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Editorials

The architecture of the corneal stroma

In recent years the evolution of modern refractive surgery has focused attention on the architecture and biological properties of the cornea. In this issue of the *BJO* (p 437) Müller *et al* address the differential behaviour of the anterior and posterior stroma during corneal swelling and draw interesting conclusions about the factors maintaining corneal shape.

Transparency of the corneal stroma depends particularly on the degree of spatial order of its collagen fibrils which are narrow in diameter and closely packed in a regular array.[1–8] The collagen fibrils themselves are weak scatterers, since their fibril diameter is less than the wavelength of light, and fibril refractive index is close to that of the ground substance. There is little variation in fibril diameter and separation between the anterior and posterior cornea.

The stromal fibrils are further organised into bundles, or lamellae, of which there are approximately 300 in the central cornea and 500 close to the limbus.[9] The posterior lamellae course directly across the full width of the cornea without a break, having their origins in fibres which wind around the limbus at the corneoscleral junction[10–12] or, according to Radner,[9] have a pseudocircular organisation at the limbus, forming the ligamentum circulare corneae. On the basis of *x* ray diffraction studies, about 49% of the stromal lamellae are preferentially aligned orthogonally, along the vertical and horizontal meridians, while about 66% lie within a 45° sector.[11 12] Fibrils within a lamella are in parallel array, except where branching of lamellae occurs. Branching in the horizontal plane occurs throughout the stroma, whereas anteroposterior branching is found only in the anterior third.[13]

The anterior and posterior stroma differ in specific ways. In general the posterior stroma is more ordered,[14] more hydrated,[15] more easily swollen, and has a lower refractive index[16] than the anterior stroma. The posterior lamellae are also wider and thicker (100–200 μm wide and 1.0–2.5 μm thick) than the anterior (0.5–30 μm wide and 0.2–1.2 μm thick).[13] There are also differences in keratocyte morphology.[17] It has long been established that the posterior lamellae of the human corneal stroma are arranged parallel to the plane of the corneal curvature[13] and this feature is recognised to facilitate dissection in lamellar corneal grafting.[18 19] Dissection of the cornea is, however, not resistance free, suggesting that there are elements which bind the collagen lamellae together.[20] Part of this resistance is likely be due to attachments between the collagen fibrils on the one hand and other matrix proteins such as the proteoglycans[21 22] or keratoepithelin.[23]

In the anterior stroma, an additional contribution is made by the marked anteroposterior lamellar interweave which has been recognised to be a feature of the corneal architecture since the early part of the century.[9 13 24–33] Here, lamellae can be shown to pass obliquely from one layer to another, sometimes passing across several lamellae to reach their destination.[13] It is likely that such obliquely disposed lamellae have their peripheral origins in the limbus, although this specific question has never been explored directly. A proportion of the anterior lamellae are known to be inserted directly into Bowman's layer and it has been suggested that the latter contribute to the formation of the anterior corneal mosaic, a normal architectural feature seen at the corneal surface.[30 35–38] The anterior corneal mosaic is visible in all normal corneas as a broad polygonal pattern which can be observed after instillation of fluorescein, simply by exerting pressure on the cornea through the closed lids, and observing the fluorescein distribution when the eyes open. This polygonal pattern can be regarded as the most superficial manifestation of a more complex, three dimensional "chicken wire" arrangement of the anterior stromal lamellae.[30]

In this issue, Müller *et al* elaborate at ultrastructural level, an older, light microscopic observation, that human anterior stroma swells considerably less than the posterior stroma, when corneas are immersed for a prolonged period in saline.[39–46] In non-nutrient media, at room temperature, where there are no cellular barriers, and no viable cells capable of deswelling the stroma, stromal swelling is due almost entirely to the gel pressure exerted by the stromal proteoglycans, acting as a polyelectrolyte gel.[47] It is the high, negative charge of the glycosaminoglycan (GAG) components of the proteoglycans, that is responsible for this property. Müller *et al* claim that the anterior stroma, 100–120 μm deep to Bowman's layer, does not swell perceptibly when the cornea is immersed in water or saline for prolonged periods and that swelling is confined to the posterior stroma. This is a remarkable observation that implies that the anterior stroma has special features which constrain swelling in these conditions, despite the presence of negatively charged proteoglycans here, as in the posterior stroma. These observations are important and need to be confirmed by morphometric measurements of fibril number density (fibril number per unit area) in the respective zones, with special attention to the presence or absence of stromal "lakes".

There are a number of factors that could explain the findings of Müller *et al.* As noted above, the morphology of the anterior and posterior stroma differs considerably. Müller *et al* suggest that the anterior stromal interweave is the chief architectural factor determining the differential swelling behaviour of the stroma. They also suggest that it is

responsible for the structural stability of this region of the cornea, a feature which is of importance to refractive surgery and possibly in such conditions as keratoconus. However, as these and other authors have observed, additional factors may contribute to the differential swelling. The GAGs of the corneal stroma are keratan sulphate (a component, for instance, of the proteoglycan lumican), dermatan sulphate (DS), and chondroitin sulphate (CS) (components of the small proteoglycan CS/DS proteoglycan, decorin). Keratan sulphate makes up about 50% of the corneal GAGs. In bovine corneal stroma, the keratan sulphate/chondroitin-4-sulphate ratio is higher posteriorly than anteriorly.[42 44] If this is the case for human cornea, then since keratan sulphate has a higher water affinity than chondroitin-4-sulphate, this could explain, in part, the greater degree of posterior stromal swelling on immersion. Another factor, which should be kept in mind, is the possibility of a differential leaching of GAGs from the stroma during prolonged immersion. Although only about 1% of keratan sulphate is lost from corneas held in closed culture[48] a significant loss of proteoglycans from swollen corneas has been recorded by others[45 49] with a preferential loss of keratan sulphate from oedematous rabbit corneas.[50] Differential loss has not been studied, but a greater loss of GAGs from the anterior stroma could reduce its "swellability".

This behaviour of human anterior stroma is reminiscent of that of the stroma of the cartilaginous fishes (Chondricthyses, which includes the subclass of elasmobranchs). The stromal lamellae in such fish are well defined and run in the plane of the cornea, but are crossed at right angles by anteroposterior bundles of "sutural" fibres or complexes, which connect the basal lamina of the corneal epithelium to a posterior collagenous layer, resembling Descemet's layer in location but not structure.[51] The sutural fibres were first described by Ranvier[52] and subsequently by Payrau *et al*,[53] Goldmann and Benedek,[54] and Faure.[55] The corneas of the cartilaginous fish swell little when immersed in water, and retain their transparency[56] apparently in the absence of a functional endothelial layer.[51 57 58] It has been suggested that the sutural fibres provide a restraining action on corneal swelling, possibly assisted by an interaction between stromal collagen and stromal matrix materials,[54] which are abundant, for instance, in the dogfish cornea. It appears that the anterior interweave of the stromal lamellae of the human cornea and, possibly, differences in proteoglycan composition and attachment may play a similar part to that of the sutural fibres in the cartilaginous fish, whose lamellae show little or no anteroposterior interweave.

The anterior stromal interweave has other structural implications for the cornea. It can be conceived that while the limbus to limbus arrangement of the posterior lamellae offers a singular advantage with respect to strength, the interweave of the anterior lamellae, and the insertion of lamellae into Bowman's layer, offers opportunities to confer a variable shape to the anterior corneal surface. Although the insertions of lamellae into Bowman's layer might seem to offer less structural strength than the limbus to limbus arrangement of the posterior stroma, loss of strength would be minimised if anterior insertions extended from the limbus to Bowman's layer, beyond the corneal centre. This might also afford better opportunities to determine shape. Since corneal shape is to some extent hereditable, the inference would be that the anterior obliquities are under genetic control and regulated by proteins whose spatiotemporal distribution during development determine corneal shape. It is relevant that the developmental origin of the anterior third of the corneal stroma is thought to differ from that of the posterior.[25]

Müller *et al* suggest that the structural stability of the anterior stroma under conditions of extreme hydration imply an important role for this zone in the maintenance of corneal curvature and that this stability is determined by the tight interweave of the stromal lamellae here. It seems a reasonable proposition that the interweave is important in maintaining shape and it seems likely too that is a determinant of shape, probably by distributing tension over the corneal surface in a manner which could not be achieved by an interlimbal arrangement alone.

One final implication of the human anterior stromal interweave should be considered. It is generally accepted that anterior stromal keratocytes die shortly after the induction of a corneal abrasion. It has reasonably been proposed, by Wilson,[60] that this is due to a FAS-FAS ligand mechanism, in response to IL-1 release from damaged epithelium. However, an alternative explanation could be advanced, that corneal abrasion, by exposing the corneal stroma to the tears, tends to cause stromal swelling. If gel swelling of the anterior stroma is restricted by the stromal interweave, then a rise in anterior stromal hydrostatic pressure would result. We may at least ask ourselves the question, could keratocyte loss be caused by such a rise in pressure—that is, do the keratocytes die because they are "strangled" by the stromal interweave? This could also explain the preferential loss of anterior stromal keratocytes which is said to occur in bullous keratopathy.

What influence does the anterior stromal architecture have on refractive procedures? Müller *et al* caution that removal of this critical, stable zone of the stroma during photorefractive keratectomy (PRK), could lead to later optical problems. This may not be the case for most PRK ablations, since the depth of ablation, say 70 μm deep to the surface of Bowman's layer, may leave untouched a 50–60 μm zone of the interwoven, anterior region of the stroma, capable of providing some structural rigidity to the newly sculpted zone. As noted by Müller *et al*, since the combined thickness of the epithelium and Bowman's layer together, is about 60 μm, a LASIK flap of 160–180 μm will just encompass the interwoven anterior stromal layer (100–120 μm thick). A deeper plane could cut into the interlimbal lamellae of the posterior stroma and, potentially, interfere with the stability of the procedure, much as Müller *et al* propose. It may be noted, in passing, that Munoz *et al*[59] devised a method for dealing with wrinkling of the LASIK flap, which involves "rehydration" of the flap with distilled water. It must be supposed that the distilled water swells and stretches a hydratable posterior lamella of the flap to achieve this effect.

In summary, Müller *et al* have drawn our attention to important structural and functional features of the cornea which are not only important in maintaining corneal curvature, but may also play an important part in determining corneal shape. The realisation of this may have far reaching consequences for our understanding of the corneal response to injury and of the biological response to refractive corneal procedures. It is clearly an area that deserves further attention.

A J BRON

Nuffield Laboratory of Ophthalmology, University of Oxford, Walton Street, Oxford OX2 6AW, UK

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Discontinuing anticytomegalovirus therapy in patients with cytomegalovirus retinitis and AIDS

Cytomegalovirus (CMV) retinitis is the most common opportunistic ocular infection in patients with the acquired immune deficiency syndrome (AIDS).[1] Before the advent of highly active antiretroviral therapy (HAART), CMV retinitis affected 30% of patients with AIDS at some time during the course of their disease.[2] Cytomegalovirus retinitis is a late stage complication associated with low CD4+ T cell counts, typically less than 50 cells $\times 10^6$/l.[3 4] Cytomegalovirus retinitis was rare at CD4+ T cells >100 cells $\times 10^6$/l.[3 4] All of the available anti-CMV therapies suppress viral replication, but do not eliminate the virus. Unless immune reconstitution occurs, prolonged suppressive anti-CMV therapy (maintenance therapy) is required.[1 5] Without immune reconstitution or maintenance therapy, CMV retinitis relapses within 3 weeks. As such, in the pre-HAART era, patients with CMV retinitis required lifetime maintenance anti-CMV therapy.

HAART consists of combination therapy for the human immunodeficiency virus (HIV), with at least three drugs, typically two nucleoside reverse transcriptase inhibitors and either a protease inhibitor or a non-nucleoside reverse transcriptase inhibitor. HAART can result in marked suppression of HIV replication, improvement in immune function, increases in CD4+ T cells, decreases in opportunistic infections, and improved survival.[6] With HAART, there has been a 55%–95% reduction in the number of new cases of the CMV retinitis, and the decrease varies depending upon the population being served.[6–8] However, CMV retinitis continues to occur, albeit at a reduced incidence, and there remains an increasing prevalent population of patients with AIDS and CMV retinitis who have experienced immune reconstitution as a consequence of HAART and are living for substantially longer times.

In addition to the increase in CD4+ T cell counts and the decrease in the incidence of CMV retinitis with HAART, studies have demonstrated the restoration of specific anti-CMV immunity in patients with CMV retinitis who have had immune reconstitution as a consequence of HAART.[9] As such, several investigators have discontinued anti-CMV maintenance therapy in patients with immune reconstitution from HAART. These case series have reported that, as long as immune reconstitution is maintained, CMV retinitis does not relapse, and that the anti-CMV therapy can be discontinued safely.[10–15]

In this issue of the *BJO* (p 471), Curi *et al* have reported their experience with discontinuing anti-CMV therapy in 41 patients with CMV retinitis who had immune reconstitution. CD4+ T cell counts at the time of diagnosis of the CMV retinitis typically were low, with a median CD4 + T cell count of 42 cells $\times$ 10^6/l, and all of the patients experienced immune reconstitution, with a median CD4+ T cell count of 238 cells $\times$ 10^6/l at the time when anti-CMV therapy was discontinued. The lowest CD4+ T cell count at that time was 143 cells $\times$ 10^6/l. None of the patients suffered relapses of the retinitis, and immune reconstitution was maintained throughout. The median final CD4+ T cell count in this population was 427 cells $\times$ 10^6/l, and the lowest was 181 cells $\times$ 10^6/l. These results are in accord with other published studies, which have reported that as long as the CD4+ T cell count increases to over 100 cells $\times$ 10^6/l, and is maintained over 50 cells $\times$ 10^6/l, anti-CMV maintenance therapy can be discontinued safely.[10–15] In the series by Curi *et al* the median follow up off anti-CMV therapy was nearly 2 years (21 months), suggesting that as long as immune reconstitution is maintained anti-CMV therapy can be withheld for prolonged periods of time.

Although it is clear that anti-CMV therapy may be discontinued safely in patients who experience immune reconstitution, there still are several issues. The first is the level of CD4+ T cell count to use for discontinuation of anti-CMV therapy. Most investigators have used a level of at least 100 cells $\times$ 10^6/l, although some have used 150 cells $\times$ 10^6/l. One centre used 50 cells $\times$ 10^6/l.[15] The report by Curi *et al* does not enable us to better refine the estimate, as all but one patient had a CD4+ T cell count over 150 cells $\times$ 10^6/l at the time of discontinuation of anti-CMV therapy. However, because some series have reported safe discontinuation of anti-CMV therapy in patients with at least 100 cells $\times$ 10^6/l, it appears that this level is reasonable. Whether lower levels are as safe remains uncertain.

The second issue is the duration of immune reconstitution before discontinuation of anti-CMV therapy. Restoration of CD4+ T cell counts may occur before the restoration of specific CMV immunity, and cases of CMV retinitis have been reported to occur immediately after introduction of HAART.[16] Although investigators have suggested a restoration of CD4+ T cell counts for at least 3–6 months, based on estimates of the time to restore specific anti-CMV immunity, most patients in the reported case series have been on HAART for longer time periods before discontinuing anti-CMV therapy. In the series by Curi *et al* the shortest time on HAART was 5 months, and the median time was 13 months. The third issue is the role of HIV viral load in monitoring patients of anti-CMV therapy.[11–13] Although suppression of HIV replication to undetectable levels is the goal of antiretroviral therapy, several case series of patients with CMV retinitis have suggested that low levels of HIV replication, as long as the CD4+ T cell count has increased, are not associated with relapse. As such, it appears that immunological reconstitution is necessary for discontinuation of anti-CMV therapy, but that complete suppression of HIV replication may not be. Although ongoing low level HIV replication will probably result in loss of immune reconstitution long term, in the short term it appears that level of immune function is the superior way to follow patients when discontinuing anti-CMV therapy. The fourth issue is when to reinstitute anti-CMV therapy. Patients who have experienced an immune reconstitution and had successful discontinuation of anti-CMV therapy have been reported to relapse when immune reconstitution is lost and the CD4+ T cell count falls to <50 cells $\times$ 10^6/l.[15] As such, it would appear to be prudent to consider reinstitution of anti-CMV therapy when the CD4+ T cell count falls to <50 cells $\times$ 10^6/l.

In conclusion, it appears that among patients who experience immune reconstitution as a consequence of HAART, anti-CMV therapy can be discontinued safely for prolonged periods of time. A threshold level of 100–150 cells $\times$ 10^6/l for a duration of 3–6 months appears to be a reasonable guideline for discontinuing anti-CMV therapy. Because of the occasional patient who will not recover specific CMV immunity despite an increase in CD4+ T cells, these patients will continue to need regular ophthalmological follow up. In addition, the CD4+ T cell count will need to be followed, as patients may relapse when the CD4+ T cell count falls below 50 cells $\times$ 10^6/l. However, as shown in the paper by Curi *et al*, prolonged immune reconstitution and prolonged periods off anti-CMV maintenance therapy are achievable.

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DOUGLAS A JABS

Departments of Ophthalmology and Medicine, Johns Hopkins University School of Medicine, and the Department of Epidemiology, Johns Hopkins University School of Hygiene and Public Health

Correspondence to: The Wilmer Eye Institute, The Johns Hopkins Hospital, 550 North Broadway, Suite 700, Baltimore, MD 21205, USA
djabs@jhmi.edu

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