# Retinal Progenitor Cells in Regeneration and Repair Highlight New Therapeutic Targets

Eric J Mayer, Balini Balasubramaniam, Debra A Carter and Andrew D Dick

*Academic Unit of Ophthalmology, University of Bristol*

## Abstract

The quest to understand the ability of the retina to not only sustain its function throughout life but also, as a result of pathological degeneration, to promote repair via stimulating endogenous regenerative capacity or via cell replacement is nearing clinical assessment. However, we still need to understand the kinetics and dynamics of cell replacement in healthy or ageing retina. This would lead to the possibility of manipulating endogenous ocular progenitor cells towards facilitating cell replacement when degeneration has ensued. Arguably, the most clinically immediate benefits will arise from cell-based therapies. However, the questions of which cells to use to maximise clinical outcome – including ocular sources or manipulation of non-ocular cell sources, including embryonic stem cells, as neuralised progenitor cell sources – and how best to deliver therapy remains unqualified. Ultimate success will depend on integration into damaged host tissue, prevention of gliosis and knowing which cells to target to replace.

## Keywords

Prognitor cells, retina, stem cells

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**Correspondence:** Eric J Mayer or Andrew D Dick, Bristol Eye Hospital, Lower Maudlin Street, Bristol BS1 2LX, UK. E: e.mayer@bristol.ac.uk or a.dick@bristol.ac.uk

## Retinal Repair and Regeneration

Throughout life, tissues and organs constantly repair and replace cells to maintain optimal function. The central nervous system (CNS) – the brain, retina and spinal cord – was conventionally thought, because of poor response to damage, to have only limited ability to repair; however, even without damage how can a set of neuronal cells last a lifetime? It is clear that when a tissue is structurally damaged (see *Figure 1*) there is progressive loss of function over time and recovery is far more limited. This happens following trauma (surgical or accidental) or with certain diseases that mechanistically have in common direct cell death, ischaemia and inflammation (diabetes, stroke, autoinflammatory disease and degenerative conditions).

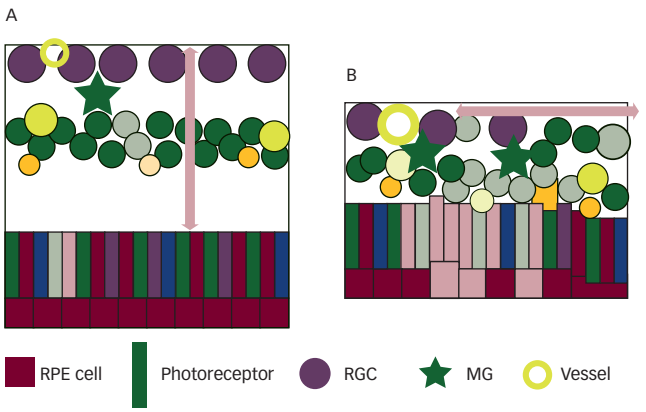
Any recovery of function after successful control of ischaemia, inflammation or cell death may occur via cell replacement. However, to this end there remains controversy over whether neuronal progenitor cells (cells able to divide and generate new cells of neuronal lineage) reside within adult retina.[1,2] Pertinently, this debate raises the possibility of neuronal replacement in man, as observed in experimental mammals,[3–5] even though single cell cultures confirm that intra-retinal progenitor cells (RPCs) are uncommon.[6] One should therefore question whether the presence of retinal neural progenitor cells or recent progenitor immigrants into the retina explains the functional recovery observed after cell loss without extensive destruction of extracellular tissue architecture from macular light toxicity[7] or after macula-off retinal detachment.[8–10]

## Adult Tissue Progenitor Cells and Their Detection

To maintain tissue and cellular homeostasis and function, cellular replacement is likely to be ongoing, inconspicuous and, possibly, stochastic. It is unlikely to occur more quickly in adults than during development. Retinal cell replacement occurs within myeloid cell populations (where perivascular macrophages and microglia are replaced over six months),[11] and is likely to be much quicker than neural cell replacement. Establishing their connections and explaining recovery, if present, is a slow process. So, what is the rate of cell replacement in normal retina? While it is possible to see cells dying in the retina,[11] the rate of cell loss in the normal retina is difficult to establish as there is no analogous method for seeing new cells added in the steady state, unless they are directly labelled and transferred.[12] Replacement from *in situ* mitosis is uncommon as few cells divide in the normal mammalian retina,[12] although dividing cells increase in stressed tissue such as Chx10-null mutants.[13]

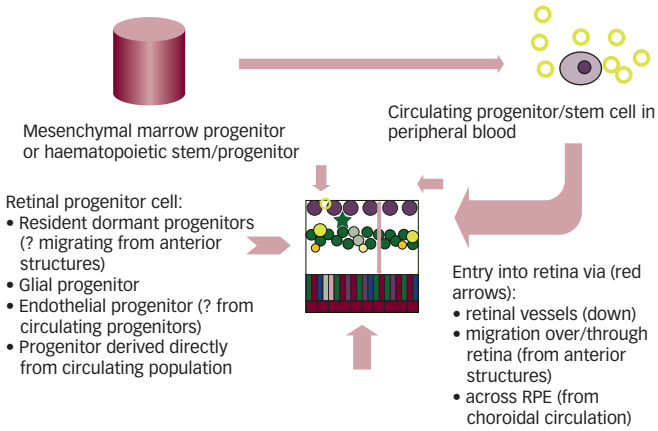
RPCs are a majority cell type during development and are easy to isolate and study. Progenitor cells from either developing or adult tissues will potentially be dividing and differentiating (in various stages and pathways of differentiation), and many markers are used to positively or negatively select cell populations of interest (see *Table 1*[14–18]). To date, this has been best achieved using cell-surface flow cytometric phenotypic analysis and isolation via flow cytometric cell sorting or, more commonly, magnetic bead cell sorting. Another

**Figure 1: Difficulties Facing Progenitor Cell Replacement of Damaged Tissue**




*Schematic representation of the challenges faced in cell replacement in standard cell turnover (A) resulting from cell death (represented by colours not defined in the key above) and in diseases where there is significant disruption of tissue architecture and accompanying cell death (B). Cellular replacement is more complex and less likely to be successful in the scenario represented in B because more cells are needed; tissue is disrupted and therefore so are the signals that guide cells to their targets. Concomitantly, there is activation and increased numbers of microglial cells (MGs) and changes at blood ocular barrier sites (retinal pigment epithelium and inner retinal vasculature), whereupon associated inflammatory signals disturb the fate decisions made by retinal progenitor cells. With gross tissue disruption, it is more likely that tissue synthesisers and engineering will become necessary.*
*RGC = retinal ganglion cell.*

**Figure 2: Possible Origins of Stem/Progenitor Cells in the Retina**




*Progenitor cells may arise from mesenchymal/marrow progenitor cells or from resident progenitor cells (retinal progenitors or glial progenitors, endothelial progenitors or others derived directly from the circulation).*

cell-sorting approach utilises progenitor cell or tissue-specific gene promoters to drive fluorescent expression (green fluorescent protein [GFP]).[19] Other intracellular markers that may be used in this way include: brain and acute leukaemia cytoplasmic (BAALC), a neuro-ectodermal marker found in the CNS and haematopoietic stem cells (HSCs) in the retina; cytoskeletal proteins such as intermediate filaments (nestin and double cortin); and markers of cell division (cyclin D1). The extent of expression and/or promoter activity may vary according to the stage of the cell cycle, and for RPCs there is significant variation in the genes used to exit from the cell cycle and during different stages of cell development.[20] The overlap of expression of cell-surface phenotypic markers and nuclear transcription factors or forkhead gene expression between different populations of stem/progenitor cells emphasises common features in this heterogenous group of cells, with potential to understand the cellular pathways involved (see *Figure 2*).

## Origin and Lineage of Adult Human Retinal Progenitor Cells

There is no definitive direct comparison between progenitor cells in developing and adult retina. It is probable that adult retinal progenitors follow the same differentiation sequence as those in developing retina. Retinal progenitors may arise from recruited bone marrow progenitor cell populations (mesenchymal or haematopoietic), from resident tissue progenitors (of unspecified, glial or endothelial lineage) stimulated by damage or disease (creating a need for new cells) or from transdifferentiation of one of the known retinal cell types (as discussed below), particularly as in mammals retinal damage stimulates repair and is associated with cell division.[21] Following retinal ischaemia there is a massive increase in cell proliferation, but only if the ischaemia is followed by reperfusion.[22] This indicates the need to stimulate the regenerative process and the possible recruitment of dividing cells from the circulation. Regardless of lineage or degree of differentiation, progenitor cells must respond to environmental cues in tissue to generate the right cell type. For example, the fate of dividing retinal cells in culture is affected by differentiated retinal cell types,[23] and the integration of transplanted progenitor cells depends on the host tissue's need for them.[24]

> *Our understanding of retinal degeneration is that cells die progressively in life and faster in disease, eventually leading to functional loss.*

In general, although not exclusively, cells with stem or progenitor properties show evidence of pre-programming or regional specification.[25] The lineage or origin of retinal progenitors therefore remains an open question, with iris pigment epithelial cell,[26] retinal pigment epithelial cell[27] or circulating progenitor cell lineages all possible, in addition to resident tissue populations. Alternatively, retinal progenitors may arise from a glial lineage, as O2A progenitor cells (O4+ and platelet-derived growth factor [PDGF]-αR+) may develop into cells of astrocytic or oligodendrocytic lineage, which are present in the retina.[28] These cells are also A2B5+ (see *Table 2*) and behave differently from similar cell phenotypes in the CNS.[29]

It is generally felt that in the adult eye RPCs reside within the ciliary body[30] or its epithelium, and to reach the retina they would need to migrate posteriorly. If this is the case, there should be a system to support their migration, such as the network of nestin-positive cells within the adult retina.[31] Primate ciliary body epithelium contains cellular and nuclear features compatible with dividing RPCs,[32] and in humans occasional cells of the inner non-pigmented epithelium of the pars plana are nestin-positive (see *Figure 3*). This niche of progenitor cells is supported by the increased rate at which nestin-positive cells are generated from anterior adult human retina (see *Figure 4*).[1]

## Progenitor Cells and Retinal Degeneration

Our understanding of retinal degeneration is that cells die progressively in life and faster in disease, eventually leading to

**Table 1: Cell Surface Markers Used to Identify Stem or Progenitor Cells**

| Marker | Properties and Cell Expression | Expression in Posterior Segment |
|---|---|---|
| A2B5 | Cell surface ganglioside. Expressed on developing thymic epithelial cells, bone marrow mesenchymal cells, NPCs, oligodendrocyte progenitor cells and neuroendocrine cells. | Retinal neurones. |
| ABCG2 (ATP-binding cassette superfamily | Excludes DNA-intercalating dyes. Determinant of Hoescht-dye negative-side population cells. Present in HSCs and committed HPCs. Good potential positive marker for adult progenitors from multiple sources. | Retinal stem cells, influencing G member 2) lineage commitment.[14] |
| AC133 antigen, CD133, prominin-1, prominin-2, prominin family | Pentaspan membrane glycoprotein present on many types of stem and progenitor cell in human and other species. | Enriches retinal progenitor cells. Photoreceptor cell outer segments. |
| CD34 | Heavily glycosylated cell surface molecule with multiple epitopes. Expressed on HSCs/progenitor cells, as well as many acute leukaemias and neoplasms of vascular origin. | Retinal vascular endothelium, endothelial progenitor cells, dendritic cells, myeloid cells (including mast cells). |
| CD43 | Mucin-like transmembrane sialo-glyco-protein adhesion molecule. High expression on HPCs (possibly regulates proliferation) and also on mature blood cells (lymphocytes, neutrophils, monocytes and platelets), but not on non-haematopoietic cells. | Lymphocytes, neutrophils, monocytes and platelets. Retinal lymphoma. |
| CD45 | Transmembrane tyrosine phosphatase involved in lymphocyte activation and differentiation. Expressed on all nucleated haematopoietic lineage cells and mature HSCs. | Microglia, dendritic cells of iris, ciliary body and choroid. Pericytes and early progenitor endothelial cells. |
| CUB-domain-binding protein 1 (CDCP1) | Transmembrane protein – cadherin family. Expressed on $CD34^+$ and $CD133^+$ HSCs, as well as metastatic tumours (colon and breast), mesenchymal stem cells and NPCs. | RPE. Mutations associated with juvenile macular dystrophy. |
| c-Kit/CD117 | HSC. | CD117+ generates RPE when transplanted.[15] |
| Endomucin | CD-34-like sialo-mucin. Expressed on HSCs throughout development, as well as early endothelium. photoreceptors in developing retina. | Present in adult and developing choroidal and retinal vessels. Present around |
| Foetal liver kinase-2 (Flk-2), CD135, fms-like tyrosine kinase receptor-3 (flt-3) | Stimulates proliferation of stem cells and differentiation of lymphoid progenitor lineages. Expressed on HSCs. | Expressed in all layers of the neural retina.[16] |
| Stem cell antigen 1 (Sca1) | Type V glyco-phosphatidyl-inositol anchored cell surface proteins, upregulated on cell activation. Expressed on multipotent HSCs/progenitor cells in bone marrow. | Marrow-derived cells of this lineage give rise to retinal vessels[17] and RPE.[18] |
| Thy-1/CD90 | Expressed on $CD34^+$ HPCs (with high proliferative potential) and primitive cord blood progenitors, possibly mediating a negative signal that results in inhibition of primitive cell proliferation. | Retinal ganglion cell neurones. |
| O4 | Earliest marker of cells of oligodendrocyte lineage. (unpublished observations). | Cultured retinal progenitor cells |
| Growth factor receptors | Including FGFR-1, EGFR, PDGF-$\alpha$R and SCGF. glia, microglia, lymphocytes and RPE. | Multiple on retinal neurones, endothelia, |

*Cell surface markers are useful for isolating stem/progenitor cells. Intracellular markers are useful for characterising cells and identifying them in tissue sections, but are not used in antibody-mediated methods of live cell separation. Using growth factor receptors to sort cells with specific antibody binding has the complication that these antibodies may then inhibit or stimulate that receptor and so may have a functional effect.*
*ATP = adenosine triphosphate; EGFR = epidermal growth factor receptor; FGFR-1 = fibroblast growth factor-1; HPC = haematopoietic progenitor cell; HSC = haematopoietic stem cell; NPC = neural progenitor cell; PDGF = platelet-derived growth factor; RPE = retinal pigment epithelium; SCGF = stem cell growth factor.*

functional loss. In this context, neuroprotection involves the prevention of apoptosis. If we accept that some cellular replacement occurs, degeneration becomes a product of the relative rate of cell loss and the rate of (and potential for) cellular replacement; if deficits in cell replacement occur, the functional result is cellular degeneration. In the normal adult retina the few proliferating cells observed (of neural, glial or vascular lineage) are principally non-myeloid, as the majority of retinal microglia/macrophages that are replaced do not proliferate *in situ*.[12] However, in transplant chimaeras, marrow-derived cells home to damaged areas in the eye and regenerate retinal pigment epithelium (RPE)[15] as well as retinal vessels.[17]

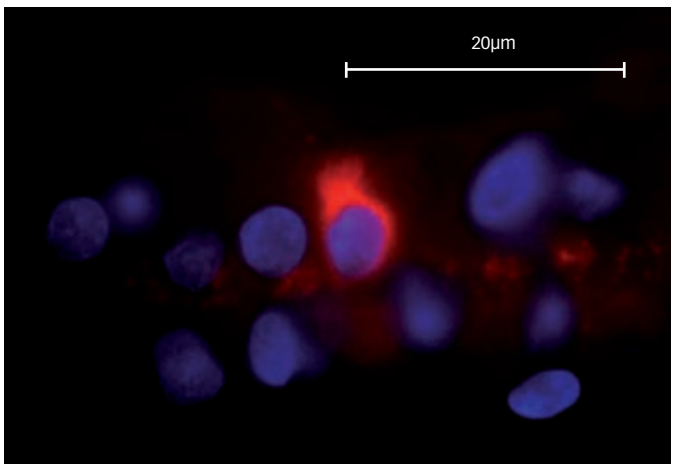
In animal models of inherited retinal degeneration, retinal degeneration is usually the result of apoptosis due to a gene deficit.[33,34] Are replacement cells more vulnerable to apoptosis because their connections are less well established within a tissue? This would certainly explain the sudden functional loss that is manifest in some degenerative diseases. Treatments that protect cells from apoptosis are as likely to work on older cells as on those that have been more recently laid down. Mouse or human bone-marrow-derived stem cells injected into mouse eyes undergoing retinal degeneration attenuate retinal photoreceptor degeneration and loss of vasculature and alter electroretinograms, although they lead to a predominance of cone instead of rod photoreceptors.[35] Cell transplants have multiple potential effects. In animal models, rescue of degeneration occurs by cell, matrix or growth factors, suggesting that degeneration occurs because of defects in any of these elements. Indirect evidence for this comes from RCS rats, where a defective gene (*Mertk*) expressed in the RPE for phagocytic function causes photoreceptor degeneration to occur. Degeneration can be delayed by retinal haemorrhage, trauma or

**Table 2: Growth Factors Rescuing Degeneration in Retina and Their Effects on Neural Progenitors**

| Growth Factor | Rescue of Retinal Cell Degeneration | Effect on Retinal Progenitor Cells |
|---|---|---|
| FGF | Retinal degeneration slowed.[49] | General effect on progenitor and stem cell populations, e.g. retinal progenitors.[1,50] |
| VEGF | | Role in early developmental programme of retinal neurogenesis.[51,52] |
| Epo | Rescue of RGC after chronic ocular hypertension.[53] | Retinal CD133+ vascular progenitors are responsive to Epo.[54] |
| IGF-1 | Rescues axotomised retinal ganglion cells.[55] | Differentiation decisions influenced.[56] |
| CNTF | Rescue of degeneration in rd1 mice and excitatory amino-acid-induced degeneration.[57–59] | Differentiation of retinal progenitor cells.[60] |
| BDNF | Rescue of degeneration in rd1 mice.[57] | Role in photoreceptor progenitor fate decision.[61] |
| GDNF | Protects from photoreceptor loss in RCS rats.[62] | Not known. |
| BMP4 | Reduces retinal degeneration induced by amino acids.[59] | Probable role in early forebrain development.[63] |
| EGF | | Important for developmental retinal progenitors and in fate decisions.[5,56] |

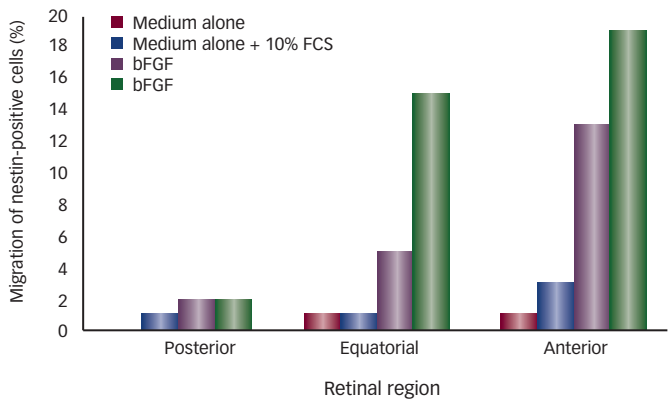
*These common roles may imply common influences of the two. It is possible that recently replaced cells might be more vulnerable to degeneration and therefore more in need of rescue because their connections are less well established than those within a tissue.*
*BDNF = brain-derived neurotrophic factor; BMP = bone morphogenetic protein; CNTF = ciliary neurotrophic factor; EGF = epidermal growth factor; Epo = erythropoietin; FGF = fibroblast growth factor; GDNF = glial-cell-line-derived neurotrophic factor; IGF = insulin-like growth factor; RGC = retinal ganglion cell; VEGF = vascular endothelial growth factor.*

**Figure 3: Immunohistochemistry of Nestin-positive Cells in Ciliary Body**



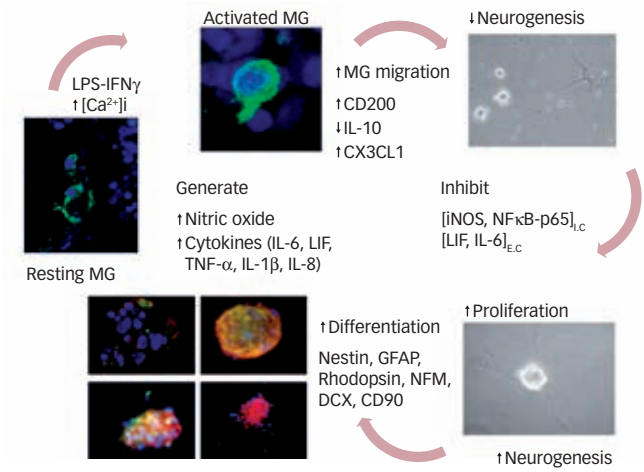

*Nestin-positive (red) cell in the inner non-pigmented pars plana epithelium of the adult human eye. DAPI counterstain shows the nuclei of this bilayered epithelium in blue.*

**Figure 4: Gradient of Nestin-positive Cells Generated from Different Regions of Adult Human *Post Mortem* Retina**




*Average percentages of migrant nestin-positive cells are expressed according to retinal region under different culture conditions.[1]*
*bFGF = basic fibroblast growth factor (now FGF2); FCS = foetal calf serum; medium alone = DMEM-HAMS F12;*

**Figure 5: The Interaction of Retinal Neural Progenitors and Microglial Cells**




*Cell interaction is important in resting retinal tissue and in retinal disease. Both microglia and neural progenitor cells are potential targets for regenerative therapies. Resting microglia (MG) become motile activated microglia (increasing their CD200 and CX3CL1 expression and decreasing interleukin [IL]-10) following stimulation with lipopolysaccharide and interferon-gamma (LPS-IFNγ) and increased intracellular calcium. Activated MG increase nitric oxide and pro-inflammatory cytokine production, particularly IL-6, which inhibits the formation of neurospheres in neural progenitor cultures.*
*GFAP = glial fibrillary acidic protein; iNOS = inductible nitric oxide synthase; LIF = leukaemia-inhibitory factor; NF-κB = nuclear factor kappa B; TNF = tumour necrosis factor.*

the injection of growth factors, e.g. basic fibroblast growth factor (bFGF or FGF-2).[36] The same is also true of light-induced retinal damage.[37,38] Other growth factors are also able to rescue retinal degeneration.[39–48] The growth factors that rescue degenerating cells also potentially influence progenitor cell fate (see *Table 2*[1,5,49–63]).

## Cell-based Therapy

When transplanting dissociated cells, for example transplanting immature retinal cells into the retina to replace damaged photoreceptors, the cellular complement of the diseased tissues is increased to a level compatible with regaining function. In the retina, this was initially demonstrated by del Cerro in 1989,[64] who transplanted zero- to two-day-old rat retinal cell suspensions into recipient animals with light-induced retinal degeneration, demonstrating survival and anatomical integration of these

transplants. Survival of cells transplanted into the subretinal space is now accepted.[65] More recently, electrophysiological and behavioural evidence has been presented of functional integration of these transplants in mice with inherited retinal degeneration;[66] therefore, cellular transplantation in the retina is clearly possible. It is a question of identifying the correct cell to transplant in sufficient numbers to justify the surgical trauma involved. To be effective we need to better understand the nature and behaviour of intrinsic cells and find cells that integrate safely and in sufficient numbers and generate the appropriate lineage. Cells will integrate into a tissue if the right cell is generated in an environment where there is a need, such that the tissue can accept the integrating cells.[25,67] Transplantation requires an understanding of cell biology, the isolation of cells able to integrate into the retina and the extra- and intra-cellular signalling that guides these events. There is a need to study single cells (from developing tissue); where this has been possible, gene expression is heterogenous[20] due to the cells studied being at different stages of the cell cycle, as well as RPCs using different genes to exit the cell cycle.[20] This suggests that cell fate and lineage decisions are made early in cellular differentiation – before cells have ceased dividing – as by definition all RPCs in this study were identified as expressing cyclin D1.[20] Importantly, RPCs are not a single cell type but rather a variety of cells at various stages along several as yet incompletely characterised differentiation pathways. Comparing the transcriptional profiles of retinal progenitors between adult and developing tissue will establish whether adult progenitors mirror those in development. For example, transcriptomics will predict and focus therapies.

*The treatment of diseases with destruction of tissue architecture represents a special challenge as cellular replacement may require accompanying tissue synthesiser technology if it is to lead to functional recovery.*

## Do Cellular Interactions Influence Progenitor Cell Fate?

Functions such as vision are testimony to the interaction of cells. Of critical importance in this respect is the interaction of RPCs with microglia. RPCs are key to the retina's potential for cellular replacement, and microglial cells are central to regulating the response of tissues to various diseases. Microglia express CD200R and other inhibitory receptors that control activation status, with its ligand expressed on the retinal neurone CD200.[68,69] Despite this cognate receptor control, signals (such as interferon-gamma) will promote classic macrophage activation of microglial cells, with subsequent release of pro-inflammatory cytokines, growth factors and proteases and increased cell migration (see *Figure 5*). IL-6 directly inhibits neurosphere formation *in vitro* and thus has potential for progenitor cell division, migration, differentiation and, ultimately, functional integration.

## Towards Therapy – The Caveats

In the study of tissue repair and regeneration, cell lines offer some advantages, with cells being available in large numbers, cells having reproducible properties and reduced numbers of unwanted cell types compared with primary cell cultures. However, with respect to therapy, the accumulation of karyotypic abnormalities and certain mutations[70] in embryonic stem cells cultured *in vitro* after more than 20 passages[71] is of concern and correlates with a loss of toti-potency,[71] which needs to be refined before therapy can be considered. Similar concerns arise for tissue-specific cell lines (e.g. Müller cells),[72] where cells become near-triploid.[73] Transplanted primary cells and *in situ* progenitor populations are important new therapeutic targets for optimising repair and regeneration in the retina, for example via gene therapy. In this respect it is encouraging that human embyonic stem cells reliably generate RPCs,[74] making them useful for screening primary cell interventions. Although primary cells from *post mortem* retina are subject to variations in tissue[2] and adult human cells in culture show slow growth[1,2] compared with immature retinal cells[50] and animal cells,[4,5] adult human retinal progenitors are a target for human therapy and a possible source of the retina's inherent regenerative potential.

The treatment of diseases with destruction of tissue architecture (see *Figure 1*) represents a special challenge as cellular replacement may require accompanying tissue synthesiser technology if it is to lead to functional recovery. The understanding of retinal cell biology in health and disease from all of these areas of research combined with advances in gene therapy offers a future with realistic hope of treating what is currently considered to be untreatable blindness. ■

Eric J Mayer is a Consultant Senior Lecturer at the University of Bristol and a National Career Scientist Award Holder from the UK Department of Health Research and Development. His research and publications focus on retinal cell biology, central nervous system repair and regeneration, stem/progenitor cells and clinical medical and surgical retinal disease. He is a Fellow of the Royal College of Ophthalmologists and a Member of the British and Eire Association of Vitreo-Retinal Surgeons and Oxford Congress of Ophthalmology. Dr Mayer serves as an Examiner for the International Council of Ophthalmology.

Andrew D Dick is Chair and Head of the Academic Unit of Ophthalmology and Head of Research for Clinical Sciences at the University of Bristol. His research interests include immunobiology of inflammation of the eye, identifying targets for immune intervention and how the retina responds to inflammation and injury and interaction with retinal progenitor cell turnover and tissue repair. He has served as UK Editor of the *British Journal of Ophthalmology*. Dr Dick is Director of Research for the UK National Eye Research Centre and sits on numerous Editorial Boards of international journals. He was recently awarded Fellowship of the UK Academy of Medical Sciences.

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