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Anatomy and development of the macula: specialisation and the vulnerability to macular degeneration

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The central retina in primates is adapted for high acuity vision. The most significant adaptations to neural retina in this respect are: 1. The very high density of cone photoreceptors on the visual axis; 2. The dominance of Midget pathways arising from these cones and 3. The diminishment of retinal blood supply in the macula, and its absence on the visual axis. Restricted blood supply to the part of the retina that has the highest density of neural elements is paradoxical. Inhibition of vascular growth and proliferation is evident during foetal life and results in metabolic stress in ganglion cells and Müller cells, which is resolved during formation of the foveal depression. In this review we argue that at the macula stressed retinal neurons adapt during development to a limited blood supply from the choriocapillaris, which supplies little in excess of metabolic demand of the neural retina under normal conditions.

We argue also that while adaptation of the choriocapillaris underlying the foveal region may initially augment the local supply of oxygen and nutrients by diffusion, in the long term these adaptations make the region more vulnerable to age-related changes, including the accumulation of insoluble material in Bruch's membrane and beneath the retinal pigment epithelium. These changes eventually impact on delivery of oxygen and nutrients to the RPE and outer neural retina because of reduced flow in the choriocapillaris and the increasing barriers to effective diffusion. Both the inflammatory response and the sequelae of oxidative stress are predictable outcomes in this scenario.

Key words: choroid, fovea, photoreceptors, retinal vessels

The macula comprises less than four per cent of total retinal area in humans but is responsible for almost all of our useful, photopic vision. Within the macula, a two millimetre lesion centred on the fovea will affect an estimated 225,000 cones in the average individual, 25 per cent of the total ganglion cell output to the brain, and result in legal blindness. In this review, we will focus on the anatomical specialisations of the macula and its development, and explore the reasons why the region is vulnerable to degeneration associated with aging. Finally, we identify key areas for research to deepen our understanding of mechanisms leading to macular degeneration, to develop strategies for intervention and to promote healthy aging in the population.

KEY FEATURES OF THE MACULA

A range of adaptations, including eye position and size, confer considerable optical advantage and are significant factors in the enhanced visual performance of primates. However, the most significant adaptive advantages are to the neural environment of the retina and its circuitry within the macular region.

The term macula derives from the presence of the xanthophyll pigments, lutein and zeaxanthin, in a region five to six millimetres in diameter at the posterior pole of the eye, appearing as a yellow spot (macula lutea), when viewed in red-free

light. Concentrations of these pigments decrease with eccentricity from the fovea,[1] with zeaxanthin being the most abundant pigment within the fovea of monkeys.[2] Initially, Wald[3] proposed that the role of these pigments was to eliminate harmful effects of blue and violet wavelengths not absorbed by the lens, a proposition supported by later research.[4,5] More recently, it has been suggested that xanthophyll pigments act as antioxidants by quenching reactive oxygen species,[2,6] thus playing a role in prevention or delay of photoreceptor death.[7]

Specialisation of the neural retina

The macula corresponds roughly to the area covered by the four anatomical regions, originally defined by Polyak[8] and named in relation to, and including, the fovea centralis (fovea), as shown in Figure 1. The outermost region—the perifovea—can be viewed as a transition zone, between the highly specialised central zones and the periphery. The high density of the retinal vasculature (Figure 1A), as well as the high rod:cone ratio (Figures 1B and 2)[9] are features common to the perifovea and periphery. The above average cone[9] and ganglion cell[10] densities distinguish the perifovea from the retinal periphery (Figures 1B and 2). The parafovea bounds the foveal region and is characterised by a relatively low density of retinal vessels (Figure 1A), a very high density of ganglion cells[10] and below average spatial densities of rods[9] (Figures 1B and 2), so that the rod:cone ratio is around 4:1, compared with 33–130:1 in the perifovea. These trends are developed further in the foveal region, which is best considered in two parts—the foveal slope and the foveola (asterisk, Figure 1 A, B and Figure 2). On the foveal slope, there are two significant transitions: from rod- to cone-dominated retina and from vascularised to avascular retina. As shown in Figure 1B, rods still dominate the photoreceptor mosaic on the foveal rim (~1mm eccentricity) and only the lower part of the foveal slope is 'cone dominated' (Figure 2). Similarly, the upper part of the slope contains the perifoveal capillary plexus, the three layers of which anastomose toward the base of the slope, forming a single vascular ring that surrounds the foveola. Rods, ganglion cells and all inner nuclear layer neurons are absent from the foveola, so that cone cell bodies—with the exception of a few parasol (P-) ganglion cells—form the innermost cell layer (Figure 2). A second striking feature is the gradient of cone densities within the foveola, such that the peak density (zero eccentricity) is approximately three times the density at the base of the foveal slope[9] (Figure 1B).

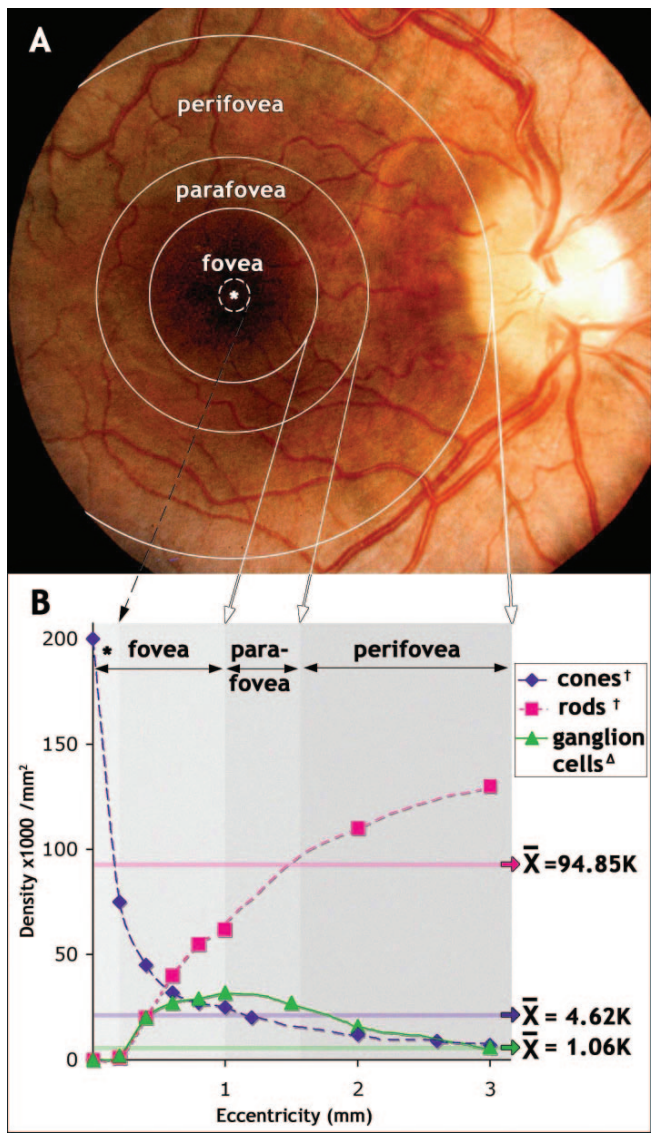

Figure 1. A: The anatomical regions of the fundus, according to Polyak;[8] B: graphs showing the densities of rods,[118] cones[118] and ganglion cells[10] in the fovea, parafovea and perifovea

A further characteristic of the central regions of primate retina, not apparent using simple histology, is the preponderance of 'midget' (M-) pathways in the retinal circuitry.[11,12] M-pathways originate from both long (LWS) and medium (MWS) wavelength-sensitive cones (the only types present in the foveola), each one establishing two 'private lines' to the brain, through hyperpolarising (OFF-) and depolarising (ON-) midget bipolar cells, then via OFF- and ON- midget ganglion cells,

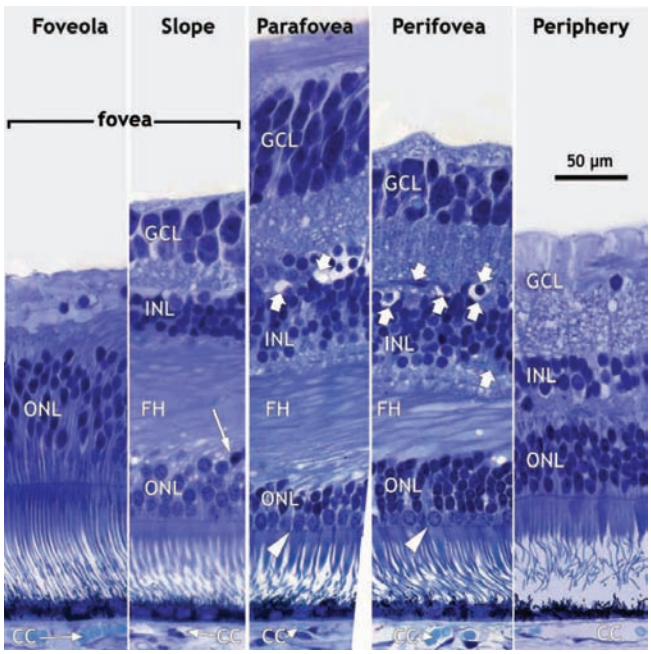

Figure 2. Photomicrographs showing the relative thickness of the adult human retina, and its constituent layers, at different locations. The GCL and INL are absent at the foveola, where only cones are present; all layers are present on the foveal slope, where rods are also found (long arrow). The retina is thickest in the parafovea where there is a dense capillary plexus in the INL (thick arrows). Cone nuclei form a single layer along the external limiting membrane (arrowhead), the darker-staining rod nuclei being stacked several cells deep on the inner aspect of the cones. The retina has a similar appearance in the perifovea but the cones are thicker, rods more numerous and the overall retinal profile thinner. Peripheral retina is about one-third thinner than the parafovea; the GCL and INL are considerably reduced compared with other locations. GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer.

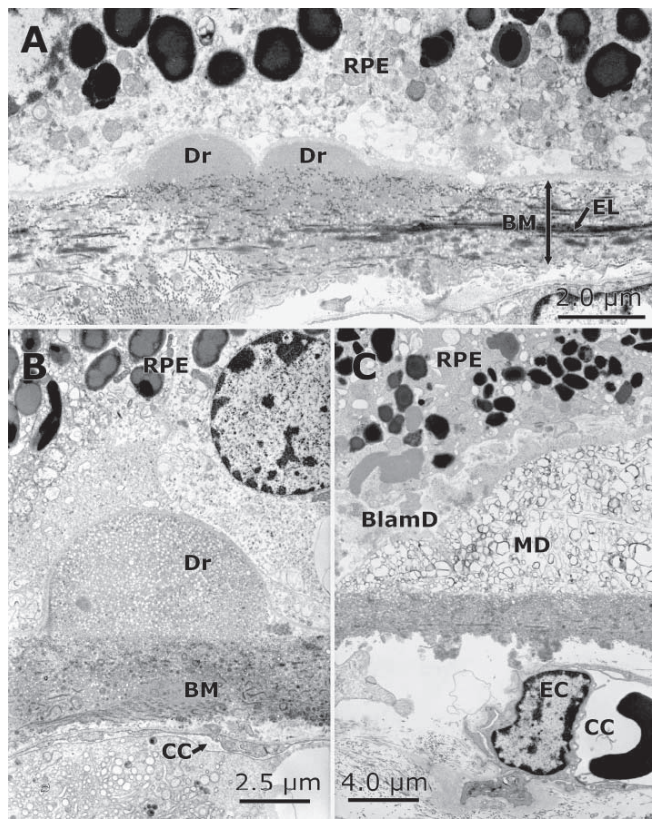

Figure 3. Electron micrographs showing sub-retinal structures and some examples of pathological deposits in aged retinae.
A. Two drusen (Dr) sitting on the inner aspect of Bruch's membrane (BM). Towards the right, BM is relatively normal having a distinct elastic lamina (EL) dividing it into inner (above) and outer (below) collagenous zones. The EL is fragmented beneath the drusen and to the left of the image and contains a large amount of vesicular material.
B. A single druse (Dr) associated with a badly deteriorated BM, which has no EL and many different types of extraneous material. The choriocapillaris (CC) is a slit-like space (arrowed) and considerable amounts of membranous material are present in the interstitial space (lower left).
C. Showing membranous (MD) and basal laminar deposits (BlamD) associated with pathology of Bruch's membrane (BM). EC, endothelial cell.

respectively. These M-pathways are distinctive in the absence of convergence of photoreceptor signals onto bipolar and ganglion cells—which accounts for the very high densities of cells in the inner, central retina.[13-15] More importantly, the predominance of M-pathways in the central few millimetres of retina makes possible the formation of a foveal depression. Because the M-pathways run essentially in parallel to one another they can be drawn apart—without losing contacts or remaking synapses—by whatever forces promote the cell displacements that lead to formation of the foveal depression during development. This would be impossible if 'parasol' (P)-pathways, in which inputs from several cones converge onto diffuse bipolar cells, which in turn converge onto the P-ganglion cell, were predominant. Indeed, it has been shown in macaque that some P-ganglion cells are trapped in the fovea,[16] their complex connections making displacement along with the other M-ganglion cells an impossibility.

Specialisation of sub-retinal structures

A dual mechanism has evolved to supply nutrients to the retina. The choroid and

its capillary bed—the choriocapillaris—are present in all mammalian species, indirectly supplying oxygen and nutrients to the photoreceptors. The retinal vasculature has evolved separately, to supply the inner retina in species where the retina has increased in thickness, by the expansion of cell populations in the inner nuclear (INL) and ganglion cell (GCL) layers.[17]

The choriocapillaris is separated from the neural retina by the retinal pigment epithelium (RPE) and Bruch's Membrane (Figures 2 and 3). Bruch's membrane is a specialised extracellular matrix complex that lies between the basement membranes of the RPE and the endothelial cells of the choriocapillaris and comprises two collagen-rich layers—the inner and outer collagenous layers—with a central elastic lamina rich in elastin and elastin-associated proteins (Figure 3). The choriocapillaris is a labyrinth of thin-walled, fenestrated, capillaries with a high flow rate that delivers oxygen by diffusion to the RPE and to the inner segments of photoreceptors, fuelling the oxidative metabolism that drives phototransduction. In the foveola, oxygen diffuses about 100 µm to reach the inner segments of the foveal cones, and there is no alternative supply from retinal vessels. In other parts of the retina oxygen diffuses 50 to 60 µm from the choriocapillaris to reach photoreceptor inner segments, in addition to oxygen and other nutrients being available from the deep retinal capillaries over a distance of 50 µm (periphery) to 100 µm (parafovea). Experimental studies in the macaque indicate that under dark adaptation photoreceptors 'draw down' 10 per cent of their oxygen requirements from the retinal blood supply,[18] although how cones in the foveola obtain their full quota of oxygen is not known.

One adaptation of sub-retinal structures is that in young healthy retinas, the choriocapillaris has a wider bore within the labyrinth at the fovea—there is less 'intervascular' space.[19] A second area of specialisation is within Bruch's membrane itself. Several studies suggest that the structure and composition of Bruch's membrane varies with topography (centre versus periphery) and with age. Post mortem studies indicate that the elastic lamina at the macula is significantly thinner and more porous than in other regions at all ages.[20] The study also indicates that its integrity is reduced in donor eyes with incipient macular degeneration (AMD) or active forms of the disease, compared to normal age-matched controls.[20]

KEY EVENTS IN DEVELOPMENT OF THE MACULA

Differentiation of the foveal region

The retina develops in centro-peripheral sequence so that during development, central regions are developmentally more advanced than peripheral.[21-23] At any location in the retina, there is a fixed birth sequence of retinal cells, so that ganglion cells, horizontal cells and cone photoreceptors are born first, followed by amacrine and bipolar cells, with rod photoreceptors and Müller cells being the last to differentiate.[23,24] In humans, a region in the outer nuclear layer of central retina that comprises only differentiating cone photoreceptors is the first indication that at 10 to 11 weeks' gestation (WG) retinal cells are exiting the cell cycle, and is the first indicator of the location of the developing foveal region.[22,25] Weeks later, when rod photoreceptors are fitted into the outer nuclear layer (ONL) surrounding the foveal cone mosaic (15 to 16 WG), the rod free region that characterises the fovea is defined.[22,26]

From the outset the foveal region of the primate retina is different. First, its very early differentiation makes the region distinctive. Second, some cell types are excluded from the foveal region or at least reduced in number. Rods are excluded from the photoreceptor mosaic at the fovea in both humans and macaques;[22,26-29] short-wavelength-sensitive (SWS) cones are excluded from the foveal cone mosaic in humans and are present in reduced numbers in the macaque retina.[27] By implication, rod- and blue-cone bipolar cells are also absent. Third, non-neuronal cells behave differently in the developing foveal region; microglial cells, which initially are spread evenly throughout the incipient fovea, migrate out of the foveal region[30] and at later stages of development, both endothelial cells and astrocytes are prevented from migrating into the developing fovea (see below).[31] Finally, while initially the rule of a centro-peripheral maturation gradient is adhered to, the latter stages of maturation of the fovea are very protracted, so that it is the last region to mature, between two and four years of age in humans[32,33] (Figure 4).

The protracted development of the foveal region means that while foveal cones are among the first cells in the retina to differentiate, they are the last to attain adult morphological characteristics. The slender morphology and stacked nuclei of cones in the foveola and on the foveal slope (Figure 2) develop entirely in the postnatal period,[32,33] from a monolayer of short, thick, cone photoreceptors in the fovea of the late-stage foetal/early postnatal retina (Figure 5). In comparison, cones in the periphery are mature in the perinatal human retina (Figure 5).

Development of the perifoveal vasculature

Despite its early differentiation, the central retina develops a blood supply rather late, compared with other regions.[34-36] Retinal vessels appear at the optic disc at 14 WG and grow rapidly into the superior and inferior quadrants of temporal and nasal retina. By 22 WG, the retina is greater than 50 per cent covered with a retinal plexus that supplies the inner retina but the retinal vessels are still several millimetres from the 'foveal' ganglion cell layer—that is, the ganglion cells that will later be displaced to form the foveal depression. These few millimetres of retina are very slowly vascularised over the next three to four weeks, forming a ring of vessels around an avascular area, in which the fovea will form, at 25 to 26 WG.[31,36] Formation of the deeper layers of retinal vessels is similarly retarded. While precise data from human retinas is not available, in macaques the deep layers of capillaries assemble around the foveal depression in the perinatal period[31]; assuming developmental equiva-

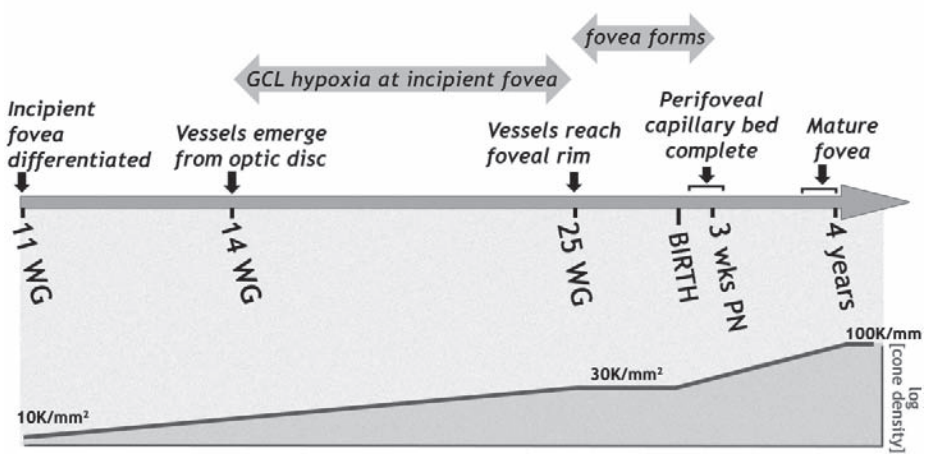

Figure 4. A timeline showing key events in development of human cental retina. WG: weeks gestation; PN: postnatal

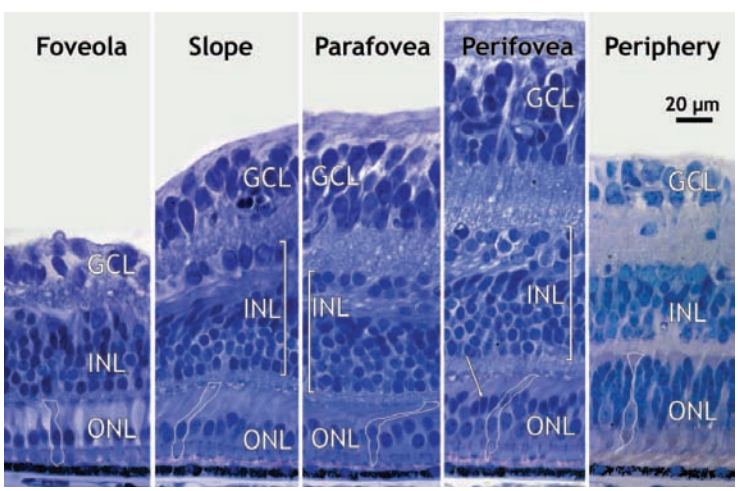

Figure 5. Photomicrographs showing the relative thickness of the newborn human retina and its constituent layers at different locations. All retinal layers are present in the foveola at birth; radial displacement of cells in the GCL and INL takes place entirely within the postnatal period in humans. Note the immature morphology of cones in the foveola (example outlined) and the lack of elongated axons (fibres of Henle; compare Figure 2). Cones on the foveal slope have started to develop elongated axons but these are more pronounced in the parafovea. The central-most rods are encountered in the perifovea (compare with the foveal slope in the adult). Note also that the thickest part of the newborn retina is at the perifovea (compare with the parafovea in the adult). These differences are due to the protracted period of centripetal photoreceptor displacement that takes place in the fist few years postnatal (see cone density graph, Figure 2). GCL: ganglion cell layer; INL: inner nuclear layer; ONL: outer nuclear layer.

lence in the two species, this would suggest that in human infants the perifoveal capillary plexus is not complete until three to four weeks postnatally.

The paradox is that while the central region of the retina is the first to differentiate and is developmentally advanced compared to the rest of the retina throughout most of development, it is the very last region of the retina to develop a blood supply to supplement delivery of nutrients and oxygen from the choriocapillaris. It is possible that this is the cause of, or at least related to, the retarded differentiation of cone photoreceptors in the central retina.[37] Lack of a retinal blood supply is also likely to cause metabolic stress in neurons of the central retina prior to formation of the foveal depression and the establishment of the perifoveal capillary plexus.[38]

Stress at the incipient fovea

Two lines of evidence indicate that at the incipient fovea retinal cells are in a state of stress; expression of glial fibrillary acidic protein (GFAP) in Müller cells and of vascular endothelial growth factor (VEGF) by ganglion cells.

Immunoreactivity (-IR) to the intermediate filament protein, GFAP, in Müller cells is an indicator of incipient pathology and/or 'stress' in those cells.[39-41] In a normal environment GFAP-IR is confined to retinal astrocytes, while Müller cells are vimentin-IR. GFAP-IR in Müller cells is commonly regarded as a response to injury[39,42] and is detected in AMD retinae,[43] diabetic retinopathy,[44] in animal models of retinal degenerations[45], in light-damaged retinae[46] and in response to FGF2 injected intravitreally,[47] although the mechanism mediating the response is not understood.[48]

In developing retina, GFAP-IR is detected in 'foveal' Müller cells just prior to and during formation of the fovea[31] (Figure 6), where it persists postnatally for an undetermined period. The source of stress is not known but we hypothesised that, due to lack of a retinal blood supply in the developing central retina, the inner retina (particularly ganglion cells) experiences metabolic stress and used expression of vascular endothelial growth factor mRNA

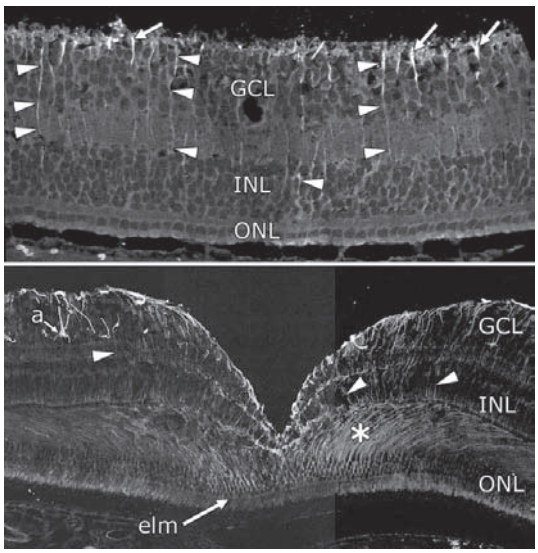

Figure 6. Images of immunofluorescence indicating GFAP-immunoreactivity in Müller cells in the foetal macaque retina.
A: Foetal day 95, at the foveal cone mosaic, prior to formation of the foveal depression. Oblique arrows indicate strong immunoreactivity in the endfeet of Müller cells, while arrowheads indicated weaker immunoreactivity in the inner and outer processes.
B: At postnatal day 1, Müller cells at the fovea are intensely immunoreactive to GFAP (arrowheads), including the outer processes that separate the cone fibres of Henle, (asterisk). Some immunoreactive astrocytes are also present in the inner retina (a). elm: external limiting membrane; GCL: ganglion cell layer; INL: inner nuclear layer; ONL: outer nuclear layer.

as an indicator of developmental hypoxia. VEGF is the major angiogenic growth factor in the retina, is expressed by retinal astrocytes,[49,50] as well as some neuronal cell types,[51] and mediates the differentiation, proliferation, migration and survival of retinal endothelial cells.[52-57] VEGF expression is mediated by the hypoxia-induced transcription factor, HIF-α (hypoxia inducible factor). During development of the retinal vasculature, VEGF is localised to astrocytes migrating ahead of endothelial cells to promote their differentiation and/or proliferation and their migration along the astrocyte template[50] (Figure 7B), adherence to which is mediated by R-cadherin.[58-60]

In situ hybridisation for VEGF mRNA confirmed expression by astrocytes guiding retinal capillaries towards the foveal region of macaque retina[38] (Figure 7C) and in human foetal retina (T Sandercoe, unpublished data). We also found high levels of VEGF expression in the ganglion cell layer overlying the pure cone mosaic of the foveal region, suggestive of hypoxic conditions.[38] The findings indicate that in macaques, hypoxic conditions prevail at the incipient fovea until a ring of capillaries forms around the foveal avascular zone (by Fd105, equivalent to about 25 WG in humans) and are further ameliorated as the retinal profile thins through formation of the foveal depression[38] (Figure 4).

Evidence for expression of inhibitory molecules

If expression of VEGF mediates development of retinal blood vessels and VEGF is expressed in the foveal region at high levels during early development, why does the fovea remain avascular? The answer appears to be that (as yet unidentified) inhibitory factors are expressed at the incipient and developing fovea. Three lines of evidence indicate the expression of inhibitory factor/s.

ASTROCYTES DO NOT ENTER THE INCIPIENT FOVEA

Astrocytes lead the migration of vessel-associated cells across the retina, the retinal vessels forming to an astrocytic template. During development of retinal vessels in the periphery, astrocytes lead endothelial cells by a margin of about 200 µm but as vessels approach the incipient fovea this margin is reduced, so that at Fd 105 in the macaque the leading endothelial cells are in contact with the leading astrocytes, which form a ring around but do not enter the incipient fovea[31] (Figure 7A). Our evidence indicates that astrocytes are prevented from entering the incipient fovea and that as a consequence, retinal vessels do not form there.

ASTROCYTES AND MICROGLIA ARE REPELLED FROM THE INCIPIENT FOVEA

Shortly after the perifoveal ring of capillaries is established, astrocytes retreat from central retina, so that by birth very few astrocytes remain centrally.[31,61,62] While the evidence is not clear-cut, it appears that astrocytes withdraw from the central region rather than being eliminated by apoptosis.[62] Microglia also withdraw from the foveal region during development but at a much earlier stage. In human retina at 14 WG, we found parenchymal microglia regularly distributed throughout central retina but by 20 WG a microglial-free region is approximately centred on the incipient fovea.[30]

LOW RATES OF ASTROCYTE AND ENDOTHELIAL CELL PROLIFERATION

Evidence for expression of an anti-proliferative factor in central retina and along

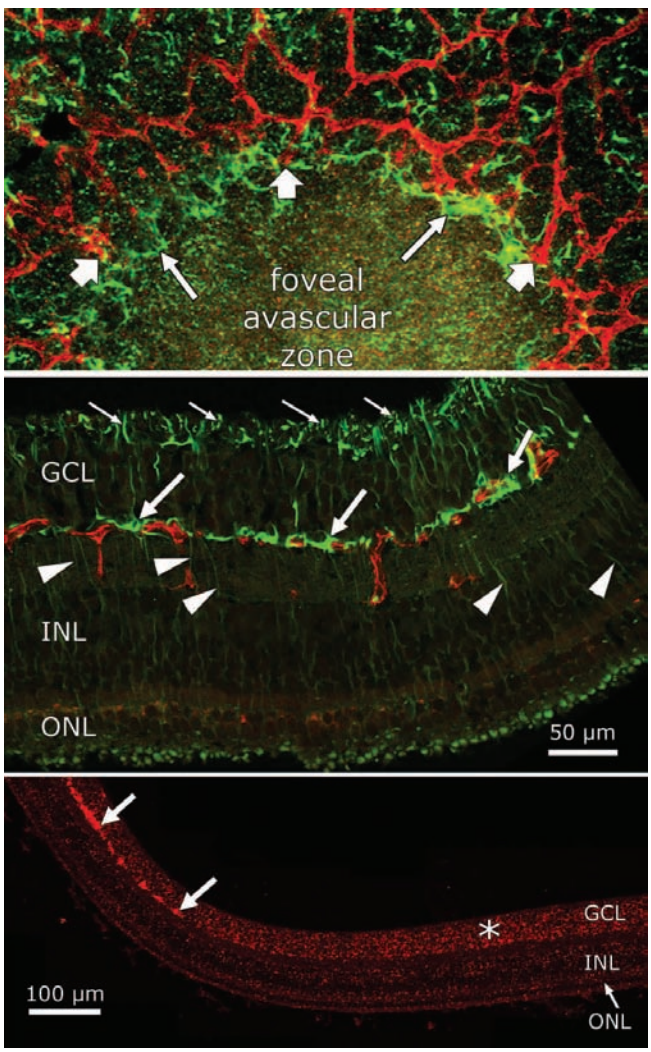

Figure 7. Development of perifoveal capillaries and VEGF expression in developing monkey retina.
A: Astrocytes (GFAP positive, green) and retinal vessels (CD31 positive, red) in a macaque at foetal day 105. The leading astrocytes (thin arrows) stop short of the central fovea and the usually 'trailing' endothelial cells come into contact with the leading astrocytes (thick arrows).
B: Capillaries (red) growing towards the fovea are placed deep in the ganglion cell layer (GCL) and closely associate with deeply placed astrocytes (green, large arrows). GFAP-immunoreactive Müller cell processes are also present (arrowheads) and in inner retina, both astrocytes and Müller cell endfeet are GFAP immunoreactive (small arrows).
C: Foetal day 100 (macaque) *in situ* hybridisation for VEGF mRNA, using Fast Red (Roche). Expression of VEGF mRNA is high in the ganglion cell layer overlying the foveal cone mosaic (asterisk), prior to formation of the foveal depression. Despite this, vessels never enter the region of the central fovea. The very high levels of mRNA labelling to the left are associated with perifoveal capillaries growing towards the central fovea (arrows). INL: inner nuclear layer; ONL: outer nuclear layer.

the horizontal meridian comes from analysis of developing macaque retina. Using a marker of proliferating cells (Ki67) and double immunolabelling to identify proliferating endothelial cells (Ki67-, CD31-IR), we found that the frequency of cell proliferation associated with vascular growth along the horizontal meridian at foetal day (Fd) 105, and around the incipient fovea, is at the most only about 50 per cent the frequency in vessels growing towards the periphery. Similarly at Fd 142 during early stages of formation of the deep layers of the perifoveal plexus, the rate of cell proliferation is less than half that seen in sample areas at comparable stages of formation in the periphery.[63] These data suggest expression of an anti-proliferative factor at the fovea, and along the horizontal meridian, in a gradient resembling the density distribution of cone photoreceptors. The source and identity of the factor/s is not known.

THE UNIQUE ENVIRONMENT OF THE FOVEA

The evidence suggests that the foveal region is distinct from other parts of the retina in a variety of ways. The most prominent characteristics are the exceptionally high density of neural elements and the absence of retinal vasculature in the central fovea. The more subtle characteristics include expression of one or more factors that promote the absence of astrocytes and microglia, either by egress or death, and the sequelae of a long period of exposure of cells to an hypoxic environment during development.

In most systems (including peripheral retina) developing tissues demand the energy they require for continued growth and development. This demand is usually met by increased vascular growth and possibly increased glycolytic metabolism, both mediated by HIF1α.[64-66] At the developing fovea, such a demand is signalled, as evidenced by expression of VEGF, but the response is limited—appearing to be partially inhibited in central retina, and completely blocked at the incipient fovea, by an unidentified inhibitory/repellent factor, which appears to act on astrocytes and

endothelial cells. Under experimental conditions retardation of retinal vascular development in rats is correlated with thinning of the retina,[67] although the mechanisms accounting for reduced thickness (reduced neurogenesis or increased cell death) have not been identified. Neurons in central primate retina survive prolonged absence of retinal blood supply with no evidence of increased cell loss[68]—suggesting perhaps a greater dependence of these neurons on glycolysis as an energy source, and the possibility that the VEGF expressed by ganglion cells of the incipient fovea is neuroprotective, as reported in other parts of the CNS.[69,70]

In contrast with development of most other tissues, what appears to happen at the developing fovea is that the neurons and glia adapt to their limited blood supply, rather than being provided with the supply that is demanded. The metabolic crisis triggered by the retarded vascular development is slowly resolved as the capillary plexus forms around the foveal region, and the depression, which thins the retina, begins to form soon after. Therefore, the foveal region should be understood as an environment in which neurons and glia are in critical balance with their blood supply—a balance easily disturbed by changes in blood flow, oxygen and nutrient delivery.

AGE-RELATED MACULOPATHY: WHY THE MACULA?

Macular degeneration is a disease that targets the critical central few millimetres of retina, causing degeneration of photoreceptors in the presence (the 'wet' form of the disease) or absence ('dry' form) of neovascularisation by vessels that originate from the choroid. Why is the macula the target of these disease processes? Here we suggest that the two adaptations of the macular region described above:

1. the critical balance between metabolic rate and blood supply
2. modifications of structure in the choriocapillaris and Bruch's membrane to facilitate nutrition of the macula—ultimately resulting in a predisposition of the region to degenerative change.

The critical balance: metabolic rate and blood supply

We outlined above the argument for a critical relationship between the neural retina and its blood supply (the unique environment of the fovea). Throughout most of the macula, photoreceptor density is above average (cones and rods—Figure 1). Photoreceptors consume more oxygen than any other cell type in the body but as we described, their oxygen sources are limited. Furthermore, it is understood that photoreceptors derive most of the oxygen they require from the choriocapillaris[71] and this supply is not autoregulated; that is, there is no autonomic control that might increase flow on demand. Studies of oxygen profiles in the macaque retina indicate that near the fovea, photoreceptors draw some supply from the retinal vessels, but in the foveola, where photoreceptor density peaks, there are no retinal vessels.[18] In summary, photoreceptors in the macula are highly dependent on oxygen supplied by the choroid and the evidence suggests that under normal circumstances oxygen is not available in excess. If choroidal blood flow were reduced, even by a narrow margin, the result would be oxidative stress and its sequelae in photoreceptors, including release of stress, survival factors and angiogenic factors by retinal cells.

The evidence supporting the hypothesis that metabolic stress is a significant factor in development of macular degeneration is growing. First, laser Doppler flowmetry studies have shown that decreased choroidal blood flow is associated with aging and is correlated with decreased density and volume of the choriocapillaris.[72] Investigations of AMD patients with large drusen show they have 33 per cent less choroidal volume and 37 per cent lower choroidal blood flow than age-matched normal controls.[73] Furthermore, choroidal volume and flow also decline with increasing severity of features predictive of choroidal neovascularisation.[74] Second, it has been shown that when choroidal blood flow is reduced experimentally, retinal stress is induced, including expression of GFAP by Müller cells—an established histopathological feature of AMD.[75]

The extra-retinal environment: Bruch's membrane and the choriocapillaris

Increased calibre of choriocapillaris vessels[19] and reduced thickness of the elastic lamina of Bruch's membrane[20] are two adaptations that in young, healthy individuals might secure a marginally higher rate of oxygen and nutrient delivery to photoreceptors at the macula per unit time, compared with the periphery. Ironically, over a lifetime these adaptations result in the macula becoming a repository for a large variety of blood-borne substances that diffuse out or possibly are forced out of the choriocapillaris under hydrostatic pressure, into Bruch's membrane and the sub-RPE space. Accumulation of lipids and other materials in Bruch's membrane increases with age and is more prevalent at the macula; moreover, a systemic, rather than local, origin for the constituents of drusen and other sub-RPE deposits can be argued. Accumulation of foreign substances both in Bruch's membrane and sub-RPE, as well as the possibility of low-grade choroidal vascular disease, also implicate local inflammatory responses.

LIPID DEPOSITION IN BRUCH'S MEMBRANE

Accumulation of extracellular lipids in the inner collagenous layer of Bruch's membrane has been reported as a function of age.[76,77] In the macular region of aged retinae, esterified cholesterol in Bruch's membrane is sevenfold higher than in peripheral retina.[77] Other age-related changes to Bruch's membrane include a two-to-threefold increase in thickness from childhood to adult life,[78] decreased collagen solubility[79] and metalloproteinase (MMP) activity[80] and accumulation of advanced glycation end products.[79] All of these age-related changes are likely to reduce the capacity of the choroid to deliver adequate oxygen and nutrients (including vitamin A) to the neural retina and RPE. *In vitro* studies show that hydraulic conductivity of Bruch's membrane decreases with age[81,82] and that lipid accumulation is a significant factor affecting this decrease.[83] These findings are supported by independent obser-

vations that diffusion of hydrophilic substances is reduced in explants of Bruch's membrane as donor age increases.[84-86] Decreased diffusion of substances, including vitamin A, will have consequences for continued normal photoreceptor[87,88] and RPE function.[89] Therefore, the findings suggest that while there are modest adaptations to sub-retinal structures that might enhance oxygen and nutrient delivery to macular photoreceptors in the normal retina, these same adaptations may make the macular structures, including photoreceptors, more vulnerable to the aging process.

These observations suggest a link between systemic vascular disease and macular degeneration. Epidemiologically, there is no clear-cut relationship. While the Rotterdam Study, for example, reports that measures of subclinical atherosclerosis identify individuals with increased risk of AMD,[90] the findings from Beaver Dam are negative in this respect.[91] A difficulty is that the measures of vascular disease used in the analyses are different in the two studies.

INFLAMMATORY EVENTS

Leukostasis, associated with ICAM-1 expression by choroidal endothelial cells, is a likely cause of closure of the choriocapillaris—a histopathological feature of dry AMD that correlates topographically with areas of photoreceptor degeneration.[92] Leukostasis is only one of several lines of evidence that now point to a significant inflammatory element in AMD pathogenesis. Autoantibodes against retinal cells have been reported in independent studies in AMD.[93-96] The presence of such autoantibodies, against antigens inside the blood-retinal-barrier, suggests barrier breakdown—at either the retinal vascular or choroid-RPE loci. To date, little effort has been directed towards investigation of the integrity of the retinal vasculature in aging and/or AMD, but there is strong evidence of breach of the outer retinal barrier in both dry and wet forms of AMD. In wet AMD, a clear breach occurs when choroidal vessels penetrate Bruch's membrane and RPE (classical wet AMD), although choroidal neovascularization can be restricted to the sub-retinal space.[97] On histopathological grounds, breaches of the outer blood-retinal-barrier appear to be mediated by macrophages[98,99] and dendritic cells.[100-103] In the dry form, breakdown of Bruch's membrane is associated with the formation of giant, multinucleated cells that are seen at the level of Bruch's membrane near the edges of ONL degeneration.[97,104,105]

Hageman[103] proposed that drusen formation reflects barrier breakdown initiated by RPE cell failure, triggering a cell-mediated response involving dendritic, antigen-presenting cells. Supporting his hypothesis is the presence of the monocyte-derived CD antigens within drusen, including HLA-DR-positive 'core-like' domains that appear to comprise processes of choroidal dendritic cells.

An alternative hypothesis, proposed here, is that ICAM-1-mediated choroidal leukostasis reduces blood flow, particularly in efferent vascular elements, causing local increases in hydrostatic pressure in the choroidal vascular bed, forcing blood-borne elements through the thin-walled fenestrated vessels and across Bruch's membrane into the sub-RPE space, where they coalesce as drusen. Consistent with this, drusen deposition has long been observed to occur across intercapillary spaces[106,107] (Figure 3). Therefore, the hypothesis predicts that many of the constituents of drusen, including complement components, are systemically, rather than locally, derived. Drusen and accumulation of other deposits (like basal laminar deposit) diminish supply to the retina by acting as diffusion barriers, and by increasing the distance over which nutrients and oxygen must diffuse to supply RPE cells and photoreceptors (Figure 3). Our hypothesis predicts that deterioration of RPE cells overlying drusen and sequelae in the retina[108] result from diminished access to oxygen and nutrients normally delivered by diffusion from the choroid. The hypothesis is consistent with findings showing reduced choroidal flow in AMD patients with large drusen[73] and the substantial immunological literature documenting the presence of blood-borne soluble forms of immunoglobulin superfamily members,[109] including major histocompatibility complex antigens.[110-112]

Presence of activated complement chains in drusen is also proposed as histopathological evidence of immune mechanisms in AMD.[113,114] More convincing experimental evidence now shows that complement, and more specifically, membrane attack complex (MAC) deposition at the site of injury, is essential for the development of choroidal neovascularisation in the mouse laser-model. Moreover, this study shows that MAC is involved in the induction of angiogenic factors including VEGF, FGF and TGFß.[115] In addition, three independent studies report polymorphisms in the complement factor H gene in humans that associate highly with risk of AMD and may account for up to 50 per cent of cases.[116-118]

It is important to note that inflammatory events are not independent of oxidative stress mechanisms. Inflammatory cells release both reactive oxygen and nitrogen species;[119,120] reactive species also promote adhesion between blood cells and endothelial cells,[121] contributing to leukostasis. Therefore, it appears that these interacting pathways contribute to pathogenesis of AMD at several stages of the disease.

LIMITED RESPONSIVENESS—THE ABSENCE OF ASTROCYTES

Scarcity of astrocytes in the macular region may exacerbate responses of the retina to the stresses described above. Müller cells and astrocytes both maintain the retinal extracellular environment, regulating ion homeostasis and neurotransmitter uptake, using energy-dependent mechanisms. Outside the macula, both cell types are present, suggesting division of labour between inner (mainly astrocytes) and outer (Müller cells) retina. In the macula, astrocytes occur at a greatly reduced density[31] and in aging retina the majority of those present are undergoing apoptosis (JM Provis and PL Penfold; unpublished data). In the macular region, it appears that Müller cells maintain the extracellular environment across the full thickness of retina, virtually exclusively. Therefore, in the macula, neuronal dysfunction lead-

ing to oxidative stress impacts directly on Müller glia, which function to neutralise reactive species. Although protected to some extent by their high rates of glycolytic and relatively low levels of oxidative metabolism,[122] Müller cells in the macula cannot rely on the support of fellow astrocytes to deal with the consequences of oxidative stress. Furthermore, the presence of reactive species at the macula may promote immune cell activation, including additional release of reactive species by leucocytes and microglia[119,120] and increased adhesion in blood-endothelial cell interactions,[121] further exacerbating conditions.

SUMMARY: WHERE TO FROM HERE?

We have argued that adaptation of the central retina for high acuity vision has led to compromise of vascular supply, which is manifest during foetal life in the slow development of central retinal vasculature and related metabolic stress in central retina. Because vascular development is retarded centrally, by unknown factors, stressed retinal neurons adapt to the limited blood supply from the choriocapillaris; a condition ameliorated by thinning of the retina associated with formation of the foveal depression. Further, while adaptation of the choriocapillaris underlying the foveal region may initially augment the local supply of oxygen and nutrients by diffusion, in the long term, these adaptations make the region more vulnerable to age-related changes, including an apparently higher rate of accumulation of insoluble material. Eventually, these changes impact on delivery of oxygen and nutrients to the RPE and outer neural retina because of reduced flow in the choriocapillaris and the accumulation of barriers to effective diffusion. Both the inflammatory response and the sequelae of oxidative stress are predictable outcomes in this scenario.

Recent findings concerning the special role of complement in AMD and experimental choroidal neovascularisation[115-118] provide a major step forward in understanding the aetiology of AMD. A next important step will be to characterise the events leading to complement activation in cases where aberration in the sequence coding for complement factor H is not implicated. Therefore, more work is required to characterise events that trigger the immune response, and better understand interaction with mechanisms of oxidative stress. Another area where studies are lacking concerns the biology of cone photoreceptors, in particular their metabolism and the factors required for cone maintenance throughout life. Similarly, while much has been done to further understanding of rod photoreceptor function and metabolism, more work is required to improve understanding of RPE cell function, the metabolic requirements of the RPE and the many roles of retinoic acid.

REFERENCES

1. Bone RA, Landrum JT, Tarsis SL. Preliminary identification of the human macular pigment. *Vision Res* 1985; 25: 1531-1535.
2. Snodderly DM. Evidence for protection against age-related macular degeneration by carotenoids and antioxidant vitamins. *Am J Clin Nut* 1995; 62: 1448S-1461S.
3. Wald G. Human vision and the spectrum. *Science* 1945; 101.
4. Ham WT Jr, Mueller HA, Sliney DH. Retinal sensitivity to damage from short wavelength light. *Nature* 1976; 260: 153-155.
5. Ham WT Jr, Mueller HA, Ruffolo JJ Jr, Guerry D 3rd, Guerry RK. Action spectrum for retinal injury from near-ultraviolet radiation in the aphakic monkey. *Am J Ophthalmol* 1982: 93: 299-306.
6. Kirschfeld K. Carotenoid pigments: their possible role in protecting against photooxidation in eyes and photoreceptor cells. *Biological Sci* 1982; 216: 71-85.
7. Thomson LR, Toyoda Y, Delori FC, Garnett KM, Wong ZY, Nichols CR, Cheng KM, Craft NE, Dorey CK. Long term dietary supplementation with zeaxanthin reduces photoreceptor death in light-damaged Japanese quail. *Exp Eye Res* 2002; 75: 529-542.
8. Polyak SL. The Retina. Chicago: University Chicago Press; 1941.
9. Curcio CA, Allen KA, Sloan KR, Lerea CL, Hurley JB, Klock IB, Milam AH. Distribution and morphology of human cone photoreceptors stained with anti-blue opsin. *J Comp Neurol* 1991; 312: 610-624.
10. Curcio CA, Allen KA. Topography of ganglion cells in human retina. *J Comp Neurol* 1990; 300: 5-25.
11. Wassle H, Boycott B. Functional architecture of the mammalian retina. *Physiol Rev* 1991; 71: 447-480.
12. Martin PR, Grünert U. Spatial density and immunoreactivity of bipolar cells in the macaque monkey retina. *J Comp Neurol* 1992; 323: 269-287.
13. Dacey DM. The mosaic of midget ganglion cells in the human retina. *J Neurosci* 1993; 13: 5334-5355.
14. Dacey DM. Primate retina: cell types, circuits and color opponency.[erratum appears in *Prog Retin Eye Res* 2000 Sep; 19(5): following 646]. *Prog Ret Eye Res* 1999; 18: 737-763.
15. Dacey DM. Parallel pathways for spectral coding in primate retina. *Ann Rev Neurosci* 2000; 23: 743-775.
16. Grünert U, Greferath U, Boycott BB, Wässle H. Parasol (Pa) ganglion cells of the primate fovea: Immunocytochemical staining with antibodies against $GABA_A$-receptors. *Vision Res* 1993; 33: 1-14.
17. Buttery RG, Hinrichsen CF, Weller WL, Haight JR. How thick should a retina be? A comparative study of mammalian species with and without intraretinal vasculature. *Vision Res* 1991; 31: 169-187.
18. Ahmed J, Braun RD, Dunn RJ, Linsenmeier RA. Oxygen distribution in the macaque retina. *Invest Ophthalmol Vis Sci* 1993; 34: 516-521.
19. Fryczkowski AW, Sherman MD. Scanning electron microscopy of human ocular vascular casts: the submacular choriocapillaris. *Acta Anat (Basel)* 1988; 132: 265-269.
20. Chong NH, Keonin J, Luthert PJ, Frennesson CI, Weingeist DM, Wolf RL, Mullins RF, Hageman GS. Decreased thickness and integrity of the macular elastic layer of Bruch's membrane correspond to the distribution of lesions associated with age-related macular degeneration. *Am J Pathol* 2005; 166: 241-251.
21. Stone J, Rapaport DH, Williams, R.W, Chalupa L. Uniformity of cell distribution in the ganglion cell layer of prenatal cat retina: implications for mechanisms of retinal development. *Dev Brain Res* 1982; 2: 231-242.
22. Provis JM, van Driel D, Billson FAB, Russell P. Development of the human retina: Patterns of cell distribution and redistribution in the ganglion cell layer. *J Comp Neurol* 1985; 233: 429-451.
23. Rapaport DH, Rakic P, LaVail MM. Spatiotemporal gradients of cell genesis in the primate retina. *Perspect Dev Neurobiol* 1996; 3: 147-159.
24. Sidman RL. Histogenesis of the Mouse Retina Studied with Thymidine 3-H. New York: Academic Press; 1961.
25. Linberg KA, Fisher SK. A burst of differentiation in the outer posterior retina of the

eleven-week human fetus: an ultrastructural study. *Vis Neurosci* 1990; 5: 43-60.
26. Diaz-Araya CM, Provis JM. Evidence of photoreceptor migration during early foveal development: A quantitative analysis of human fetal retinae. *Vis Neurosci* 1992; 8: 505-514.
27. Bumsted K, Hendrickson A. The distribution and development of short wavelength cones differs between Macaca monkey and human fovea. *J Comp Neurol* 1999: 403; 502-516.
28. Xiao M, Hendrickson A. Spatial and temporal expression of short, long/medium, or both opsins in human fetal cones. *J Comp Neurol* 2000; 425: 545-559.
29. Dorn EM, Hendrickson L, Hendrickson AE. The appearance of rod opsin during monkey retinal development. *Invest Ophthalmol Vis Sci* 1995; 36: 2634-2651.
30. Provis JM, Diaz CM, Penfold PL. Microglia in human retina: A heterogeneous population with distinct ontogenies. *Perspect Dev Neurobiol* 1996; 3: 213-221.
31. Provis JM, Sandercoe T, Hendrickson AE. Astrocytes and blood vessels define the foveal rim during primate retinal development. *Invest Ophthalmol Vis Sci* 2000; 41: 2827-2836.
32. Hendrickson AE, Yuodelis C. The morphological development of the human fovea. *Ophthalmology* 1984; 91: 603-612.
33. Yuodelis C, Hendrickson A. A qualitative and quantitative analysis of the human fovea during development. *Vision Res* 1986; 26: 847-855.
34. Gariano RF, Iruela AM, Hendrickson AE. Vascular development in primate retina: 1. comparison of laminar plexus formation in monkey and human. *Invest Ophthalmol Vis Sci* 1994; 35: 3442-3455.
35. Gariano RF, Provis JM, Hendrickson AE. Development of the foveal avascular zone [letter; comment]. *Ophthalmology* 2000; 107: 1026.
36. Provis JM. Development of the primate retinal vasculature. *Prog Ret Eye Res* 2001; 20: 799-821.
37. Cornish EE, Madigan MC, Natoli RC, Hales A, Hendrickson A, Provis JM. Gradients of cone differentiation and FGF expression during development of the foveal depression in macaque retina. *Vis Neurosci.* In press.
38. Sandercoe TM, Geller SF, Hendrickson AE, Stone J, Provis JM. VEGF expression by ganglion cells in central retina before formation of the foveal depression in monkey retina: evidence of developmental hypoxia. *J Comp Neurol* 2003; 462: 42-54.
39. Erickson PA, Feinstein SC, Lewis GP, Fisher SK. Glial fibrillary acidic protein and its mRNA: ultrastructural detection and determination of changes after CNS injury. *J Struct Biol* 1992; 108: 148-161.
40. Mizutani M, Gerhardinger C, Lorenzi M. Müller cell changes in human diabetic retinopathy. *Diabetes* 1998; 47: 445-449.
41. Wahlin KJ, Campochiaro PA, Zack DJ, Adler R. Neurotrophic factors cause activation of intracellular signaling pathways in Müller cells and other cells of the inner retina, but not photoreceptors. *Invest Ophthalmol Vis Sci* 2000; 41: 927-936.
42. Bignami A, Dahl D. The radial glia of Müller and their response to injury: An immunofluorescence study with antibodies to the glial fibrillary acidic protein. *Exp Eye Res* 1979; 28: 62-69.
43. Madigan MC, Penfold PL, Provis JM, Balind TK, Billson FA. Intermediate filament expression in human retinal macroglia: Histopathological changes associated with age-related macular degeneration. *Retina* 1994; 14: 65-74.
44. DiLoreto DJ, Martzen MR, del Cerro C, Coleman PD, del Cerro M. Müller cell changes precede photoreceptor cell degeneration in the age-related retinal degeneration of the Fischer 344 rat. *Brain Res*1995; 698: 1-14.
45. Lewis GP, Matsumoto B, Fisher SK. Changes in the organization and expression of cytoskeletal proteins during retinal degeneration induced by retinal detachment. *Invest Ophthalmol Vis Sci* 1995; 36: 2404-2416.
46. Sarthy V, Egal H. Transient induction of the glial intermediate filament protein gene in Müller cells in the mouse retina. *Dna Cell Biol* 1995; 14: 313-320.
47. Lewis GP, Erickson PA, Guerin CJ, Anderson DH, Fisher SK. Basic fibroblast growth factor: a potential regulator of proliferation and intermediate filament expression in the retina. *J Neurosci* 1992; 12: 3968-3978.
48. Verderber L, Johnson W, Mucke L, Sarthy V. Differential regulation of a glial fibrillary acidic protein-LacZ transgene in retinal astrocytes and Müller cells. *Invest Ophthalmol Vis Sci* 1995; 36: 1137-1143.
49. Stone J, Itin A, Alon T, Pe'er J, Gnessin H, Chan-Ling T, Keshet E. Development of retinal vasculature is mediated by hypoxia-induced vascular endothelial growth factor (VEGF) expression by neuroglia. *J Neurosci* 1995; 15: 4738-4747.
50. Stone J, Maslim J. Mechanisms of retinal angiogenesis. *Prog Ret Eye Res* 1997; 16: 157-181.
51. Donahue ML, Phelps D, Watkins RH, LoMonaco MB, Horowitz S. Retinal vascular endothelial growth factor (VEGF) mRNA expression is altered in relation to neovasculatization in oxygen induced retinopathy. *Curr Eye Res* 1996; 15: 175-184.
52. Alon T, Hemo I, Itin A, Pe'er J, Stone J, Keshet E. Vascular endothelial growth factor acts as a survival factor for newly formed retinal vessels and has implications for retinopathy of prematurity. *Nature Med* 1995; 1: 1024-1028.
53. Carmeliet P, Ferreira V, Breier G, Pollefeyt S, Kieckens L, Gertsenstein M, Fahrig M, Vandenhoeck A, Harpal K, Eberhardt C, Declercq C, Pawling J, Moons L, Collen D, Risau W, Nagy A. Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. *Nature* April 4 1996; 380: 435-439.
54. Ferrara N, Carver-Moore K, Chen H, Dowd M, Lu L, O'Shea KS, Powell-Braxton L, Hillan KJ, Moore MW. Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene. *Nature* 1996; 380: 439-442.
55. Gerber HP, Dixit V, Ferrara N. Vascular endothelial growth factor induces expression of the antiapoptotic proteins Bcl-2 and A1 in vascular endothelial cells. *J Biol Chem* 1998; 273: 13313-13316.
56. Gerber HP, McMurtrey A, Kowalski J, Yan M, Keyt BA, Dixit V, Ferrara N. Vascular endothelial growth factor regulates endothelial cell survival through the phosphatidylinositol 3'-kinase/Akt signal transduction pathway. Requirement for Flk-1/KDR activation. *J Biol Chem* 1998; 273: 30336-30343.
57. Thakker GD, Hajjar DP, Muller WA, Rosengart TK. The role of phosphatidylinositol 3-kinase in vascular endothelial growth factor signaling. *J Biol Chem* 1999; 274: 10002-10007.
58. Gerhardt H, Rascher G, Schuck J, Weigold U, Redies C, Wolburg H. R- and B-cadherin expression defines subpopulations of glial cells involved in axonal guidance in the optic nerve head of the chicken. *Glia* 2000; 31: 131-143.
59. Dorrell MI, Aguilar E, Friedlander M. Retinal vascular development is mediated by endothelial filopodia, a pre-existing astrocytic template and specific R-cadherin adhesion. *Invest Ophthalmol Vis Sci* 2002; 43: 3500-3510.
60. Dorrell MI, Otani A, Aguilar E, Moreno SK, Friedlander M. Adult bone marrow-derived stem cells use R-cadherin to target sites of neovascularization in the developing retina. *Blood* 2004; 103: 3420-3427.
61. Distler C, Weigel H, Hoffmann KP. Glia cells of the monkey retina. I. Astrocytes. *J Comp Neurol* 1993; 333: 134-147.
62. Distler C, Kopatz K, Telkes I. Developmental changes in astrocyte density in the macaque perifoveal region. *Europ J Neurosci* 2000; 12: 1331-1341.
63. Stone J, Sandercoe T, Provis J. Mechanisms of the formation and stability of retinal

blood vessels. Ocular angiogenesis: diseases, mechanisms and therapeutics. In: Tombran-Tink J, Barnstable C, eds. Ocular Angiogenesis: Diseases, Mechanisms and Therapeutics. Totowa, NJ: Humana Press. In press.
64. Obach M, Navarro-Sabate A, Caro J, Kong X, Duran J, Gomez M, Perales JC, Ventura F, Rosa JL, Bartrons R. 6-Phosphofructo-2-kinase (pfkfb3) gene promoter contains hypoxia-inducible factor-1 binding sites necessary for transactivation in response to hypoxia. *J Biol Chem* 2004; 279: 53562-53567.
65. D'Angio CT, LoMonaco MB, Johnston CJ, Reed CK, Finkelstein JN. Differential roles for NF-kappa B in endotoxin and oxygen induction of interleukin-8 in the macrophage. *Am J Physiol Lung Cell Mol Physiol* 2004; 286: L30-L36.
66. Josko J, Mazurek M. Transcription factors having impact on vascular endothelial growth factor (VEGF) gene expression in angiogenesis. *Med Sci Monit* 2004; 10: RA89-RA98.
67. Haigh JJ, Morelli PI, Gerhardt H, Haigh K, Tsien J, Damert A, Miquerol L, Muhlner U, Klein R, Ferrara N, Wagner EF, Betsholtz C, Nagy A. Cortical and retinal defects caused by dosage-dependent reductions in VEGF-A paracrine signaling. *Dev Biol* 2003; 262: 225-241.
68. Georges P, Madigan MC, Provis JM. Apoptosis during development of the human retina: relationship to foveal development and retinal synaptogenesis. *J Comp Neurol* 1999; 413: 198-208.
69. Storkebaum E, Lambrechts D, Carmeliet P. VEGF: once regarded as a specific angiogenic factor, now implicated in neuroprotection. *Bioessays* 2004; 26: 943-954.
70. Sun FY, Guo X. Molecular and cellular mechanisms of neuroprotection by vascular endothelial growth factor. *J Neurosci Res* 2005; 79: 180-184.
71. Linsenmeier RA, Padnick-Silver L. Metabolic dependence of photoreceptors on the choroid in the normal and detached retina. *Invest Ophthalmol Vis Sci* 2000; 41: 3117-3123.
72. Grunwald JE, Hariprasad SM, DuPont J. Effect of aging on foveolar choroidal circulation. *Arch Ophthalmol* 1998; 116: 150-154.
73. Grunwald JE, Hariprasad SM, DuPont J, Maguire MG, Fine SL, Brucker AJ, Maguire AM, Ho AC. Foveolar choroidal blood flow in age-related macular degeneration. *Invest Ophthalmol Vis Sci* 1998; 39: 385-390.
74. Grunwald JE, Metelitsina TI, DuPont JC, Ying GS, Maguire MG. Reduced foveolar choroidal blood flow in eyes with increasing AMD severity. *Invest Ophthalmol Vis Sci* 2005; 46: 1033-1038.
75. Fitzgerald ME, Vana BA, Reiner A. Evidence for retinal pathology following interruption of neural regulation of choroidal blood flow: Muller cells express GFAP following lesions of the nucleus of Edinger-Westphal in pigeons. *Curr Eye Res* 1990; 9: 583-598.
76. Newsome DA, Huh W, Green WR. Bruch's membrane age-related changes vary by region. *Curr Eye Res* 1987; 6: 1211-1221.
77. Curcio CA, Millican CL, Bailey T, Kruth HS. Accumulation of cholesterol with age in human Bruch's membrane. *Invest Ophthalmol Vis Sci* 2001; 42: 265-274.
78. Hogan MJ, Alvarado J. Studies on the human macula. IV. Aging changes in Bruch's membrane. *Arch Ophthalmol* 1967; 77: 410-420.
79. Farboud B, Aotaki-Keen A, Miyata T, Hjelmeland LM, Handa JT. Development of a polyclonal antibody with broad epitope specificity for advanced glycation endproducts and localization of these epitopes in Bruch's membrane of the aging eye. *Mol Vis* 1999; 5: 11.
80. Guo L, Hussain AA, Limb GA, Marshall J. Age-dependent variation in metalloproteinase activity of isolated human Bruch's membrane and choroid. *Invest Ophthalmol Vis Sci* 1999; 40: 2676-2682.
81. Starita C, Hussain AA, Marshall J. Decreasing hydraulic conductivity of Bruch's membrane: relevance to photoreceptor survival and lipofuscinoses. *Am J Med Genet* 1995; 57: 235-237.
82. Starita C, Hussain AA, Patmore A, Marshall J. Localization of the site of major resistance to fluid transport in Bruch's membrane. *Invest Ophthalmol Vis Sci* 1997; 38: 762-767.
83. Starita C, Hussain AA, Pagliarini S, Marshall J. Hydrodynamics of ageing Bruch's membrane: implications for macular disease. *Exp Eye Res* 1996; 62: 565-572.
84. Moore DJ, Hussain AA, Marshall J. Age-related variation in the hydraulic conductivity of Bruch's membrane. *Invest Ophthalmol Vis Sci* 1995; 36: 1290-1297.
85. Moore DJ, Clover GM. The effect of age on the macromolecular permeability of human Bruch's membrane. *Invest Ophthalmol Vis Sci* 2001; 42: 2970-2975.
86. Hussain AA, Rowe L, Marshall J. Age-related alterations in the diffusional transport of amino acids across the human Bruch's-choroid complex. *J Opt Soc Am A Opt Image Sci Vis* 2002; 19: 166-172.
87. Curcio CA, Saunders PL, Younger PW, Malek G. Peripapillary chorioretinal atrophy: Bruch's membrane changes and photoreceptor loss. *Ophthalmology* 2000; 107: 334-343.
88. Jackson GR, Curcio CA, Sloan KR, Owsley C. Photoreceptor degeneration in aging and age-related maculopathy. In: Penfold P, Provis J, eds. Macular Degeneration. Berlin-Heidelberg: Springer; 2005. p 45-62.
89. Winkler BS, Boulton ME, Gottsch JD, Sternberg P. Oxidative damage and age-related macular degeneration. *Mol Vis* 1999; 5: 32.
90. Vingerling JR, Dielemans I, Bots ML, Hofman A, Grobbee DE, de Jong PT. Age-related macular degeneration is associated with atherosclerosis. The Rotterdam Study. *Am J Epidemiol* 1995; 142: 404-409.
91. Klein R, Klein BE, Tomany SC, Cruickshanks KJ. The association of cardiovascular disease with the long-term incidence of age-related maculopathy: the Beaver Dam Eye Study. *Ophthalmology* 2003; 110: 1273-1280.
92. Penfold P, Wong J, van Driel D, Provis J, Madigan M. Immunology and age-related macular degeneration. In: Penfold P, Provis J, eds. Macular Degeneration. Berlin-Heidelberg-New York: Springer; 2005.
93. Penfold PL, Provis JM, Furby JH, Gatenby PA, Billson FA. Autoantibodies to retinal astrocytes associated with age-related macular degeneration. *Graefes Arch Clin Exp Ophthalmol* 1990; 228: 270-274.
94. Gurne DH, Tso MO, Edward DP, Ripps H. Antiretinal antibodies in serum of patients with age-related macular degeneration. *Ophthalmology* 1991; 98: 602-607.
95. Chen H, Wu L, Pan S, Wu DZ. An immunologic study on age-related macular degeneration. *Yen Ko Hsueh Pao* 1993; 9: 113-120.
96. Gu X, Meer SG, Miyagi M, Rayborn ME, Hollyfield JG, Crabb JW, Salomon RG. Carboxyethylpyrrole protein adducts and autoantibodies, biomarkers for age-related macular degeneration. *J Biol Chem* 2003; 278: 42027-42035.
97. Sarks JP, Sarks SH, Killingsworth MC. Morphology of early choroidal neovascularisation in age-related macular degeneration: correlation with activity. *Eye* 1997; 11: 515-522.
98. Penfold P, Killingsworth M, Sarks S. An ultrastructural study of the role of leucocytes and fibroblasts in the breakdown of Bruch's membrane. *Aust J Ophthalmol* 1984; 12: 23-31.
99. Penfold PL, Killingsworth MC, Sarks SH. Senile macular degeneration: the involvement of immunocompetent cells. *Graefes Arch Clin Exp Ophthalmol* 1985; 223: 69-76.
100. Mullins RF, Hageman GS. Human ocular drusen possess novel core domains with a distinct carbohydrate composition. *J Histochem Cytochem* 1999; 47: 1533-15340.
101. Mullins RF, Russell SR, Anderson DH, Hageman GS. Drusen associated with aging and age-related macular degenera-

tion contain proteins common to extracellular deposits associated with atherosclerosis, elastosis, amyloidosis, and dense deposit disease. *Faseb J* 2000; 14: 835-846.
102. Mullins RF, Aptsiauri N, Hageman GS. Structure and composition of drusen associated with glomerulonephritis: implications for the role of complement activation in drusen biogenesis. *Eye* 2001; 15: 390-395.
103. Hageman GS, Luthert PJ, Victor Chong NH, Johnson LV, Anderson DH, Mullins RF. An integrated hypothesis that considers drusen as biomarkers of immune-mediated processes at the RPE-Bruch's membrane interface in aging and age-related macular degeneration. *Prog Retin Eye Res* 2001; 20: 705-732.
104. Penfold PL, Killingsworth MC, Sarks SS. Senile macular degeneration. The involvement of giant cells in atrophy of the retinal pigment epithelium. *Invest Ophthalmol Vis Sci* 1986; 27: 364-367.
105. Dastgheib K, Green R. Granulomatous reaction to Bruch's membrane in age-related macular degeneration. *Arch Ophthalmol* 1994; 112: 813-818.
106. Friedman E, Smith TR, Kuwabara T. Senile choroidal vascular patterns and drusen. *Arch Ophthalmol* 1963; 69: 220-230.
107. Lengyel I, Tufail A, Hosaini HA, Luthert P, Bird AC, Jeffery G. Association of drusen deposition with choroidal intercapillary pillars in the aging human eye. *Invest Ophthalmol Vis Sci* 2004; 45: 2886-2892.
108. Johnson PT, Lewis GP, Talaga KC, Brown MN, Kappel PJ, Fisher SK, Anderson DH, Johnson LV. Drusen-associated degeneration in the retina. *Invest Ophthalmol Vis Sci* 2003; 44: 4481-4488.
109. Hope SA, Meredith IT. Cellular adhesion molecules and cardiovascular disease. Part II. Their association with conventional and emerging risk factors, acute coronary events and cardiovascular risk prediction. *Intern Med J* 2003; 33: 450-462.
110. Heiligenhaus A, Rebmann V, Neubert A, Plewa S, Ferencik S, Vogeler U, Steuhl KP, Grosse-Wilde H. Soluble HLA class I and HLA-DR plasma levels in patients with anterior uveitis. *Tissue Antigens* 2004; 63: 369-375.
111. Rebmann V, Ronin-Walknowska E, Sipak-Szmigiel O, Miklaszewicz A, Czajkowska E, Grosse-Wilde H. Soluble HLA-DR and soluble CD95 ligand levels in pregnant women with antiphospholipid syndromes. *Tissue Antigens* 2003; 62: 536-541.
112. Esteban O, Zhao H. Directed evolution of soluble single-chain human class II MHC molecules. *J Mol Biol* 2004; 340: 81-95.
113. Johnson LV, Ozaki S, Staples MK, Erickson PA, Anderson DH. A potential role for immune complex pathogenesis in drusen formation. *Exp Eye Res* 2000; 70: 441-449.
114. Anderson DH, Mullins RF, Hageman GS, Johnson LV. A role for local inflammation in the formation of drusen in the aging eye. *Am J Ophthalmol* 2002; 134: 411-431.
115. Bora PS, Sohn JH, Cruz JM, Jha P, Nishihori H, Wang Y, Kaliappan S, Kaplan HJ, Bora NS. Role of complement and complement membrane attack complex in laser-induced choroidal neovascularization. *J Immunol* 2005; 174: 491-497.
116. Edwards AO, Ritter Iii R, Abel KJ, Manning A, Panhuysen C, Farrer LA. Complement factor H polymorphism and age-related macular degeneration. *Science* 2005; 308: 421-422.
117. Haines JL, Hauser MA, Schmidt S, Scott WK, Olson LM, Gallins P, Spencer KL, Kwan SY, Noureddine M, Gilbert JR, Schnetz-Boutaud N, Agarwal A, Postel EA, Pericak-Vance MA. Complement factor H variant increases the risk of age-related macular degeneration. *Science* 2005; 308: 419-421.
118. Klein RJ, Zeiss C, Chew EY, Tsai JY, Sackler RS, Haynes C, Henning AK, Sangiovanni JP, Mane SM, Mayne ST, Bracken MB, Ferris FL, Ott J, Barnstable C, Hoh J. Complement factor H polymorphism in age-related macular degeneration. *Science* 2005; 308: 385-389.
119. Emerit J, Edeas M, Bricaire F. Neurodegenerative diseases and oxidative stress. *Biomed Pharmacother* 2004; 58: 39-46.
120. Splettstoesser WD, Schuff-Werner P. Oxidative stress in phagocytes—the enemy within. *Microsc Res Tech* 2002; 57: 441-455.
121. Cooper D, Stokes KY, Tailor A, Granger DN. Oxidative stress promotes blood cell-endothelial cell interactions in the microcirculation. *Cardiovasc Toxicol* 2002; 2: 165-180.
122. Winkler BS, Arnold MJ, Brassell MA, Puro DG. Energy metabolism in human retinal Muller cells. *Invest Ophthalmol Vis Sci* 2000; 41: 3183-3190.

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