
Recognition mechanism	direct	indirect
Onset of response	immediate (hours)	delayed (days)
Clonal expansion of responder cells	no	yes
Induces inflammation	always	often
Memory (to prevent reinfection)	no	yes



tion, an ever-growing number of soluble factors with this capacity has been identified in AqH (fig. 3). TGF-β_2 and vasoactive intestinal peptide (VIP) are present in AqH at concentrations that completely shut down T-cell proliferation in response to antigen or stimulation with anti-CD3 antibodies. α-Melanocyte-stimulating hormone (α-MSH) is also present, and although this neuropeptide does not suppress T-cell proliferation, it selectively inhibits activated T cells from producing either IFN-γ or IL-4. Of even greater interest is the recent discovery by Taylor and his colleagues that immune T cells exposed to antigen in the presence of α-MSH are converted into regulator T cells. By secreting TGF-β, these T cells can suppress the activities of other T cells activated in their microenvironment.

It is important to point out that AqH is not able to prevent cytotoxic T cells from lysing their specific targets. This is important because it indicates that the negative effects of AqH on adaptive immune effectors are not global; rather, they are selective, targeting the T cells most likely to induce inflammation.

AqH also prevents antibodies from triggering complement activation. To date, AqH is known to contain (a) a very small molecular weight factor (<1,000 daltons) that prevents C1q from binding to the Fc portion of IgG antibody molecules, and (b) a larger molecular weight factor (>30,000 daltons) that inhibits the generation of C3b from C3. Once again it is important to note that AqH does not interfere with the effectiveness of antibodies that neutralize viruses, indicating that the immunoglobulin target of AqH factors are adaptive immune effectors (complement-fixing antibodies) that threaten to cause inflammation.

The sources of the factors in AqH that modulate T-cell and antibody functions are not completely defined. Since supernatants of cultures of explanted iris and ciliary body and of cultures of explanted cornea inhibit T-cell activation in a manner similar to AqH it is likely that pigment epithelial cells, as well as corneal endothelium, secrete some of the immunomodulatory factors. Another likely source are terminii of autonomic nerves found within the iris and ciliary body.

Not all local factors that inhibit adaptive immune effectors are soluble. Yoshida and coworkers recently reported that pigment epithelial cells, cultured from the iris and ciliary body, suppress activation of naïve and immune T cells through a direct cell-to-cell contact mechanism. Moreover, T cells that have made contact in this manner with cultured pigment epithelial cells are, on the one hand, spared from apoptosis when given a signal that promotes programmed cell death, and, on the other, converted into regulatory cells that suppress bystander T cells through the secretion of TGF-β. The cell surface molecules responsible for the interaction between pigment epithelia and T cells remain elusive. Nonetheless, a theme emerges from these considerations.

The ocular microenvironment (anterior chamber) is protected from the ravages of inflammation triggered by adaptive immune effectors at two biologic levels: Architecturally, a blood:tissue barrier acts to limit access to the compartment of blood-borne cells and molecules with receptors specific for antigens expressed locally. Molecularly, the AqH (through soluble factors) and the cells that surround the anterior chamber (through cell surface molecules) prevent adaptive T cells and antibodies from triggering inflammatory cells and molecules directly, and even endow the T cells with the property of further suppressing inflammation. Together, these forces help to explain why it is virtually impossible to elicit delayed hypersensitivity reactions within the anterior chamber of fully sensitized mice, and they make it possible to understand why corneal allografts and xenografts are completely impervious to antibody-mediated rejection mechanisms. These are powerful expressions of the existence of adaptive immune privilege at this site.

Innate Immune Privilege in the Eye

Until very recently, the notion that privilege directed at innate immunity exists was not seriously considered. However, within the past few years, reports from several laboratories have produced irrefutable evidence that the ability of the innate immune system to express itself in the eye is impaired and modified. Perhaps the most dramatic example comes from the laboratory of Dr. J.Y. Niederkorn where work was carried out with a mouse tumor cell line that is susceptible to lysis by NK cells. When this tumor cell line was injected into the flank of mice with a severe deficit in adaptive immunity, the tumor was rejected – because these mice still possess an intact innate immune system. This tumor rejection was shown to be mediated by NK cells. When these same tumor cells were injected into the anterior chamber of the eye of adaptive immunodeficient mice, no rejection occurred. Instead, the injected cells formed progressively growing tumors. This result formally demonstrates the existence of innate immune privilege in the eye.

It is of interest that the tissues within the eye display considerable vulnerability to the deleterious effects of effectors of innate immunity. Corneal endothelial cells express very low levels of MHC class I molecules; NK cells are particularly equipped to recognize and kill cells with low class I MHC expression. Thus, corneal endothelium is vulnerable to lysis by NK cells. Corneal endothelial cells also express CD14, the receptor for the LPS-binding protein. This receptor targets LPS to the endothelium, thereby inviting LPS to recruit inflammatory cells that could harm this delicate, crucial inner layer of the cornea. The corneal endothelium is exquisitely sensitive to reactive oxygen intermediates and nitric oxide (NO), and these toxic molecules are the products of LPS-activated macrophages and neutrophils. Most ocular parenchymal cells express CD95 ligand constitutively. Reciprocally, neutrophils constitutively express CD95, and when neutrophils interact with CD95 ligand-bearing cells, the neutrophils are triggered to release pro-inflammatory mediators and destructive hydrolytic enzymes. Since these mediators additionally attract neutrophils and macrophages, and since both cells quickly release TNF-α when activated, the high level of expression of receptors for TNF-α on ocular cells renders these cells vulnerable to the deleterious consequences of this powerful, pleiotropic cytokine. It is a small wonder that the eye has acquired a protective adaptation that enables it to blunt the harmful effects of innate immunity.

Factors That Modify Expression of Innate Ocular Immunity

Niederkorn and his associate Apte demonstrated that AqH from normal eyes can prevent the lysis of susceptible target cells by NK cells in vitro. Apte determined that at least two factors in normal AqH mitigate NK cell killing: TGF-β and macrophage migration inhibitory factor (MIF). TGF-β's inhibitory activity on NK cells is delayed in time, whereas MIF acts quickly to disarm NK cells. These are the factors in the ocular microenvironment that permit tumor cells to grow progressively in the anterior chamber of mice with an intact innate immune system. Immigration of neutrophils and macrophages into the ocular microenvironment from the blood is inhibited by the presence of α-MSH and TGF-β, and both of these factors interfere with the activation of these inflammatory cells within the anterior chamber. In addition, the ability of activated macrophages to produce NO is inhibited by calcitonin gene-

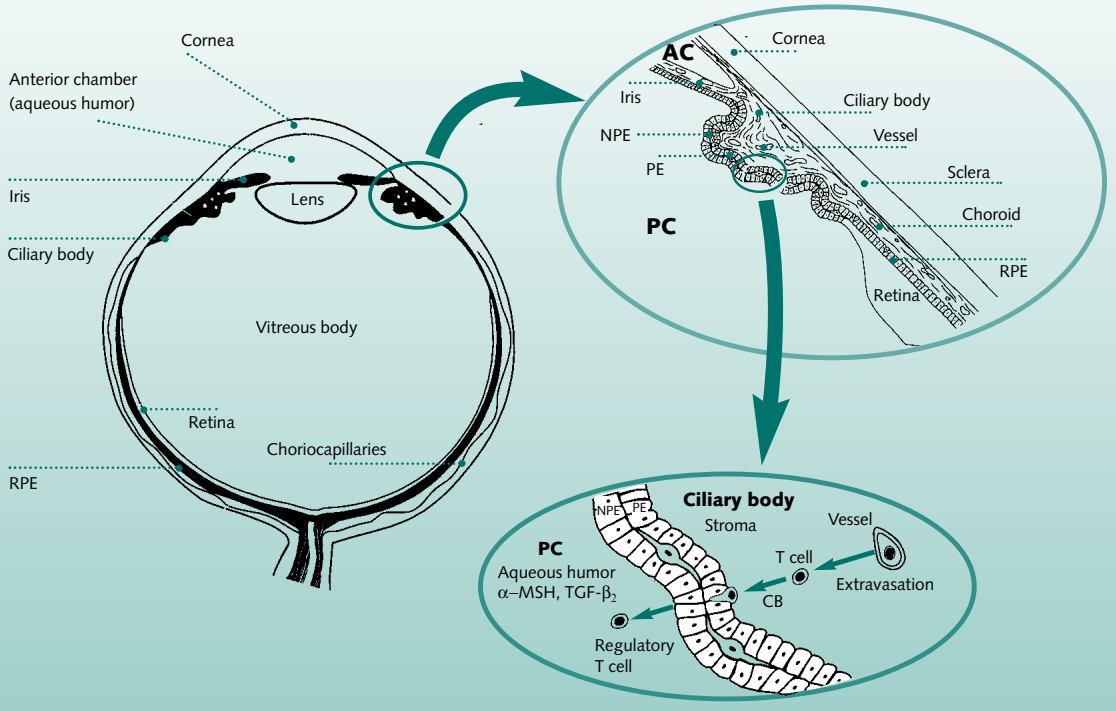


Fig. 3. Immunosuppressive ocular microenvironment where leukocytes that penetrate through the microvessels encounter immunomodulatory forces: pigmented epithelium of iris, ciliary body, retina, and AqH containing soluble immunosuppressive factors. T cells that enter this microenvironment acquire regulatory properties that suppress inflammation.
RPE = Retinal pigment epithelium; PE = pigmented ciliary epithelium; NPE = nonpigmented ciliary epithelium; AC = anterior chamber; PC = posterior chamber; CB = ciliary body.

related peptide (CGRP), another normal constituent of AqH. Soluble factors in AqH also inhibit the activation of complement via the alternative pathway. As of yet, the identity of the inhibiting molecule is unknown. Undoubtedly, other factors exist in AqH that contribute to the suppression of innate immune cells and molecules; so much remains to be learned.

Molecules expressed on cells lining the anterior chamber also inhibit innate immune effectors. Several membrane-associated inhibitors of complement activation (CD55, CD59, CD46) are constitutively expressed, and act to inhibit complement-dependent inflammation. Very recently, Kaplan and his associates have discovered that a fourth membrane complement inhibitor (Scrry) that is expressed in the normal eye plays a key role in inhibiting LPS-induced inflammation in the eye. Mice in which the gene for this inhibitor has been transgenically knocked out have a heightened susceptibility to LPS-induced uveitis.

Clinical Meaning of Ocular Immune Privilege

At the beginning of this article, the argument was advanced that immune privilege in the eye is designed to limit the intraocular expression of inflammation, primarily because inflammation in this organ disrupts the visual axis and causes blindness. In addition to the extraordinary success of orthotopic corneal transplants in humans, are there other clinical situations in which ocular immune privilege might be implicated? A few possible examples are advanced below:

Sympathetic Ophthalmia

Trauma to the eye that penetrates the globe and disrupts intraocular tissues can cause sympathetic ophthalmia. Experimental evidence indicates that the uveitis that develops in the contralateral eye is directed at retinal autoantigens, implying that these powerful antigens were released by the trauma and sensitized the patient. Yet, only a small minority of individuals suffering such an ocular wound actually develop sympathetic ophthalmia. Perhaps a reason why more patients don't develop this autoimmune complication is that antigen released by the trauma induces ACAID. If true, this is an example where the physiologic existence of immune privilege proves advantageous to the eye.

Recurrent Herpes Uveitis

Clearance of the herpes simplex virus from infected tissue (such as in herpes labialis) depends upon immunity mediated by CD4+ T cells of the delayed hypersensitivity type. In experimental animals, ACAID can be induced to HSV antigens, i.e. the animals fail to acquire HSV-specific delayed hypersensitivity. Transient inhibition of CD4+ T-cell immunity early in ocular herpes infection markedly reduces the incidence of stromal keratitis in mice, indicating that ocular immune privilege protects vision. A similar protective effect of ACAID might also occur in humans. However, some patients recover from an acute ocular herpes infection only to develop recurrent herpes uveitis during which live virus can be recovered from the afflicted eye. The possibility exists that in these subjects ACAID may persist indefinitely and thereby prevent immune elimination of virus from ocular tissues, thus promoting persistent, recurrent infections. If true, this would be an example where the existence of immune privilege has a disadvantageous outcome.

It remains to be determined experimentally whether innate and adaptive immune privilege, or its loss, plays a role in eye diseases. One can imagine that the incidence and severity of acute anterior uveitis might be reduced because of the existence of immune privilege, and one can speculate that rapid growth of spontaneous intraocular tumors might depend upon the integrity of immune privilege. Considering the power of ocular privilege to suppress innate immune effectors, the possibility even exists that immune privilege may have a role in the pathogenesis of diseases with inflammatory components – such as age-related macular degeneration, optic neuropathy of glaucoma, and even diabetic retinopathy. Working out the details of the molecular basis for ocular immune privilege promises a cornucopia of new approaches to the many multifactorial diseases that are currently untreatable and that cause significant loss of sight.

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Face to Face

with an Untreatable Disease

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Zürich

Patients with retinal degenerative diseases have one thing in common: We are all faced with the threat of blindness, and, in our hearts, we all carry the hope that one day a treatment for our condition will be found which may halt or cure the process. Ophthalmologists call us patients, but as a matter of fact we are 'impatients', going steadily blind day by day without a cure in sight. Researchers are walking step by step beside us along the stony path towards an understanding of the causes of retinal degenerative diseases and towards our mutual goal of finding a cure.

We all remember the moment when we or one of our loved ones was first diagnosed with the disease. I was only 13 years old when they told me I had retinitis pigmentosa (RP). My parents were very open and tried to explain the extent of the disease to me, gently but without hiding the fact that one day this condition would leave me blind. Contrary to common expectations, this news did not really bother me at the time. The explanation of my night blindness as well as the fact that my field of vision had decreased to about 8 degrees helped me understand the many strange things that had been happening to me: I always wondered why other people could find things so quickly in the dark or why they didn't seem to have any problems catching a ball or jumping over an obstacle. The diagnosis somehow gave me a new freedom – I no longer had to play volleyball and other competitive games; games in which I could never fully participate and which embarrassed me each time none of the other children wanted to have me play on their team.

The first time I realized the impact of the disease on my personal life was when I was ready to choose a profession. My interests were in science or medicine, but being severely visually impaired these dreams were not to come true. Realizing that my future was not in my own hands sometimes made me frustrated and angry, and I kept asking myself: Why me? However, I was lucky to have grown up in a very supportive family who have a positive attitude towards the future. I decided to go into advertising where I gave my best and was successful. Since I had so-called tunnel vision and a very good central visual acuity, reading was not a problem for me and helped me continue to live my life as normally as possible. Driving was never an option and I sometimes missed the free mobility other young people normally enjoy. Since I had no other choice, I was open about my visual impairment, but I knew that my peers could never guess how severely impaired I really was. As we all learn to do, I invented a lot of different strategies to help me overcome or avoid uncomfortable or difficult situations.

RP causes a gradual loss of vision and the sight that you do have is never really stable. Depending on the lighting, you may see everything very clearly, if it is good but if it is bad, you may see nothing at all. This means that from one moment to the other, I had to perform first as a sighted person and then behave as a perfectly trained blind person. Looking back, I would describe this period of slow vision loss (about 15 years) as the most difficult time of my life. Being in this no-man's-land between the world of the sighted and the world of the blind was an enormous stress. I always believed what I saw at first sight, but soon came to realize that this was not always correct and that I could not necessarily trust my own eyes. This was especially difficult in social interaction: Family members and friends never knew when I actually needed help and when not. Today, I am completely blind and for both my-

self and others it is obvious when help is needed.

Although it might seem paradoxical, it was almost a relief when, 8 years ago at the age of 42, I finally lost my vision completely. At last I knew exactly where I stood. And, despite the pain of this loss, going blind can also have some interesting aspects. I am by nature a very visual person, and the fact that my brain still works with images despite the lack of visual input fascinates me. All the things that I had been able to see before now serve as precious treasures for my imagination. Going blind also meant that I had to explore my own limits and overcome my personal anxieties. I always hated being dependent on others and my wish for independence spurred on my will to quickly master the different rehabilitation techniques such as walking with a white cane as well as the simple tasks and skills one needs just to get through the day.

The two things I miss most of all though are not being able to see the expression on people's faces when I speak to them and the ability to read print. Being unable to see who is around means that I have to depend on others to approach me and start any social interaction that may take place. It has happened that I had attended an event and only realized later that people were there who I would have loved to meet again. I used to read everything that came into my hands. Now, even though a great number of books are available on tape or via talking computers, nothing can really compensate for the pleasure you get from visiting a book shop, taking a book in your hands, and browsing through the new books on the shelf. Still, I believe that there has never been as good a time as today to be blind: Technology is making immense progress. Computer technology, for example, has opened up totally new possibilities to acquire information independently. With the aids available 20 years ago, I would never have been able to work in such an interesting and rewarding profession as the one I am in today.

To be faced with an untreatable disease is difficult to cope with and each of us deals with it in different ways. For me it was the reason I decided to join the RP organization of my country 20 years ago and to take an active part in the fight for sight. Despite the fact that there is still no treatment available, I can see the difference between now and then: Today there is well-founded hope that this situation will change sooner or later. Through my work in the organization I have had the opportunity of getting to know many wonderful people – patients and researchers – who I would never have met otherwise. Should this be the deeper sense of my having RP? If so, what better reward could I get than friendship?

Fig. 1.

Normal vision.

Loss of peripheral vision ('tunnel vision') in RP. In some cases small patches of retinal activity on the periphery are preserved, making it possible to detect movement and objects that help improve orientation.

Fig. 2.

Normal vision.

Loss of central vision in macular degeneration. Affected individuals have difficulty reading and recognizing faces, but enough peripheral vision is retained for good orientation and mobility.

Retina International

Retina International (formerly International Retinitis Pigmentosa Association) is a voluntary charitable umbrella organization of more than 40 national patient organizations for people with retinal degenerative diseases (see below) such as retinitis pigmentosa (RP), macular degeneration, Usher syndrome and allied retinal dystrophies, as well as their families and friends. The two main objectives of Retina International are:

1. To promote and fund research directed at finding the cause and developing a treatment and, ultimately, a cure for retinal degenerative diseases, especially the inherited forms.

2. To foster mutual support among its members, families and friends.

Furthermore, the organization strives to promote public awareness by providing and exchanging information on retinal degenerative diseases, and to support the establishment of new patient societies.

The first two RP organizations were founded simultaneously and independently in Finland and the USA. People felt the need for more communication and were concerned that not enough research was being done to find a cure for RP. A worldwide movement was thus born and, in 1978, nine national organizations joined together and founded the International Retinitis Pigmentosa Association. Later on, the organization decided to broaden the scope of its activities to include all forms of retinal degenerative conditions and changing the name to Retina International was the logical consequence. The Retina International member organizations represent over 140,000 members worldwide and raise more than USD 25 million per year, which is invested in research. Each member association and Retina International are advised by their own scientific and medical advisory boards.

Impact of Retinal Degenerative Diseases

Retinal degenerative diseases affect over 10 million people in Europe. Age-related macular degeneration is the major cause of severe visual impairment in the population over 60 years of age and is on the increase. The reasons for this increase are unknown. Inherited forms of retinal degenerative diseases such as RP and Usher syndrome usually affect peripheral vision causing night-blindness and tunnel vision. The age of onset in the majority of cases is during the second decade of life. This has severe implications during the patient's most active years, resulting in underemployment, early retirement and severe financial hardship for the affected families.

The Current State of Research

Over the last few years, research into the cellular and genetic defects that cause retinal cell death has made great progress. For some inherited retinal diseases, the affected genes and proteins have been identified. The challenge now is to understand the connection between the abnormal function of individual proteins and the death of photoreceptor cells as well as to develop drug therapies that prevent or delay this process. Several new forms of biological treatment approaches are currently being developed which have shown some efficacy in animal models but have still to be tested for long-term effectiveness and safety before they can be studied in humans. These include the use of growth factors to delay cell death; cell transplantation, i.e. replacing photorector cells or retinal pigment epithelium cells with new healthy ones; and gene therapy which aims at replacing the mutated genes with nondefective genes. With more and more gene mutations being identified, many researchers consider gene therapy a promising approach for the future; however, there are still many obstacles to overcome, e.g. how to transfer DNA of the healthy gene into the diseased cells. Further investigations focus on the development and assessment of neuroretinal implants (microchips), which would receive visual information transmitted from a camera mounted on a pair of glasses and thus restore rudimentary vision. The next big step will be the transfer of these basic findings into clinical trials.

Challenges for the Member Organizations

Retina International has emerged into the 21st century ready for change and adaptation. This century promises new and exciting discoveries thanks to the recent progress of research, and there is optimism that the next few decades will bring about a cure for retinal degenerative diseases. However, this is not the time to sit back! On the contrary, all efforts must be accelerated to meet the following challenges:

- Bringing research from university laboratories into those of the pharmaceutical industry: It is important to develop strategies to lobby for the necessary funds for clinical trials which swallow up huge sums of money.
- Patient recruitment: To access those patients who fit the criteria for the first clinical trials, national member organizations must actively promote reliable and updated patient registries.
- Improved cooperation and solidarity between the member organizations: Retina International is a growing organization with members from different economic and cultural backgrounds whose needs will be even more diverse in the future. The experienced, well-organized and well-established national organizations should support the newly emerging ones.

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Retinal Degenerative Diseases

The retina is a specialized light-sensitive tissue at the back of the eye that contains photoreceptor cells (rods and cones) and neurons connected to a neural network for the processing of visual information. The rods function in conditions of low illumination whereas cones are responsible for color vision and all visual tasks that require high resolution (e.g. reading). The rods are mostly located away from the center of the eye in the retinal periphery. The highest concentration of cones is found at the center of the retina, the macula, which is necessary for visual acuity. For support of its metabolic functions, the retina is dependent on cells of the adjacent retinal pigment epithelium.

Retinitis pigmentosa (RP) designates a group of inherited diseases that affect the retina and are characterized by a gradual destruction of the rods and cones, resulting in a progressive loss of vision and, possibly, blindness. Usually, the rod cells are the first to degenerate, causing night blindness and 'tunnel vision' (fig. 1). RP is most often diagnosed during childhood or early adulthood. Depending on the type of RP, the rate of progression varies. To date, there is no known way to halt the degeneration of the retina or to cure the disease.

Macular degeneration refers to a group of disorders in which the breakdown of cells is limited to the macula, leading to a loss of central vision (fig. 2). The most common form, age-related macular degeneration (AMD), usually affects people over the age of 60. There are two types of AMD: 'dry' and 'wet'. Dry AMD accounts for about 90% of all cases. With dry AMD, yellow-white deposits called drusen accumulate in the retinal pigment epithelium tissue beneath the macula. In wet AMD, abnormal blood vessel growth forms beneath the macula. These vessels leak blood and fluid into the macula damaging photoreceptor cells. In some cases, if wet AMD is diagnosed early, laser surgery has been shown to reduce the risk of extensive macular scarring. Hereditary forms of macular degeneration with an early onset, such as Stargardt disease, Best's disease or progressive cone dystrophy, also exist. As a general rule these diseases cause severe visual impairment but rarely result in complete blindness.

For the vast majority of MD patients there is currently no effective treatment, but a number of effective visual aids and rehabilitation options are available.

Individuals with **Usher syndrome** suffer from RP and congenital deafness or progressive hearing loss.

Lasers in Eye Surgery

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Fig. 3. Peripheral YAG laser iridotomy at 12.00 h in the midperipheral iris.

The origin of light treatment to the eye dates back to 400 BC, when Plato recognized the power of light and described the dangers of staring directly at the sun during an eclipse. In 1946, Gerd Meyer-Schwickerath, a German ophthalmologist, first demonstrated the use of light to coagulate retinal tissue (photocoagulation) in humans [1]: By focusing the light of a xenon arc lamp he induced small burns on the retina to seal retinal tears – a technique which was to revolutionize the treatment of eye diseases. The first functioning laser was built by the physicist Theodore Maiman in 1960, and the first clinical ophthalmic laser treatments in humans were reported by Charles Campell and coworkers in 1963 and Christian Zweng and coworkers in 1964 [2]. Because light can reach almost any ocular structure noninvasively, lasers have had a greater impact on ophthalmology than on any other field in medicine.

'Laser' is an acronym for 'light amplification by stimulated emission of radiation'. Stimulated emission, first predicted theoretically by Albert Einstein in 1917, is the fundamental physical process that makes lasers possible. There are three basic interactions between light (photons) and electrons [3]: (1) Absorption – an atom absorbs a photon, which forces one of its electrons to move to a higher energy orbit. (2) Spontaneous emission – an atom in its excited state emits a photon when an electron falls back to a lower energy orbit. (3) Stimulated emission – a photon interacts with an atom that has energy stored and stimulates an electron to fall onto a lower energy orbit and produce a second photon, coherent with the first. There are many ways of producing light, but stimulated emission is the only known method that produces coherent light. For an output of just 1 mW, a minimum of 10^{16} stimulated emissions per second are necessary. The realization of this concept was so difficult that it took over half a century between the theoretical prediction of stimulated emission and the construction of a practical laser light source. Gas and solid-state lasers are the most widely used in clinical ophthalmology (fig. 1). In gas lasers, atoms of a working gas (e.g. argon or krypton) are enclosed in a cylindric tube and eventually one of the high-energy electrons undergoes spontaneous emission and generates a photon of the correct frequency to cause stimulated emission. By repeatedly reflecting the photons back and forth across the cylinder tube with the help of mirrors placed at opposite ends of the tube, a chain reaction of stimulated emission is produced. One mirror reflects totally and the other partially, and the relatively small amount of light that is allowed to pass through the partially reflecting mirror produces the laser beam, either continuously or pulsed.

Lasers can have various effects on the target tissue depending on the wavelength and power used:

- Thermal: Absorption of laser energy (visible or infrared light) by tissue pigment results in temperature increase (e.g. photocoagulation, Ho:YAG).
- Photochemical: Ultraviolet and visible light absorption induces the formation or destruction of chemical bonds (e.g. photodynamic therapy, excimer laser).
- Mechanical: Plasma formation (optical breakdown) leads to tissue disruption (e.g. photodisruption with Nd:YAG)
- Vaporization: Micro-explosion occurs due to the sudden rise in water temperature to above boiling point (e.g. Er:YAG)

Ophthalmology was the first medical discipline to apply lasers as surgical tools. Over the last 30 years, lasers have become the treatment of choice for disorders involving almost every part of the eye, and we can distinguish their use in anterior segment, refractive, pediatric and retinal surgery.

Anterior Segment Surgery

The use of lasers in treating anterior segment anomalies became popular in the last 30 years. As the anterior segment is easily accessible with conventional surgery, it took longer until laser surgery was used routinely in this part of the eye. Disorders for which lasers are used today are cataract (a clouding of the eye's natural lens which leads to blurred or decreased vision) and glaucoma (vision loss due to damage to the optic nerve, which is often caused by increased intraocular pressure).

Laser Surgery for Cataract

Cataract Removal

The current standard surgical technique to remove a cataract is phacoemulsification, introduced by Charles Kelman in 1967. A small incision of about 3 mm is made on the side of the cornea, the center of the lens is softened with ultrasound waves and removed, followed by the implantation of an artificial foldable intraocular lens. Removal of the human crystalline lens by laser (laser-phaco) has been a dream for a long

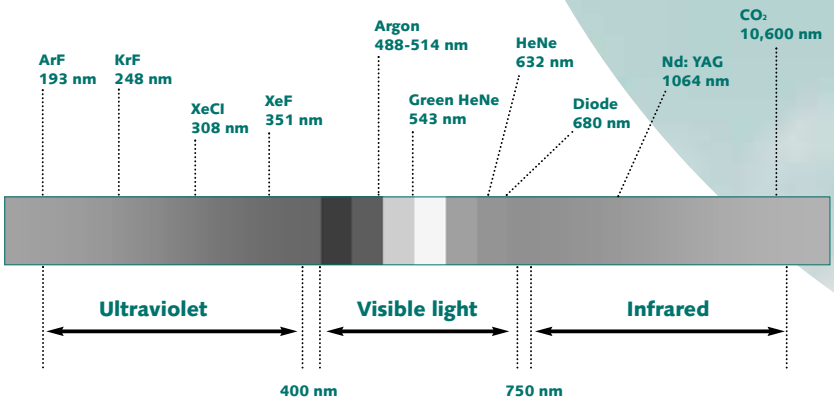
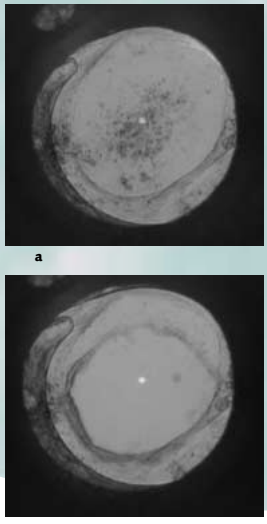


Fig. 1. Electromagnetic spectrum of lasers used in eye surgery.



a

b

Fig. 2. Posterior chamber intraocular lens implantation.
a Posterior capsule opacification.
b Nd:YAG laser posterior capsulotomy to open the posterior capsule.

time and is currently in the developmental stage. Three different lasers are being used: neodymium:yttrium-aluminum-garnet (Nd:YAG) in the near-infrared energy range with a 1.064-µm wavelength, erbium:yttrium-aluminum-garnet (Er:YAG) with a wavelength of 2.94 µm, and yttrium-lithium-fluoride (picosecond laser). Each of these lasers holds great promise as a future clinical tool, but so far hard cataracts cannot be effectively removed and the surgical time needed to remove a standard cataract is comparable with that of phacoemulsification. Although many ophthalmologists believe that some form of laser technology will be the future of cataract surgery, its putative advantages over standard ultrasonic methods still remain to be proven. More research is necessary to achieve safer and gentler cataract surgery.

Neodymium: YAG Laser

Secondary cataract formation (or after-cataract) is a significant late complication in people who undergo extracapsular cataract extraction, a procedure in which the surgeon removes the lens nucleus manually or with phacoemulsification (see above). In both types of surgery the posterior half of the capsule, the outer covering of the lens, is left behind. Following intraocular lens implantation, a formation of posterior capsular opacification (PCO) may develop (fig. 2a). The frequency of PCO is age-related. Almost all children develop PCO after extracapsular cataract extraction, whereas the incidence is much lower in adults. PCO decreases the visual acuity and therefore significantly diminishes the treatment success of cataract surgery. Patients with PCO require posterior capsulectomy, a procedure which removes the central part of the posterior capsule and instantly improves vision. Nowaday this opening of the posterior capsule (disruption of the posterior capsular bag membrane) is performed in a few seconds using an Nd:YAG laser. The procedure can be carried out on an out-patient basis at the slit lamp (fig. 2b). It has become the treatment of choice and has restored the visual acuity of millions of patients who were still unhappy after cataract removal.

Laser Surgery for Glaucoma

Lasers play an important role in modern-day treatment of glaucoma, where their main use is to lower intraocular pressure (IOP).

Argon Laser Trabeculoplasty

Argon laser trabeculoplasty (ALT) is a firmly established, well-tolerated procedure used to lower IOP in various types of open-angle glaucoma. It is performed by placing small, evenly spaced, nonpenetrating argon laser spots into the trabecular meshwork of the angle in the anterior chamber, which allow the aqueous humor to drain. A period of at least 4–6 weeks after ALT is required before the final result can be evaluated. Results show that ALT has managed to control IOP in 67–80% of eyes for 1 year, 35–50% for 5 years and 5–30% for 10 years.

Peripheral Laser Iridotomy

In 1857, von Graefe introduced surgical iridectomy for the treatment or prophylaxis of narrow-angle glaucoma. In 1956, Meyer-Schwickerath demonstrated that an iridectomy could be created without the need for an incision, using xenon arc photocoagulation. This method failed to gain popularity because of frequently occurring lens and corneal opacities. However, with modern lasers (argon and Nd:YAG), laser iridotomy has now essentially replaced surgical iridectomy in the vast majority of cases (fig. 3).

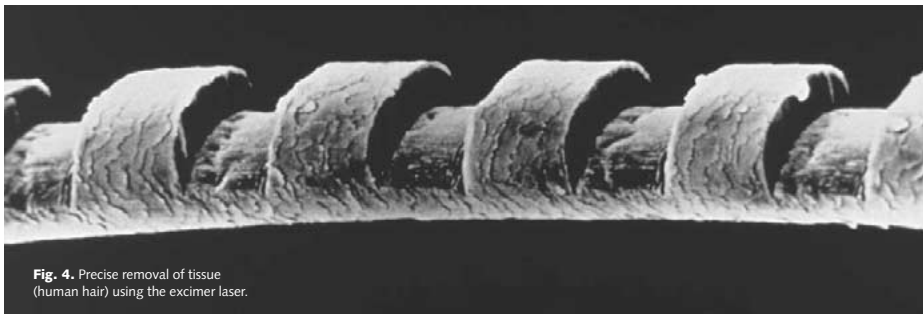


Fig. 4. Precise removal of tissue (human hair) using the excimer laser.

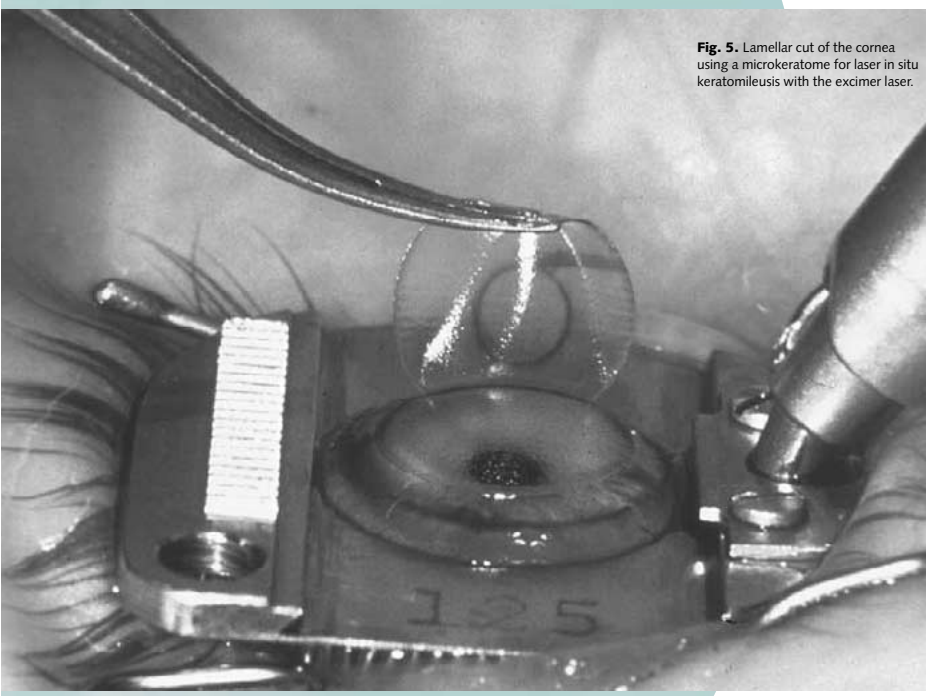


Fig. 5. Lamellar cut of the cornea using a microkeratome for laser in situ keratomileusis with the excimer laser.

Refractive Surgery

Refractive or vision correction surgery includes techniques which alter the eye's focusing power by changing its natural structures, eliminating the need for glasses and contact lenses. In the year 2000, almost 2 million refractive procedures were carried out worldwide, the vast majority (>90%) being performed using laser technology.

Excimer

Today's most advanced refractive surgical techniques are performed with the excimer (excited dimers) laser. The development of excimer lasers began in 1975, when investigators noted that under high pressure meta-stable rare gas atoms produce unstable compounds. These compounds rapidly dissociate to the ground energy state of the individual molecules with the release of an energetic ultraviolet photon. These molecules can be induced to produce light amplification by stimulated emission when they are excited by an electron beam; the ArF (argon-fluorine) molecule emits light with a wavelength of 193 nm. At this wavelength corneal tissue can be removed with extreme precision – about 0.25 µm of corneal tissue is removed with each pulse – and with minimal damage to the surrounding tissue (fig. 4). For myopic correction central tissue has to be removed causing a flattening of the cornea; for hyperopic correction peripheral tissue is ablated to cause central steepening of the cornea, and for astigmatism an elliptical shape is removed to make all meridians of the cornea equally steep or flat. Today, the two main techniques used to correct refractive errors by reshaping the cornea are photorefractive keratectomy (PRK) and laser-assisted in situ keratomileusis (LASIK).

With *PRK*, the superficial layer of the cornea, the epithelium, is removed mechanically, and then a specific amount of stromal tissue is ablated using an excimer laser. Until the epithelium is healed, which usually lasts 3–4 days, a protective soft contact lens can be placed on the eye and corticosteroid eye drops are administered for 3–6 months. Overall, the results for PRK have been acceptable and have improved with experience. Currently, PRK is in use for myopia of up to –6.0 dpt, for astigmatism, and for hyperopia of up to +5 dpt. Typical complications are over- and under-correction, central scar formation (haze) and corneal infections. The wound-healing process is prolonged and stability is mostly not achieved before 6 months.

The second technique, *LASIK*, is a combination of a lamellar cut into the cornea and corneal stromal ablation using the excimer laser. The lamellar cut is produced with a microkeratome, cutting to a depth of about 120–160 µm, and leaving the flap attached to the cornea by a hinge (fig. 5). Excimer laser ablation is performed after the flap has been lifted up, leaving the stromal tissue of the cornea uncovered. After the ablation the flap is folded back onto the stromal bed, and within minutes the flap is attached by internal corneal forces and heals without sutures. The healing process is much faster after LASIK and a visual acuity of 20/20 (1.0) uncorrected on the first postoperative day is not rare. Correction of myopia up to –10.0 dpt, hyperopia up to +5.0 dpt and astigmatism of up to –5.0 dpt is possible and successful when all the contraindications for this type of procedure (thin corneas, extremely large pupil diameter and corneal pathology) are taken into consideration. Currently, other lasers like picosecond or femptosecond laser are being evaluated for their performance in intrastromal ablation or corneal lamellar incisions. New methods which correct the aberrations of the whole optical system, e.g. wavefront aberroscopy, are also being developed to increase the best corrected visual acuity of a human eye following refractive surgery.

Laser Thermal Keratoplasty

Laser thermal keratoplasty (LTK) is a promising new technique which uses laser energy to gently heat peripheral corneal tissue, producing a change in the cornea's refractive power. 16 or 32 spots are placed on the cornea in a symmetrical, circular fashion with high accuracy (fig. 6). Absorbed laser light increases the temperature of water and adjacent collagen fibrils, thus causing them to contract. The resulting tension produces a steepening of the anterior cornea over the optical zone, which is intended to correct hyperopic refractive errors. Currently, two types of thermal lasers are being used for hyperopic LTK: the holmium:yttrium aluminum garnet (Ho:YAG) laser (fig. 7) and the continuous-wave diode laser. Laser energy is delivered by either the noncontact or the contact mode. The procedure offers a solution for people over 40 who require glasses for reading, and corrections of up to +3.0 dpt of hyperopia can be achieved. In some patients, however, the result is not permanent and retreatment might be necessary.

Pediatric Eye Surgey

A disease occurring in some premature babies is retinopathy of prematurity, which is the growth of abnormal blood vessels in the retina, induced by an ischemic, avascular retina. Over time, this vessel growth may produce a fibrous scar tissue which attaches to the retina and may cause retinal detachment and eventually blindness. The hyperopic environment of the preterm neonate is directly linked to the severity of disease. Standard treatment has been cryotherapy of the anterior avascular retina in the infant eyes that reach 'threshold retinopathy'. Recently, both argon and diode laser indirect ophthalmo-

Fig 6. Corneas with a circular ring of Ho:YAG laser spots for the treatment of hyperopia, which leads to a central steepening of the cornea.

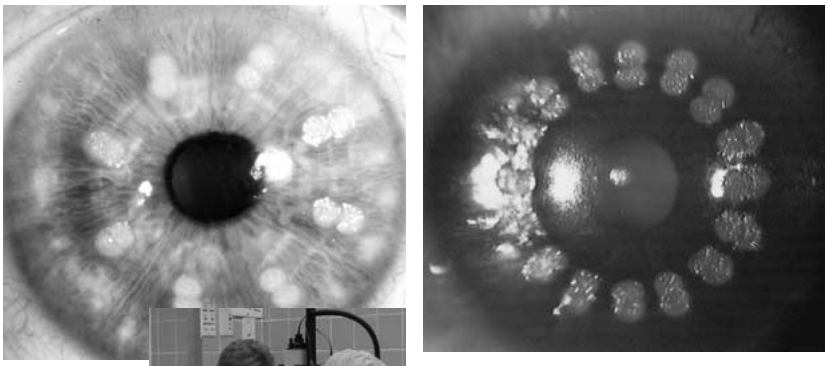


Fig 7. Laser thermal keratoplasty with the Ho:YAG laser.

scope systems have been developed for transpupillary peripheral retinal ablation. Laser treatment is at least as effective as cryotherapy if not more so [4], and it is also easier to apply, especially for posterior disease. Where available, lasers have largely supplanted cryotherapy, except in cases of severe cloudy media. Besides this main indication, retinoblastoma or posterior segment diseases in children have been treated using argon or YAG lasers.

Retinal Surgery

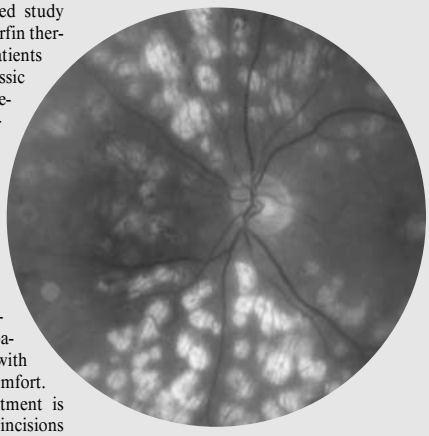
Photocoagulation of the Posterior Segment

Using topical anesthesia and a contact lens, the laser light for photocoagulation can be delivered into the eye by a slit-lamp delivery system. Diseases or conditions which are treatable by photocoagulation include diabetic proliferative retinopathy (fig. 8), diabetic macular edema, branch retinal vein occlusion, peripheral retinal and choroidal neovascularization, idiopathic central serous chorioretinopathy, vascular anomalies, nonvascular tumors, and retinal breaks. The spot size is dependent on the location of the treatment: 50–200 µm for macular photocoagulation, 200–1,000 µm for peripheral retinal spots. Power and burn duration are initially set according to desired burn intensity, and the aiming beam intensity is set to the lowest level that permits adequate beam visualization. The retinal burn, or opacification, produced during photocoagulation is due to protein denaturation in the outer retina [5].

Photodynamic Therapy

Age-related macular degeneration is a major cause of severe vision loss in people older than 65 years in North America and Europe. Loss of visual acuity results from choroidal neovascularization (CNV). The standard treatment for choroidal membranes involves photocoagulation with a hot laser, which inevitably destroys the adjacent and overlying normal retina. Now a new treatment method for CNV is available – photodynamic therapy with verteporfin (Visudyne™) – that can minimize damage to the surrounding viable retinal tissue. In this treatment, 15 min after the verteporfin has been injected intravenously, the drug is activated by delivering a cold laser light (at 689 nm) over 80 s, using a spot size with a diameter 1,000 µm larger than the greatest linear dimension of the CNV lesion. A recent comparative, placebo-controlled study has recommended verteporfin therapy for the treatment of patients with predominately classic CNV resulting from age-related macular degeneration [6].

Fig 8. Argon laser panretinal photocoagulation for the treatment of proliferative diabetic retinopathy.



Conclusion

Lasers have brought about a revolution in ophthalmic surgery: Owing to their precision and noninvasive nature, the actual surgery can be performed on an outpatient basis in a matter of minutes, with little or no pain or discomfort. Recovery time after treatment is short as there are no large incisions to heal and postoperative complications, e.g. inflammation and infection, are reduced substantially. Today, almost 40 years after the advent of the laser, medical laser technology continues to evolve rapidly and ophthalmologists continue to explore new applications for this tool, whose possibilities for saving and sharpening vision seem to be unlimited.

Thomas Kohnen is associate professor of ophthalmology ('Privatdozent') at the Johann Wolfgang Goethe University in Frankfurt where he heads the Department of Refractive Surgery. Since October 2000, he is also visiting associate professor at the Cullen Eye Institute at the Baylor College of Medicine in Houston, Texas. Dr. Kohnen is currently editing the book 'Modern Cataract Surgery Update' to be published by Karger in the second half of 2001.

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Apollo, god of light, music and the arts, medicine, reason and archery.

Plaster cast of a marble bust in the British Museum, London:
Roman copy of a Greek sculpture dating from around 470 BC (Skulpturhalle Basel, Switzerland; photo by G. Schmid).