

Tweaking the immune system as an adjuvant for the treatment of retinal degenerations

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Abstract: Blinding diseases such as photoreceptor degenerations are debilitating conditions that severely impair daily lives of affected patients. This group of diseases are amenable to photoreceptor replacement therapies and recent transplantation studies provided proof-of-principle for functional recovery at the retinal and behavioral level, though the actual mechanism of repair still needs further investigations. The immune system responds in several ways upon photoreceptor engraftment, resulting in T-cell and macrophage infiltrations and, consequently, decrease in graft survival. Most studies on the role of the immune system suggest a detrimental effect in a therapeutic setting. Conversely, the opposite idea wherein the immune system can be activated towards a protective state was also explored in other experimental paradigms. Here, Neves and colleagues explored the potential of cross-species studies and, to a certain extent, the concept of a protective immune system in retinal degeneration and therapy. Mesencephalic astrocyte-derived neurotrophic factor (MANF) was identified in this study as a novel factor that, by modulating the immune system, can slow down photoreceptor degeneration and improve transplantation outcome.

Keywords: Immune modulation; retina; retinal degeneration; photoreceptor; transplantation; retinal repair

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The mammalian retina has a very limited regenerative capacity (1) and degeneration of the main light-sensing cells—rod and cone photoreceptors—results in permanent damage and consequently vision loss as observed in diseases such as retinitis pigmentosa (2). Such debilitating condition brings a significant burden to society and economy, thus, it is important to develop strategies that allow the repair of the degenerated tissues. Gene therapy (3), retinal prosthesis (4) and cell replacement approaches (5) are promising strategies to treat blinding diseases and significant resources are being allocated to these fields. On the other hand, few studies explored the endogenous regenerative capacity of the mammalian retina (6).

Multiple studies showed a role of the immune system in promoting endogenous repair in other tissues and disease paradigms addressing the interaction between immune system and endogenous regenerative potential (7-10). Neves and colleagues adopted a novel approach to study the role of the immune system in the regenerative capacity of the mammalian retina. Two model organisms were used and their best features explored for the purpose of the study: *Drosophila* and its power for genetic studies, and the mouse that closely mimics the degenerative phenotypes present in human diseases (11).

During retinal neurodegenerative processes such as retinitis pigmentosa or age-related macular degeneration,

microglia is activated and a pro-inflammatory environment is created. Upon CNS and thus also retina injury microglia within the tissue can acquire a M1 or M2 state and, depending on such states, the affected tissue enters a degenerative process and consequently cell death or shows signs for the onset of a repair mechanisms. This M1/M2 dichotomy was initially addressed by Neves *et al.* using *Drosophila* as a model organism. In *Drosophila*, hemocytes (cells with macrophage-like activities) respond to tissue injury and coordinate a cellular response and the resolution of the inflammatory process. In their study retinal damage was caused by exposure to UV light that resulted in the degeneration of the *Drosophila*'s photoreceptors. Upon damage, photoreceptors secrete PDGF- and VEGF-related factor 1 (Pvf-1), which in turn activates hemocytes for tissue repair, by binding to the Pvr receptor (PvR). RNA sequencing analysis on isolated hemocytes during UV-mediated photoreceptor damage, revealed a significant upregulation of several factors including mesencephalic astrocyte-derived neurotrophic factor (MANF), which was further investigated. In the absence of MANF, the UV treated *Drosophila* eye showed a significant tissue loss which could be rescued by overexpressing MANF in hemocytes. Despite MANF's neuroprotective role, its mechanism was further dissected by identifying its effect on the morphology and marker expression of hemocytes. Hemocytes treated with human recombinant MANF in culture or overexpressing MANF *in vivo* resulted in an increased proportion of lamellocytes and increased positivity of the *Drosophila* homologue of the mammalian M2 marker arginase 1 (*arg1*). These changes in morphology could be correlated to the mammalian M1/M2 phenotype observed in macrophages and microglia. Besides histological evidence for the presence of a M2-like phenotype in hemocytes upon injury, future studies may be helpful to confirm the M2 phenotype in more detail. In that regard, a comparison at the transcriptional level between the M2-like phenotype and true M2 and M1-microglia would help to dissect downstream elements of MANF. The immune modulatory potential of MANF in retinal repair was then determined by manipulating the amount of secreted and membrane-bound MANF. To achieve this, Neves and colleagues overexpressed MANF in the absence of the *Drosophila* KDEI receptor homologue which resulted in a decreased number of lamellocytes formed and *arg1* expressed. Phenotypically, tissue loss was significantly higher in these conditions, showing that MANF expression is critical for tissue repair.

Interestingly, the mammalian retina displays a similar response to light injury (transient damage when exposed to 1,000 lux of light for 1.5 hours in C57BL/6 mice) with an upregulation of VEGF and PDGF-family proteins followed by increased MANF mRNA levels in dendritic cells (CD11b⁺). Curiously, crosstalk between activated microglia and Müller glia cells occurred upon damage resulting in Müller glia cells positive for MANF. The interplay between these two cell types has not been explored and might lead to novel insights in late stages of retinal degeneration. In accordance to other retinal degenerations, microglia became activated and migrated to the outer nuclear layer where degenerating cells are located. Conservation among species of the response towards retinal injury was further validated by inhibiting both PDGF signaling pathways leading to an increased number of CD11b⁺ cells in the vitreous and photoreceptor cell death.

The use of C57BL/6J mice for the retinal damage paradigm allows the retina to recover to a certain extent as shown by Neves and colleagues by transiently increasing the number of CD11b⁺ cells in the retina. Moreover, MANF's broad neuroprotective effect was further validated in three more severe models of retinal degeneration: severe light damage using BALB/cJ, the *Crx*^{trm65} and *Pde6b*^{rd1} mice. In contrast to C57BL/6 mice, BALB/cJ mice do not harbor a protective variant of the RPE65 gene which, in turn, makes an optimal model of induced retinal degeneration. A single vitreal injection of human recombinant MANF protein resulted in a significant reduction in the level of cell death in the three paradigms of retinal degeneration. The extent of this neuroprotective effect was confirmed by human fibroblasts constitutively expressing MANF-GFP fusion protein that were transplanted into the vitreous of *Crx*^{trm65} mice. Two weeks after transplantation, MANF's neuroprotective effect was still observed in this degeneration model by a significantly higher number of remaining photoreceptor rows.

The neuroprotective effect of MANF was convincingly shown in photoreceptor degenerations in general, however it would be of interest to determine whether MANF effects can specifically target also cones as in cone dystrophies. Additionally, photoreceptors are not the only cells that degenerate in retinopathies. Retinal pigment epithelium cells are one of the cell types affected in multiple retinal degenerations, such as sub-types of Retinitis Pigmentosa (2) and age-related macular degeneration (12). Determining MANF effects in RPE degenerations as it is

observed in *Ccl2/Cx3Cr1^{-/-}* mice (13) would further support the authors hypothesis for a broad spectrum application of MANF as a neuroprotective agent. Complementary to the latter, it would be of great interest to determine whether this mechanism is conserved in human retinas of patients suffering from AMD where the immune system is unbalanced. Such questions may be addressed in future studies.

A closer look into the CD11b⁺ cells (dendritic cells) in the vitreous revealed a round morphology, which reflects an alternative activation and MANF's immunomodulatory effect. Upon vitreal delivery of MANF, the recruited innate immune cells were composed of monocytes and monocyte-derived macrophages (~60% to 80% of recruited cells). Neutrophils positive for Ly6-G⁺ (Gr1^{high}) accounted for 15% and both neutrophils and macrophages expressed MANF and *arg1* suggesting an anti-inflammatory response by the immune system. Besides MANF's paracrine effect, it can partially regulate macrophages in an autocrine manner reflecting its immunomodulatory effect. To validate the dependence of MANF effects on the immune system, light-induced photoreceptor degeneration mouse models with impaired immune system, i.e., lacking CD11b⁺ cells or the *Cx3Cr1^{-/-}* mouse were used. The absence of *Cx3Cr1* protein leads to retinal degeneration and increased pro-inflammatory activation of immune cells. If MANF would be the sole effector of this immunomodulatory effect, administration of MANF would result in decreased levels of cell death. Interestingly, the authors observed the opposite effect in both mouse models: cell death remained constant. The expression levels of MANF in the *Cx3Cr1* knockout mouse remained constant, which might be due to the contribution of cell types other than macrophages for the protective effect of MANF depended immunomodulation. Deeper understanding of this complex mechanism would provide novel insight in how the immune system can be modulated to support regenerative responses that may allow broad application in several neurodegenerative diseases.

Such use of MANF was further extended to a photoreceptor replacement approach. Following transplantation of *Nrl-eGFP* rods into wild-type mice, microglia positive for MANF localized to the site of integrated photoreceptors suggesting that they might play a role in the transplantation outcome. Indeed, supplementation of MANF to rod transplantation significantly improved the transplantation success, which was determined by the number of GFP⁺ photoreceptors within the host ONL and interpreted as increased

numbers of structurally integrated donor photoreceptors. However, recently four studies unveiled that following photoreceptor transplantation donor cells mainly exchange cytoplasmic material with host photoreceptors while structural integration occurs to a minor extend (14-17). Thus, it is currently unknown whether the transplantation improvement promoted by MANF as observed by Neves and colleagues is caused by an increased donor cell integration or donor-host cytoplasmic exchange. Identifying the mechanism by which MANF supplementation promotes transplanted photoreceptors will be an important prerequisite to further develop the use of MANF for cell-based therapeutic interventions in the retina.

Aside of which mechanism is enhanced by MANF supplementation—structural integration or cytoplasmic exchange—the authors took advantage of previous data on donor photoreceptor age and its relationship to the transplantation outcome. MacLaren *et al.* [2006] followed by Bartsch *et al.* [2008] showed that young post-mitotic photoreceptors between postnatal day (P) 4–7 allow improved transplantation success when compared to their young or older counterparts (18,19). Hence, Neves *et al.*, assessed the effect of MANF immunomodulation on photoreceptor transplantation using older, i.e., mature, *Nrl-eGFP* photoreceptors (p21) and compared the transplantation outcome to the “standard” age of donor cells—this is P4–7. In fact, transplanted P21 rods showed improved transplantation outcomes when supplemented with MANF when compared to controls—P21 rods without MANF—but it still had not a similar outcome as transplanted P7 rods. However, faster functional recovery (assessed by electroretinogram) was observed when MANF was supplemented to transplanted P7 rods when compared to control conditions.

In conclusion, Neves and colleagues' study identified a key molecule involved in the immune response to retinal repair—MANF—and dissected its immunomodulatory and neuroprotective effects in different retinal disease paradigms underlining the view of a protective role of the immune system in retinal regeneration.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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