Age-Dependent Changes in the Basal Retinovitreous Adhesion

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PURPOSE. To determine the width of the posterior vitreous base in human eyes of different ages and to clarify the nature of the posterior retinovitreous adhesion that underlies the development of juxtabasal retinal tears and retinal detachment after posterior vitreous detachment (PVD).

METHODS. The posterior limit of the vitreous base was delineated with indocyanine green after mechanical peeling of the postbasal vitreous cortex from the retina in 58 pairs of donor eyes. The area of residual retinovitreous adhesion was measured by image analysis. Scanning electron microscopy was performed on the undersurface (or retinal aspect) of the inner limiting lamina (ILL) after trypsin digestion of the peripheral retina.

RESULTS. An age-dependent increase in the anteroposterior dimension of the posterior vitreous base was revealed that became progressively wider in eyes of male donors than in those of female donors and in the nasal half compared with the temporal half of the globe. Ultrastructural studies showed progressive invasion of the innermost peripheral retina by bundles of collagen fibrils, initially in the form of characteristic braids splaying out beneath the ILL and eventually as a dense sublamellar mat in the elderly. The collagen fibrils penetrated the ILL through localized defects and intertwined with those in the basal gel.

CONCLUSIONS. With aging, the posterior border of the vitreous base migrates posteriorly so that an annular band of firm adhesion eventually straddles the ora serrata eccentrically. Intraparetal synthesis of collagen fibrils, their penetration of the ILL, and their splicing with cortical vitreous fibrils, underlie the slowly evolving retinovitreous adhesion. (Invest Ophthalmol Vis Sci. 2003;44:1793–1800) DOI:10.1167/iovs.02-0802

The term vitreous base was coined by Salzmann1 to describe a band of firm developmental attachment between the collagenous framework of the vitreous gel and the posterior part of the pars plana and adjoining ora serrata. Fanning out from this ciliovitreous adhesion are unusually thick bundles of collagen fibrils.2–4 The collagen fibrils in the anterior vitreous merge with the inner limiting lamina (ILL) of the ciliary epithelium in childhood. However, progressive ILL reduplication with age eventually results in a multilayered structure in which the laminae undergo a complex interdigitation with the vitreous collagen fibrils.5,6

In 1950, Lindner7 observed histologically that, in eyes removed after severe injury, separation of the vitreous cortex from the retina frequently extends up to the ora serrata in children, but a peripheral ring of retinovitreous adhesion prevents such extreme forward extension of posterior vitreous detachment (PVD) in adult eyes. A study of 68 donor eyes with PVD by Teng and Chi8 thereafter revealed a widening of the zone of attachment up to 3.0 mm postorally with increasing age after the age of 30 years. The putative mechanism of the basal adhesion, as disclosed by transmission electron microscopy, comprises breaches in the thin ILL of the postoral retina that allow collagen fibrils in the vitreous to intermingle with fibrils located beneath the ILL and occupying clefts or crypts between the Müller cells.4,6,9,10 Traumatic avulsion of the vitreous base testifies to the strength of the basal adhesion11 wherein anteroposterior compression and equatorial expansion of the globe from a blunt impact results in the formation of a bucket-handle tear comprising a strip of posterior pars plana, ora serrata, and, in adult eyes, peripheral retina. Moreover, localized variations in the topography of the posterior border of the vitreous base can provide a focus for dynamic vitreous traction after PVD, resulting in juxtabasal formation of a retinal break and retinal detachment.8,12

As part of our ongoing investigation of vitreous structure and pathophysiology, we revisited the basal vitreoretinal interrelationship and its age-dependent changes. We used mechanical peeling of the vitreous cortex to determine the posterior limit of the retinovitreous adhesion before evaluating the area of residual attachment quantitatively. In addition, scanning electron microscopy of the undersurface (or retinal aspect) of the ILL, after trypsin digestion of the retina, was performed to gain better appreciation of the architecture of the intraretinal fibril bundles and their role in the development of postoral adhesion.

METHODS

Eye Collection

Fifty-eight pairs of human cadaveric eyes (with no evident ophthalmic disorder other than pseudophakia) were obtained from the Eye Bank at the Royal Eye Hospital (Manchester, UK). The corneas had been removed for transplantation within 48 hours of death. The globes were immersed in sterile saline and stored at 4°C before same-day collection and dissection. The donors ranged in age from 8 to 96 years; 31 were male and 27 were female. In addition, single (right) eyes were obtained from a 22-month-old (male) and a 26-year-old (male) donor. No other...
Specimen Preparation

After removal of the iris and lens (or lens implant), an open-sky vitrectomy was performed with a suction cutter to remove the central portion of the vitreous body. The interior of the eyecup (including the ciliary body, 0.2 to 0.3 mL of 0.5% indocyanine green (ICG) solution (IC-Green: Akon, Inc., Buffalo Grove, IL) was introduced onto the bare surface of the postbasal retina with a syringe, and the dye was left in place for 10 minutes. The resultant green retinal surface was more a consequence of the particulate nature of the ICG suspension rather than true staining of the ILL. When the detached vitreous was replaced onto the retina, a clear dividing line between the detached and attached vitreous was visible. The area of residual adhesion in the peripheral retina was thus delineated by the ora serrata anteriorly and by a linear green precipitate at its posterior border (Fig. 1). Subretinal pooling of dye was seen in relation to any breaks in the retina arising during peeling.

Photography and Measurement

The unfixed specimens were photographed (with a calibration bar), and the area of retinovitreous adhesion was measured by an image-analysis program (Semper V Image Analysis; Synoptics Ltd., Cambridge, UK), using a TYR910 monochrome television camera (Bosch, Germany). An average width (W) was generated by using the formula $W = A/L$, where $A$ is the area and $L$ is the length of the specimen. Similar $A/L$ calculations were used for the respective halves (nasal and temporal) or quarters of the specimen. This method overcame measurement problems, particularly in relation to “scalloping” of the ora serrata (i.e., the prominent teeth and bays seen in the nasal periphery).15

Trypsin Digestion

After the choroid was discarded, selected retinas were cut into quarters, and excess vitreous removed with scissors. The specimens were then washed gently in phosphate-buffered saline (PBS) for 5 minutes before incubation in 0.1 M Tris buffer (pH 7.8) containing 3% (wt/vol) trypsin at 37°C for 30 to 60 minutes. The incubation was terminated when the medium became cloudy and the tissue showed signs of disintegration. The specimens were then transferred to PBS and given three washes to remove any remaining retinal debris.

Specimen Preparation for Electron Microscopy

After trypsin digestion, the retinal specimens were fixed in 1% osmium tetroxide in 0.1 M sodium cacodylate buffer (pH 7.3) at 4°C for 24 hours, postfixed in 1% osmium tetroxide in 0.1 M sodium cacodylate for 1 hour, and dehydrated through graded ethanol. Retinal specimens for scanning electron microscopy (SEM) were then critical-point dried, sputter coated in gold and evaluated by scanning electron microscope (model 360; Cambridge Instruments, Cambridge, UK).

To assist in the evaluation of the scanning images, a few specimens also underwent transmission electron microscopy (TEM). Fixed, trypsin-digested specimens were dehydrated and embedded in Spur resin. Ultrathin sections were cut, contrasted with 2% uranyl acetate and Reynolds lead citrate, and then analyzed by electron microscope (model 1200, JEOL, Tokyo, Japan).

Results

Measurement of the Posterior Vitreous Base

The posterior borderline of the vitreous base as revealed by ICG was approximately parallel to the ora serrata (Fig. 1). No postoral adhesion was identified in the three children’s eyes (Fig. 1A). The border was smoothest or “sharpest” in eyes with...
The average width of the posterior vitreous base changed with age, increasing up to the age of 80 years and then decreasing slightly (Fig. 2). This relationship was well fitted with a cubic function. There was, on average, no difference between the right and left eyes of individual donors (mean difference, 0.032 mm; 95% confidence interval [CI] of the mean 

$0.0039 \text{ to } 0.040$), the best fitting cubic functions fitted with the data for the right and left eyes overlap. Eyes with preexisting PVD were not used for fitting the cubic functions shown, but virtually identical results were obtained when these data were included.

**SCANNING ELECTRON MICROSCOPY**

After trypsin digestion of the retina, the undersurface of the peripheral basal lamina was noted on SEM (Fig. 5) and TEM to be free of residual cellular elements. There was no postbasal ILL identifiable in the digested and stripped specimens—that is, the ILL was retained only where it remained adherent to the (basal) vitreous cortex (Figs. 5A, 5C). An approximate correlation was found between the width of the zone of retained postoral ILL in representative specimens and the average macroscopic width of the posterior vitreous base. In young adult eyes, the undersurface of the ILL near the ora displayed a characteristic pattern of collagenous infiltration (Fig. 5B). Specifically, “braids” of tightly associated fibril bundles were dispersed beneath the ILL. Each braid arose from a root that was sometimes tapered, at other times slightly expanded and hooked, and the braids generally followed a sinuous course. Braids then teased out and became unbraided (i.e., unreeled), forming a skein or web of thin bundles. The bare ILL between these features showed multiple dehiscences that we concluded were drying artifacts (and that revealed a matrix of collagen from the cortical vitreous in their depths). TEM analysis of these various patterns of collagenous infiltration revealed (within bundles) the presence of uniform, thin fibrils of mean diameter $16.0 \pm 1.7 \text{ nm (n = 302)}$. These fibrils were identical with those observed within the vitreous gel.

With increasing age (Fig. 6), the undersurface of the ILL became more densely populated by bundles of collagen fibrils.
Braids measuring between 10 and 35 μm in length and up to 1 μm in maximum diameter sometimes divided into two (or even three) branches, each of a similar size. Their skeins often intermingled with similar structures arising from an adjacent braid or alternatively overlapped another skein beneath the ILL (Fig. 6). In the oldest eyes, the collagen braids and skeins formed a diffuse, tangled mat in a wide postoral band beneath the basal ILL (Fig. 7).

The intraretinal collagen fibrils traversed the ILL progressively with age, and TEM and SEM both showed intermingling or "splicing" of fibrils (or bundles of fibrils) through breaks in the ILL (Figs. 6, 7). Although the ILL breaks tended to be obscured by sublaminar skeins on SEM, TEM typically demonstrated fibrils from below the ILL approaching the cortical vitreous fibrils (which were predominantly oriented parallel to the underlying ILL) at an angle (Fig. 7B). The edges of the ILL...
around such breaks were often pushed toward the vitreous and were never oriented toward the retina.

Occasionally, clusters of braids were discovered (Fig. 8) forming an interlacing mass in which the roots were typically shaped like earlobes (auricular expansions) or like tongues (lingual expansions). Arising from these clusters, stout, straightened bundles of fibrils approached perpendicular to the ILL and passed directly through it into the cortical vitreous. These structures may correspond to the intraretinal component of the “verucaee” described by Dunker et al.14

**DISCUSSION**

We demonstrated a clear widening of the posterior vitreous base with increasing age in a sample of donor eyes with no known ophthalmic disease. In 1957, Teng and Chi8 also reported a posterior migration of the borderline of the retinovitreous adhesion, basing their observations on single eye bank donor eyes that had undergone PVD (thus revealing the extent of residual vitreoretinal adhesion).8 However, there are a number of limitations to this earlier study: First, the age of onset of the PVD in the eyes used in their study is unlikely to correspond to age at death, and PVD is likely to have limited posterior migration; second, young eyes with PVD probably had underlying disease, thus introducing bias; third, Teng and Chi had no means of excluding partial PVD (as opposed to complete or total PVD) in their specimens—that is, a peripheral separation of the vitreous cortex from the retina short of the true location of the posterior border of the vitreous base15; and fourth, they could not make any interocular comparisons to demonstrate the consistency and validity of their approach. Therefore, our study was designed to redress these limitations and to reexamine this clinically important matter.

According to our observations, the posterior migration of the vitreous base commenced at least a decade earlier than demonstrated by Teng and Chi8 (i.e., by the age of 20 years). Furthermore, we observed much less scatter in the data, par-
particularly in the older age groups. They observed a narrow (<1 mm) posterior vitreous base in many of the eyes of older donors, perhaps implying that the PVD was of long standing and that this event had prevented the further posterior migration of the vitreous base. We observed prior complete PVD only in normal eyes of donors older than 70 years, in keeping with the observations in Foos\textsuperscript{15} and Foos and Wheeler.\textsuperscript{16} Nevertheless, we were unable to demonstrate that PVD contributed to the “plateau” that we observed in the width–age relationship (Fig. 2). We have no explanation for the eventual dip in the curve, other than to speculate on a possible relationship between life expectancy and mechanisms linked to the width of the vitreous base. Survival bias is often seen in age-related indices.\textsuperscript{17}

We have demonstrated a significant increase in age-related migration of the posterior border of the vitreous base in male compared with female donor eyes. The measurements taken were absolute and may simply reflect that males have larger eyes than females.\textsuperscript{4} Although PVD occurs earlier in life and more frequently in males compared with females,\textsuperscript{16,18} we were unable to demonstrate an effect of prior complete PVD on this sex difference.

Our study is the first to demonstrate definitively that the increase in the width of the posterior vitreous base with age is greater in the nasal half than in the temporal half of the eye, and maximal in the inferonasal quadrant. This is contrary to statements in earlier reports\textsuperscript{4,19} and to clinical observations that have been attributed to Schepens,\textsuperscript{20,21} to the effect that the temporal vitreous base is the wider. However, it confirms impressions reported by Foos\textsuperscript{4,6} who studied thousands of eyes obtained at autopsy. He also reported a corollary to the effect that the anterior (ciliary) component of the vitreous base is wider temporally than nasally.\textsuperscript{6} The eccentricity of the vitreous base in relation to the ora serrata which we and Foos have observed may thus be said to compensate for the greater distance of the ora serrata from the corneoscleral limbus on the temporal side. Indeed, the posterior border of the vitreous base becomes more or less concentric (in the coronal plane) with the anterior segment structures in later life.

Our SEM observations of sublaminar collagenous braids expanding into skeins and eventually investing the superficial retina with a collagenous mat correspond to earlier TEM descriptions of so-called clefts, crypts, and sclerosis of the peripheral retina.\textsuperscript{4,6,10} However, our findings provide a better topographical context and new pathophysiological insights into these structures. The collagenous motif on SEM appears to us to provide evidence of de novo synthesis of collagen fibrils, as proposed by Gartner,\textsuperscript{22} with bundles of fibrils associating in braids and then disassociating into skeins before traversing the ILL and becoming incorporated into the basal vitreous cortex. By way of an alternative explanation of the development of crypts, Foos\textsuperscript{4,6} implicated a “repairative” response to peripheral retinal degeneration arising from ischemia or from biomechanical insults. He hypothesized that collagen fibrils from the vit-
vesicular cavity are “pressed” into, and become incarcerated within, the superficial retina with increasing age—so-called degenerative remodeling.\(^4,6\) This now appears to be an unlikely explanation for so reproducible a pattern of collagen invasion beneath the ILL, and one that we have observed to precede the development of ILL defects. Furthermore, the edges of the ILL defects were directed toward the center of the eye, not toward the retina.

Vitreous collagen fibrils are composed of collagen types II, IX, and V/XI.\(^5,6\) Taken together, previous studies including in situ hybridization analyses of miRNA expression in mouse and chick eyes\(^8,9\) and biochemical analyses of bovine vitreous humor\(^24,25\) suggest that vitreous collagen synthesis mainly occurs prenatally, although there is low-level production until adulthood. During development, the (secondary) vitreous collagen is mainly synthesized by the nonpigmented ciliary epithelium, but as the secondary vitreous starts to form, there is transient widespread expression by the developing retina.\(^24\) Generally, it appears that the mature retina does not synthesize vitreous collagen, but it is possible that cells in the peripheral retina of adult human eyes undergo a phenotypic alteration into immature retinal cells or nonpigmented ciliary epithelial-like cells. The incomplete partitioning of cellular phenotypes across the ora serrata has recently been demonstrated, with adult primate pars plana containing cells that are immunoreactive for retinal cell markers, and there is much current interest in the observed presence of stem cells in the peripheral retina of mammals.\(^26,27\)

It remains to be established whether the collagen fibrils in the peripheral retina simply follow the path of least resistance between the Müller cells and between these cells and the ILL, or whether sublaminar organization of the fibrils is otherwise modulated. Similarly, the mechanism of fibrillar penetration of the ILL (whether purely mechanical or cell mediated, for example, through matrix metalloproteinase secretion) is un-
known. Whatever the mechanism, the pattern of collagen linkage across the ILL is crucial to the establishment of a smooth posterior border to the vitreous base overall, and consequently a reduced risk of a retinal break forming after PVD.

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References


