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Influence of microglia on retinal progenitor cell turnover and cell replacement

AD Dick[1,2]

Abstract

Microglia within the retina are continually replaced from the bone marrow and are the resident myeloid-derived cells within the retina. Throughout life, microglial function is conditioned by the microenvironment affording immunomodulation to control inflammation as well as functioning to enable normal development and, during adulthood, maintain normal retinal function. In adulthood, recent evidence supports the concept that the retina continues to replace cells to maintain optimal function. Although in some cases after injury, degeneration, or inflammation there remains an inextricable decline in visual function inferring a deficit in cell replacement, the deficit could be explained by microglial cell activation influencing the ability of either retinal progenitor cells or recruited progenitor cells to integrate and differentiate appropriately. Myeloid cell response differs depending on insult: it is evident that during inflammation microglia and the infiltrating myeloid cell function are conditioned by the cytokine environment. Indeed, modulating myeloid cell function therapeutically suppresses disease in experimental models of autoimmunity, whereas in non-inflammatory models microglia have little or no effect on the course of degeneration. The extent of myeloid activation can help determine retinal progenitor cell turnover. Retinal progenitor cells may be isolated from adult human retina, which, albeit limited, display mitotic activity and can differentiate. Microglial activation secreting IL-6 limits progenitor cell turnover and the extent to which differentiation to post-mitotic retinal cells occurs. Such experimental data illustrate the need to develop methods to replenish normal retinal myeloid cell function facilitating integration, either by cell transplantation or by encouraging retinal progenitor cells to recover retinal function.
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Active turnover of retinal microglia

Microglia represent the myeloid cell population of the retina and CNS. In the retina, there are two populations: perivascular macrophages anatomically situated within the glial limitans of the inner retinal vasculature and ramified retinal microglia within the tissue parenchyma.[1,2] More recently, we have understood better the dynamics of cell replacement. Although timing varies between different laboratory data, consistently the data show that bone marrow-derived monocyte precursor cells are able to migrate across the blood–retinal barrier and show complete replacement of the retinal myeloid cell population within 6 months,[3,4] with little evidence of *in situ* proliferation determining cell turnover. Phenotypic analysis during cell replacement also presents us with a further understanding of the chronology and temporal sequence of cell replacement, which closely follows that observed during retinal development.[5] There appears to be a migration pattern to cell replacement, in which the majority of the cells enter through retinal circulation, and is likely to be stochastic. Initially, cells are replaced at the peripheral, marginal, and juxtapapillary retina, followed by other retinal sites and finally the retinal parenchyma. From the inner retina, replacement

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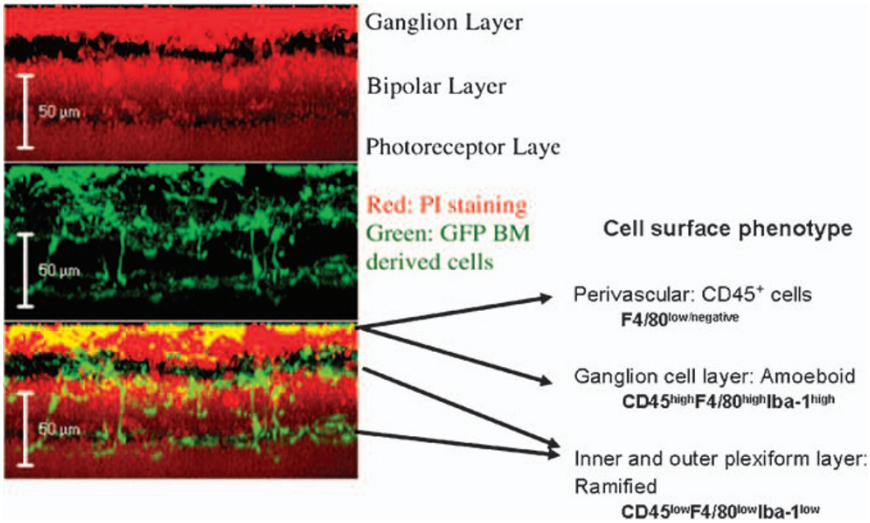

Figure 1 Phenotype of myeloid and microglial populations within mouse retina. Utilising a chimaeric bone marrow EGFP-transgenic mouse model we were able to demonstrate microglial repopulation of the retina.[4] Following reconstitution of bone marrow of normal adult C57BL/6 mice with EGFP bone marrow, we could visualise replacement of retinal microglia over a 6-month period. Further immunohistochemical analysis defined a varying microglial phenotype, as previously described by flow cytometry,[1] dependant upon anatomical residency of microglia. In the mouse, this was particularly true with respect to F4/80 expression, where amoeboid ganglion layer microglia were F4/80^{high} and ramified parenchymal microglia were F4/80^{low}.

spreads outwards to the photoreceptor layer. The change in phenotype observed (see Figure 1), from perivascular F4/80^{-} cells to F4/80^{high} cells in the ganglion cell layer and F4/80^{+} cells in the parenchyma, suggests, at least in mouse, either tissue conditioning, or less likely, preferential homing of pre-programmed sublineage myeloid F4/80^{high} *vs* F4/80^{low} cells into the retina.[6] The implications of this turnover are that although this remains a dynamic active process, the resulting microglial function will depend upon the microenvironment and signals from the retina that they find themselves (see below for myeloid cell activation). Therefore, in humans, even if we replace or, where necessary, increase the number of myeloid cells within the retina, homoeostasis will not be achieved unless cells receive signals akin to the normal 'resting' retina. Thus, a balance is required between a positive, initially beneficial response of microglial/myeloid cell activation to injury, ischaemia, degeneration, or inflammation and signals to return to the homoeostatic activation status to facilitate and maintain normal retinal function.

The interaction of microglia with other retinal cells such as glia has previously highlighted the importance of microglial behaviour/activation status and the subsequent cell–cell (directly or indirectly through soluble mediators) interaction that controls glial cell-mediated growth factor support for photoreceptor survival during degeneration.[7,8] Taking such observations further, growth factors, especially FGF2, and also regulation through neurotrophins (NT3 and cognate p75NTR and TrkC receptors expressed on Muller glia) provide survival signals directly or indirectly (through FGF2 production) not only for neuronal and photoreceptor survival but also for promoting the survival and turnover of neural progenitor cells.[9]

Myeloid cell activation: control of microglia in the retina

Macrophages are fundamental to the innate immune system possessing a rapid ability to adapt to both cognate and soluble receptor signalling (eg, through cytokines and chemokines), which determine their response. Consequential to possessing cell surface and intracellular pattern-recognition receptors (Toll-like receptors (TLR) and NLR),[10] they respond to danger signals from invading pathogens. However, their behavioural plasticity, more than just defence against microbes, is emphasised by their function during development, in wound healing, bridging and activating acquired immunity, and in degeneration.[11,12] Moreover, most tissue possess a stable population of myeloid cells facilitating homoeostasis within that tissue, as seen with retinal microglia.[13]

To understand the function of retinal microglia, briefly reviewing the activation of macrophages is important. Macrophage response and function is dependent on activation status (Figure 2). Besides recognising that *in vivo* macrophage responses display plasticity, a pragmatic classification of effector responses can be

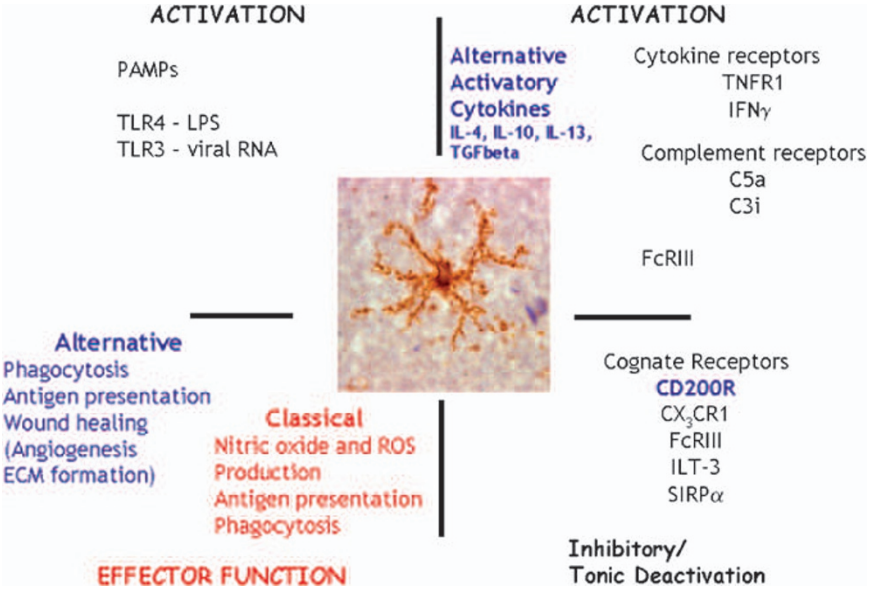

Figure 2 Schematic demonstrating control of myeloid cell activation. Myeloid cells, including microglia, show a high degree of behavioural plasticity and are able to respond rapidly as part of the danger signal generating innate immunity. Their activation is multifactorial, but principal mechanisms include ligation of PAMPs following microbial and viral infection or indirectly through tissue response and cytokines as a result of inflammation, infection, or degeneration. Both mechanisms of activation constitute pathways to mobilise acquired immunity through antigen presentation and dendritic cell activation and together form powerful mechanisms to control danger signals and insults. The effector response, therefore, of monocytes and macrophages will be conditioned by the microenvironment and the signals they receive, be it classical activation (M1 macrophage) or alternative (M2 macrophage). In the CNS and retina, there are mechanisms that refine control of unwanted cell activation through cognate control of macrophage (microglial) activation, in particular, through the inhibitory receptor CD200R while maintaining the ability to rapidly migrate and respond to insults through fractalkine and cognate receptor on microglia (CX_3CR1).

defined[14] as follows: (i) classical activation through TNF-dependent IFNγ or TLR-4-dependent lipopolysaccharide (LPS) stimulation, generating a cytolytic, highly reactive oxygen species- and nitric oxide (NO)-producing cell alongside numerous pro-inflammatory cytokines, including IL-1 and IL-6; (ii) alternative activation through IL-4, IL-10, TGFβ, or IL-13 stimulation, generating phagocytosis and proline for collagen deposition and extracellular matrix deposition; and (iii) cognate deactivation through signalling of cell surface receptors such as CD200Receptor (CD200R). Whether macrophages activate through the classical or any alternate pathway is in part dependent upon regulation of enzymes acting on a single arginine substrate through NO synthase 2 (NOS2) or arginase, respectively.[15] NOS2 or arginase activation in turn generates a polarisation of macrophage function, designated M1 or M2, respectively, that influences particularly whether Th1 or Th2 immune response occurs.

In the retina, microglia do not constitutively express NOS2, and their ramified morphology suggests a resting state because, traditionally, activation was thought to be synonymous with change to an amoeboid morphology. However, recently, elegant dynamic imaging of microglia within tissue explants has shown that microglia are active and dynamic with respect to retraction and extension of their processes and ultimately, and not previously appreciated, migration is evident even when ramified.[16] Notwithstanding current observations, microglia show little other evidences of classical activation in normal retina. Evidence suggests that the retina bestows a cognate deactivation of microglial function. The retina, in both rodents and man, is endowed with a large amount of neuronal expression of CD200,[17] a member of the immunoglobulin superfamily,[18] and microglia, like other myeloid cells, express their cognate inhibitory receptor CD200R.[19] Interaction between neuronal CD200 and myeloid CD200R helps control microglial activation by inhibiting the ability to express NOS2 and generate NO,[20] signalling not through traditional ITIM but through inhibition of MAPkinase activation by mobilising adaptor proteins Dok-1 and Dok-2.[21] Indirectly, the notion that retinal microglia are tonically suppressed *in situ* is also supported by our observation that in human retinal explants exposure to IFNγ or LPS results in a high production of IL-10 rather than NOS2 or NO, although pro-inflammatory cytokines such as IL-6 and IL-1 are generated.[22,23] This does not prevent LPS-induced microglial migration (and not inducing classical

activation), which is both fractalkine (CX_3CR1) and CD200R dependent,[24] and thus still maintains the potential of microglia to migrate in response to injury.

Microglia and macrophages during inflammation and degeneration

There are distinct responses of microglia depending on the initiation and mechanisms of retinal insult. In autoimmune inflammation of an experimental model of autoimmune uveoretinitis (experimental autoimmune uveoretinitis (EAU)), myeloid cell infiltrate occurred in response to T-cell-mediated disease, and during peak inflammation myeloid cells displayed classical activation status (NOS2 expressing and NO generating). Conversely, in this model, the activation status of myeloid cells varied during the course of the disease.[25] During resolution, macrophages were not classically activated but displayed a phenotype of alternative activation (expression of mannose receptor and MHC class II^{high} NOS^{neg}, and generation of β-glucuronidase). Importantly, further studies in mice showed that the extent of GR-$1^+$$CD11b^+$ macrophage infiltration is greatest during peak disease and, together with other myeloid cell infiltration at peak disease (neutrophils), macrophage numbers never return to normal.[26] During resolution and afterwards, there remains an increase in numbers of GR-$1^+$$Ly6G^-$$CD11b^+$ macrophages within the retina. Therefore, in autoinflammatory models, a paradigm of macrophage activation during EAU can be generated. Macrophage activation of infiltrating cells occurs secondary to the T-cell-mediated EAU resulting in a TNF-dependent classical activation (M1) secondary to T-cell production of IFNγ and, finally, tissue destruction,[27] which may be suppressed by either removal of macrophages[28] or inhibition of TNFα production,[29] that has been translated into treatments for uveitis.[30] Inducing alternative (M2) macrophage activation through gene therapy with IL-10 is another viable future therapeutic option.[31] During resolution of EAU there remains increased cell numbers and a balance between macrophage infiltration and T cells, which now demonstrate alternative activation (M2) and a regulatory T-cell phenotype, respectively.[26] Further evidence of the benefit of manipulating macrophage activation is observed on attempting to therapeutically mimic the homoeostatic state through the activation of inhibitory CD200R with agonist monoclonal antibodies (mAbs),[32] redressing the balance and controlling macrophage activation with consequent suppression of retinal inflammatory disease.

However, contrary to the macrophage function observed during T-cell-mediated inflammatory disease, during degeneration, retinal microglia display divergent functions. In RDS mice, which are homozygous for a null mutation in the *Prph2* gene that encodes a structural protein peripherin 2, essential for the formation and maintenance of normal outer photoreceptor segments, there are large increases of microglial numbers during degeneration.[33,34] However, in this model, retinal microglia do not demonstrate classical activation as they remain $NOS2^{neg}$ and without evidence of nitrotyrosine production, but, particularly in subretinal macrophages, they express sialoadhesin. Sialoadhesin (Siglec; a sialic acid-binding protein) has been implicated in a subset of macrophages activated for phagocytosis and not for antigen presentation during inflammation.[35] Although reports have generated disparity as to the extent of sialoadhesin expression, there remains consistency in the presence of sialoadhesin-positive macrophages in the subretinal space following retinal transplantation in these degenerative models.[36] Together, irrespective of whether resident microglia express sialoadhesin, neither microglia nor macrophage infiltrates appear to negatively influence the course of degeneration, although they may influence the fate of transplanted tissue or cells. Removal of macrophages by clodrinated liposome treatment does not alter the course of degeneration in RDS mice.[33]

No matter whether through inflammation or degeneration, microglial and macrophage activation is present. Although we have observed discrepancy in activation status according to the signals the cells receive, understanding what controls microglial/macrophage activation will help in redressing homoeostasis, facilitating tissue repair and cell replacement, and therapeutically increase the chances of cell or tissue transplantation for the treatment of degenerative disease.

Retinal progenitor cells

Although there remains little dispute that in rodents a retinal stem cell population exists in ciliary body/ciliary marginal zone (CMZ)[37] and that retinal progenitors/precursors are numerous during the development of the retina and cell transplantation results in integration and restoration of function,[38] their existence in adult human retina remains controversial. To maintain tissue and cellular homoeostasis and function, cellular replacement is likely to be ongoing yet inconspicuous. Progenitor cells from either developing or adult tissues are potentially dividing and differentiating (in various stages and pathways of differentiation), and many markers are used to positively or negatively select cell populations of interest. Progenitor cells divide very rarely.[39,40] These progenitor cells are thought to differ from stem cells in that their proliferation is most likely asymmetric—that is, whereas some cells retain the properties of the parent

cell and some begin the process of differentiation into mature cells,[40] and more quiescent populations of progenitor cells are described in other sites.[9,41,42] We have noted the expression of nestin (embryonic type VI intermediate filament) in adult human retina.[43] Nestin is expressed during development and is a signature of mitotically active progenitor cells, particularly neuronal, within cells in the subventricular zone of the CNS. The observation of nestin-positive cells in CMZ as well as in radial cells within the retina infers the presence of progenitor cell populations within the retina as well as that of cells capable of directing migration of cells within the tissue towards cell renewal.[44,45] Others have also described from an immortalised Muller glia cell line the potential of cell depots with progenitor cell potential.[46,47] Further, our initial observations demonstrated that cells from epiretinal membranes of surgical specimens expressed nestin.[43] We have isolated a population of nestin-positive $CD133^{+}CD90^{-}$ cells from adult human retina that show, albeit small, a significant capacity to divide and generate neurospheres and differentiate into neurones, glia, and photoreceptors (Carter *et al*, unpublished data). These cells are maintained by LIF (leukaemia inhibitory factor) in a proliferative but undifferentiated state. LIF signals through gp130 (part of IL-6 and associated with LIF receptor), driving progenitor cells to re-enter the cell cycle.[48,49] Adult retinal nestin-positive $CD133^{+}CD90^{-}$ cell subsets and behaviour should not be compared directly with the hallmarks of stem cells, which have a high rate of logarithmic expansion of totipotent cells usually associated with embryonic tissue.

Given our ability to isolate retinal progenitor cells, and ours and others' observations of microglial and macrophage activation during inflammation or degeneration, it is now possible to investigate directly the fate of retinal progenitor cells under the influence of microglial activation.

Microglia influence retinal progenitor cell function

Following our protocol that isolates retinal progenitor cells from adult human retina,[44] we further noted that with exogenous addition of pro-inflammatory cytokine IL-6, the ability to generate neurospheres, a hallmark of cell turnover and differentiation of progenitor cells (see above), is diminished. This effect was reversed when IL-6 was neutralised effectively with a specific anti-IL-6 mAb.

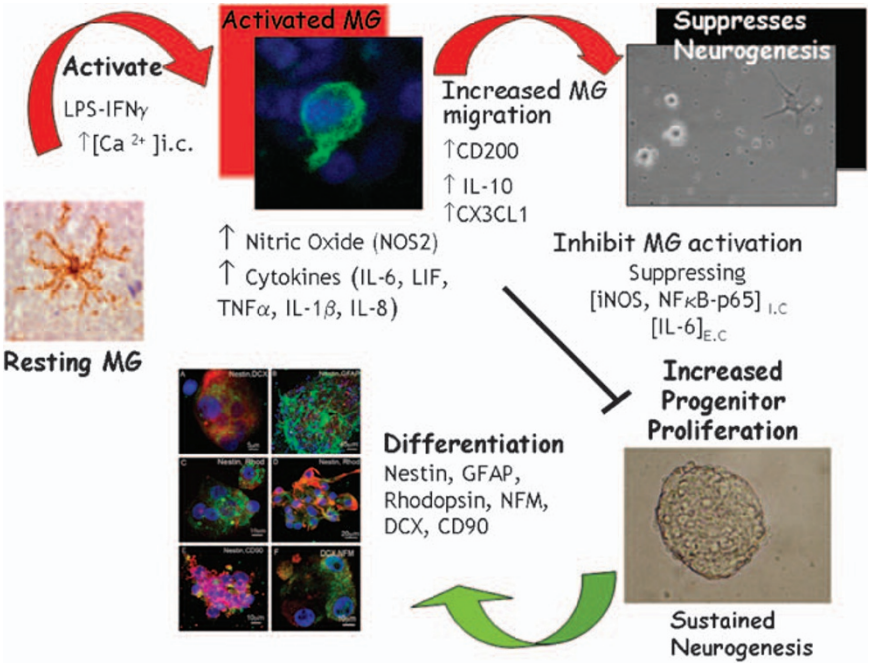

Figure 3 The interaction of retinal neural progenitors and microglial cells. Cell interaction is important in resting retinal tissue and during retinal disease. Both microglia and neural progenitor cells are potential targets for regenerative therapies. Resting microglia become motile activated microglia (increasing their CD200 and CX_3CL1 expression and increasing IL-10 initially, along with a more sustained release of IL-6, IL-1β, TNFα, and IL-8 following stimulation with lipopolysaccharide and interferon-γ, all associated with an increased intracellular calcium). Activated microglia under certain conditions also may increase nitric oxide production and, together with increases in IL-6, inhibit the generation of neurospheres in neural progenitor cultures and thus potentially *in situ* prevent progenitor cells from potential differentiation for self-renewal as demonstrated *in vitro*. (GFAP: glial fibrillary acidic protein; NFM: neurofilament M, DCX: doublecortin). This can be reversed when IL-6 is neutralised, generating proliferation of progenitor cells and differentiation (adapted from Mayer *et al*. *European Ophthalmic Review*).

Therefore, using an experimental paradigm in which adult human retinal microglia are isolated from retinal explants[22–24] and activated through exogenous LPS and IFNγ, we can generate microglial activation, generating IL-6. Under these experimental conditions, microglia displayed a cell surface phenotype of CD45, MHC class II, CD68, CD11b, CD200R, IL-6R, LIF-R, and TLR-4, and the supernatant from activated microglial cultures was able to suppress the ability of retinal progenitor cell to generate neurospheres. In the neurospheres that were formed, the capacity to differentiate was not restricted. Turnover of cells with progenitor behaviour was again restored under these experimental conditions when IL-6 was effectively neutralised within the supernatant with anti-IL-6 mAb (B Balasubramaniam *et al*, unpublished observations). Within the CNS, similar observations are noted: at sites of retinal destruction, microglia play a key role in co-ordinating tissue responses and modulating the immune response, removal of debris by phagocytosis, and tissue reconstruction. Activated MG secrete IL-6 and IL-8, which may influence retinal progenitor cell behaviour.[50] Activated microglial cells may have dual capacity to modulate neurogenesis, either by enhancing the proliferation of progenitor cells or by inhibiting neurosphere generation and, subsequently, the extent of differentiation. The balance between pro- and anti-inflammatory secreted molecules as a direct consequence of microglial or myeloid cell infiltration in disease states influences the behaviour of neural progenitors.[51] Our current preliminary observations and identification of a pro-inflammatory cytokine, IL-6, released from activated MG, with neurosuppressive potential in the adult human retina, and subsequent enhancement of neurosphere generation and maintenance in cultures following IL-6 neutralisation support a co-ordinated role for activated microglia, in turn governed by signals from the retinal microenvironment, in regulating retinal progenitor cells (Figure 3).

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References

1 Dick AD, Ford AL, Forrester JV, Sedgwick JD. Flow cytometric identification of a minority population of MHC class II positive cells in the normal rat retina distinct from CD45lowCD11b/c + CD4low parenchymal microglia. *Br J Ophthalmol* 1995; **79**(9): 834–840.
2 Provis JM, Diaz CM, Penfold PL. Microglia in human retina: a heterogeneous population with distinct ontogenies. *Perspect Dev Neurobiol* 1996; **3**(3): 213–222.
3 Kezic J, McMenamin PG. Differential turnover rates of monocyte-derived cells in varied ocular tissue microenvironments. *J Leukoc Biol* 2008; **84**(3): 721–729.
4 Xu H, Chen M, Mayer EJ, Forrester JV, Dick AD. Turnover of resident retinal microglia in the normal adult mouse. *Glia* 2007; **55**(11): 1189–1198.
5 Marin-Teva JL, Almendros A, Calvente R, Cuadros MA, Navascues J. Tangential migration of ameboid microglia in the developing quail retina: mechanism of migration and migratory behavior. *Glia* 1998; **22**(1): 31–52.
6 Martinez-Pomares L, Platt N, McKnight AJ, da Silva RP, Gordon S. Macrophage membrane molecules: markers of tissue differentiation and heterogeneity. *Immunobiology* 1996; **195**(4–5): 407–416.
7 Harada T, Harada C, Kohsaka S, Wada E, Yoshida K, Ohno S *et al*. Microglia–Muller glia cell interactions control neurotrophic factor production during light-induced retinal degeneration. *J Neurosci* 2002; **22**(21): 9228–9236.
8 Harada T, Harada C, Nakayama N, Okuyama S, Yoshida K, Kohsaka S *et al*. Modification of glial–neuronal cell interactions prevents photoreceptor apoptosis during light-induced retinal degeneration. *Neuron* 2000; **26**(2): 533–541.
9 Giordano F, De Marzo A, Vetrini F, Marigo V. Fibroblast growth factor and epidermal growth factor differently affect differentiation of murine retinal stem cells *in vitro*. *Mol Vis* 2007; **13**: 1842–1850.
10 Leber JH, Crimmins GT, Raghavan S, Meyer-Morse NP, Cox JS, Portnoy DA. Distinct TLR- and NLR-mediated transcriptional responses to an intracellular pathogen. *PLoS Pathog* 2008; **4**(1): e6.
11 Gordon S. Alternative activation of macrophages. *Nat Rev* 2003; **3**(1): 23–35.
12 Taylor PR, Martinez-Pomares L, Stacey M, Lin HH, Brown GD, Gordon S. Macrophage receptors and immune recognition. *Annu Rev Immunol* 2005; **23**: 901–944.
13 Dick AD, Carter DA, Robertson MJ, Broderick C, Hughes E, Forrester JV *et al*. Control of myeloid activity during retinal inflammation. *J Leukoc Biol* 2003; **74**(2): 161–166.
14 Martinez FO, Sica A, Mantovani A, Locati M. Macrophage activation and polarization. *Front Biosci* 2008; **13**: 453–461.
15 Mills CD, Kincaid K, Alt JM, Heilman MJ, Hill AM. M-1/M-2 macrophages and the Th1/Th2 paradigm. *J Immunol* 2000; **164**(12): 6166–6173.
16 Lee JE, Liang KJ, Fariss R, Wong WT. *Ex vivo* dynamic imaging of retinal microglia using time-lapse confocal microscopy. *Invest Ophthalmol Vis Sci* 2008; **49**(9): 4169–4176.
17 Dick AD, Broderick C, Forrester JV, Wright GJ. Distribution of OX2 antigen and OX2 receptor within retina. *Invest Ophthalmol Vis Sci* 2001; **42**(1): 170–176.
18 Barclay AN, Clark MJ, McCaughan GW. Neuronal/lymphoid membrane glycoprotein MRC OX-2 is a member of the immunoglobulin superfamily with a light-chain-like structure. *Biochem Soc Symp* 1986; **51**: 149–157.
19 Wright GJ, Cherwinski H, Foster-Cuevas M, Brooke G, Puklavec MJ, Bigler M *et al*. Characterization of the CD200 receptor family in mice and humans and their interactions with CD200. *J Immunol* 2003; **171**(6): 3034–3046.

20 Broderick C, Hoek RM, Forrester JV, Liversidge J, Sedgwick JD, Dick AD. Constitutive retinal CD200 expression regulates resident microglia and activation state of inflammatory cells during experimental autoimmune uveoretinitis. *Am J Pathol* 2002; **161**(5): 1669–1677.
21 Zhang S, Cherwinski H, Sedgwick JD, Phillips JH. Molecular mechanisms of CD200 inhibition of mast cell activation. *J Immunol* 2004; **173**(11): 6786–6793.
22 Broderick C, Duncan L, Taylor N, Dick AD. IFN-gamma and LPS mediated IL-10-dependent suppression of retinal microglial activation. *Invest Ophthalmol Vis Sci* 2000; **41**: 2613–2622.
23 Carter DA, Dick AD. Lipopolysaccharide/interferon-gamma and not transforming growth factor beta inhibits retinal microglial migration from retinal explant. *Br J Ophthalmol* 2003; **87**(4): 481–487.
24 Carter DA, Dick AD. CD200 maintains microglial potential to migrate in adult human retinal explant model. *Curr Eye Res* 2004; **28**(6): 427–436.
25 Robertson MJ, Erwig LP, Liversidge J, Forrester JV, Rees AJ, Dick AD. Retinal microenvironment controls resident and infiltrating macrophage function during uveoretinitis. *Invest Ophthalmol Vis Sci* 2002; **43**(7): 2250–2257.
26 Kerr EC, Raveney BJE, Copland DA, Dick AD, Nicholson LB. Analysis of retinal cell infiltrate in experimental autoimmune uveoretinitis reveals multiple regulatory cell populations. *J Autoimmun* 2008; **31**(4): 354–361.
27 Dick AD, Forrester JV, Liversidge J, Cope AP. The role of tumour necrosis factor (TNF-alpha) in experimental autoimmune uveoretinitis (EAU). *Prog Retin Eye Res* 2004; **23**(6): 617–637.
28 Forrester JV, Huitinga I, Lumsden L, Dijkstra CD. Marrow-derived activated macrophages are required during the effector phase of experimental autoimmune uveoretinitis in rats. *Curr Eye Res* 1998; **17**(4): 426–437.
29 Dick AD, McMenamin PG, Korner H, Scallon BJ, Ghrayeb J, Forrester JV *et al*. Inhibition of tumor necrosis factor activity minimizes target organ damage in experimental autoimmune uveoretinitis despite quantitatively normal activated T cell traffic to the retina. *Eur J Immunol* 1996; **26**(5): 1018–1025.
30 Murphy CC, Greiner K, Plskova J, Duncan L, Frost A, Isaacs JD *et al*. Neutralizing tumor necrosis factor activity leads to remission in patients with refractory noninfectious posterior uveitis. *Arch Ophthalmol* 2004; **122**(6): 845–851.
31 Broderick CA, Smith AJ, Balaggan KS, Georgiadis A, Buch PK, Trittibach PC *et al*. Local administration of an adeno-associated viral vector expressing IL-10 reduces monocyte infiltration and subsequent photoreceptor damage during experimental autoimmune uveitis. *Mol Ther* 2005; **12**(2): 369–373.
32 Copland DA, Calder CJ, Raveney BJ, Nicholson LB, Phillips J, Cherwinski H *et al*. Monoclonal antibody-mediated CD200 receptor signaling suppresses macrophage activation and tissue damage in experimental autoimmune uveoretinitis. *Am J Pathol* 2007; **171**(2): 580–588.
33 Hughes EH, Schlichtenbrede FC, Murphy CC, Broderick C, van Rooijen N, Ali RR *et al*. Minocycline delays photoreceptor death in the rds mouse through a microglia-independent mechanism. *Exp Eye Res* 2004; **78**(6): 1077–1084.
34 Hughes EH, Schlichtenbrede FC, Murphy CC, Sarra GM, Luthert PJ, Ali RR *et al*. Generation of activated sialoadhesin-positive microglia during retinal degeneration. *Invest Ophthalmol Vis Sci* 2003; **44**(5): 2229–2234.
35 Jiang HR, Hwenda L, Makinen K, Oetke C, Crocker PR, Forrester JV. Sialoadhesin promotes the inflammatory response in experimental autoimmune uveoretinitis. *J Immunol* 2006; **177**(4): 2258–2264.
36 Sancho-Pelluz J, Wunderlich KA, Rauch U, Romero FJ, van Veen T, Limb GA *et al*. Sialoadhesin expression in intact degenerating retinas and following transplantation. *Invest Ophthalmol Vis Sci* 2008; **49**(12): 5602–5610.
37 Tropepe V, Coles BL, Chiasson BJ, Horsford DJ, Elia AJ, McInnes RR *et al*. Retinal stem cells in the adult mammalian eye. *Science* 2000; **287**(5460): 2032–2036.
38 MacLaren RE, Pearson RA, MacNeil A, Douglas RH, Salt TE, Akimoto M *et al*. Retinal repair by transplantation of photoreceptor precursors. *Nature* 2006; **444**(7116): 203–207.
39 Morshead CM, Reynolds BA, Craig CG, McBurney MW, Staines WA, Morassutti D *et al*. Neural stem cells in the adult mammalian forebrain: a relatively quiescent subpopulation of subependymal cells. *Neuron* 1994; **13**(5): 1071–1082.
40 Sell S. Stem cell origin of cancer and differentiation therapy. *Crit Rev Oncol Hematol* 2004; **51**(1): 1–28.
41 Coskun V, Wu H, Blanchi B, Tsao S, Kim K, Zhao J *et al*. CD133+ neural stem cells in the ependyma of mammalian postnatal forebrain. *Proc Natl Acad Sci USA* 2008; **105**(3): 1026–1031.
42 Nickerson PE, Emsley JG, Myers T, Clarke DB. Proliferation and expression of progenitor and mature retinal phenotypes in the adult mammalian ciliary body after retinal ganglion cell injury. *Invest Ophthalmol Vis Sci* 2007; **48**(11): 5266–5275.
43 Mayer EM, Hughes EH, Carter DA, Dick AD. Nestin positive cells in adult human retina and in epiretinal membranes. *Br J Ophthalmol* 2003; **87**: 1154–1158.
44 Carter DA, Mayer EJ, Dick AD. The effect of postmortem time, donor age and sex on the generation of neurospheres from adult human retina. *Br J Ophthalmol* 2007; **91**(9): 1216–1218.
45 Mayer EJ, Carter DA, Ren Y, Hughes EH, Rice CM, Halfpenny CA *et al*. Neural progenitor cells from postmortem adult human retina. *Br J Ophthalmol* 2005; **89**(1): 102–106.
46 Lawrence JM, Singhal S, Bhatia B, Keegan DJ, Reh TA, Luthert PJ *et al*. MIO-M1 cells and similar muller glial cell lines derived from adult human retina exhibit neural stem cell characteristics. *Stem Cells* 2007; **25**(8): 2033–2043.
47 Limb GA, Salt TE, Munro PM, Moss SE, Khaw PT. *In vitro* characterization of a spontaneously immortalized human Muller cell line (MIO-M1). *Invest Ophthalmol Vis Sci* 2002; **43**(3): 864–869.
48 Davey RE, Onishi K, Mahdavi A, Zandstra PW. LIF-mediated control of embryonic stem cell self-renewal emerges due to an autoregulatory loop. *FASEB J* 2007; **21**(9): 2020–2032.
49 Smith DK, Treutlein HR. LIF receptor-gp130 interaction investigated by homology modeling: implications for LIF binding. *Protein Sci* 1998; **7**(4): 886–896.
50 Aarum J, Sandberg K, Haeberlein SL, Persson MA. Migration and differentiation of neural precursor cells can be directed by microglia. *Proc Natl Acad Sci USA* 2003; **100**(26): 15983–15988.
51 Battista D, Ferrari CC, Gage FH, Pitossi FJ. Neurogenic niche modulation by activated microglia: transforming growth factor beta increases neurogenesis in the adult dentate gyrus. *Euro J Neurosci* 2006; **23**(1): 83–93.