Editorial

The interplay between inflammation, immunity and commensal microflora in glaucomatous neurodegeneration

Christin Henein[1,2], Peng T. Khaw[1]

[1]National Institute for Health Research Biomedical Research Centre for Ophthalmology, Moorfields Eye Hospital and UCL Institute of Ophthalmology, London, UK; [2]School of Pharmacy, University College London, London, UK

Correspondence to: Peng T. Khaw. National Institute for Health Research Biomedical Research Centre for Ophthalmology, Moorfields Eye Hospital and UCL Institute of Ophthalmology, London, UK. Email: p.khaw@ucl.ac.uk.

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The immensely diverse and rich human gut microbiome has a huge potential to impact health and disease (1). Gut microbiome dysbiosis has been associated with gastro-intestinal inflammatory conditions and extra-intestinal disorders; such as stroke, depression, neurodegenerative, cardiovascular and metabolic disorders (2-6). In the September 2018 issue of nature communications, Chen and colleagues report the findings from a study that found commensal microflora-induced T cell responses mediate progressive neurodegeneration in glaucoma, in mouse glaucoma models (7).

This investigation sheds some light on intersecting mechanistic pathways in glaucomatous neurodegeneration. Progressive degeneration of retinal ganglion cells (RGCs) resulting in optic nerve head cupping and visual loss are characteristic features of glaucoma. The pathophysiological causes of this degeneration are not clearly defined but most certainly involves multifactorial and polygenic mechanisms. Large genome-wide association studies suggest a role of angiopoietin-receptor tyrosine kinase signalling, lipid metabolism, mitochondrial function, and developmental processes as underlying risks for elevated intraocular pressure (IOP) and primary open angle glaucoma (POAG) (8). Although age and raised IOP remain the most prominent risk factors for glaucoma, disease phenotypes of normal tension glaucoma suggest mechanisms beyond pressure-mediated neurodegeneration.

Elevated IOP can cause retinal ganglion degeneration via various pathways such as mechanical stress, metabolic stress, and immune response. Firstly, mechanical stress on the lamina cribosa and surrounding tissues damages retinal ganglion axons and disrupts axonal transport of trophic factors (9). Secondly, mitochondrial dysfunction in RGCs and astrocytes due to ageing may not meet metabolic demands in IOP-induced metabolic stress (10). Thirdly, elevated IOP can also trigger an immune response. It is this autoimmune response to commensal microflora that is thought to mediate neurodegeneration through the production of activated retinal T cells. Chen *et al.* showed elevated IOP induces two phases of retinal damage; the acute phase likely mediated by mechanical stress; the prolonged retinal degeneration, which continues after IOP has returned to normal, mediated by T cells (7).

There is an established body of evidence for linking gut microflora to some brain diseases. Gut dysbiosis and reduced diversity of gut microflora may be a factor in the development of neurodegeneration via neuroinflammation mechanisms in Alzheimer's disease, Parkinson's disease, multiple sclerosis and amyotrophic lateral sclerosis (ALS) (6). Although these diseases have distinct clinical manifestations, they are all associated with normal ageing. With ageing, the diversity of gut microflora reduces impacting a complex interlocking of hormonal and biochemical pathways linking gut health to the brain (11). There is a lack of evidence showing the influence of commensal microflora on ophthalmic diseases. Although it is known commensal ocular microbiome and infectious factor have a role in the development of ocular adnexal

malignancies (12,13); there is limited evidence linking commensal gut microflora and glaucoma progression.

In summary Chen *et al.* showed (I) elevation of IOP induces retinal T-cell infiltration, (II) T cells mediate prolonged retinal neurodegeneration, (III) IOP elevation activates T-cell responses to heat shock proteins (HSP), (IV) HSP-specific T cells augment glaucomatous neurodegeneration, (V) absence of glaucomatous neural damage in GF mice and (VI) HSP-specific T cells increased in patients with glaucoma.

Chen *et al.* showed short (3 weeks) elevation of IOP using microbead injection B6 mouse model and progressive elevation of IOP using genetic (DBA/2J) mice model, induces infiltration of IFN-γ-secreting T cells resulting in the reduction of RGC density and axonal loss (7). For retinal T cell infiltration to occur local inflammation is required. More specifically, T cells $CD4^+$ T cells mediate the progressive phase of neurodegeneration after the initial induction of elevated IOP with microbeads in immunocompromised mice models. T cell and B cell deficient mice ($Rag1^{-/-}$) did not exhibit progressive loss of RGCs or axons from 2 to 8 weeks post microbead injection when IOP returned to normal levels. CD4+ T cells were transferred from B6 mice into $Rag1^{-/-}$ mice via the tail vein to assess if $CD4^+$ T cells had a causal role in the progression of the glaucomatous neurodegeneration. $Rag1^{-/-}$ mice which received T cells from glaucomatous B6 mice showed a T cell infiltration and a significant further decrease in RGC density and axon numbers (7).

Elevated IOP induced IFN-γ-secreting T cells were found to be specific for heat shock proteins (HSP). T cells from GFP transgenic mice immunised with HSP-27 were transferred to immunocompromised glaucomatous $Rag1^{-/-}$ mice to confirm retinal infiltration occurs. When compared to controls, recipient $Rag1^{-/-}$ mice had an approximately 15-fold increase in GFP^+ retinas $^{-/-}T$ cells, following T cell transfer from HSP-27 immunised mice. Furthermore, HSP-27 specific T cells induced progressive glaucomatous damage. Recipient B6 mice that received $CD4^+$ T cells from HSP-27 immunised mice exhibited a significantly higher loss of RGCs and axons compared to controls. Induction of HSP specific T cells and glaucomatous neurodegeneration by elevated IOP require exposure to commensal microflora. Elevated IOP induced retinal T cell infiltration, HSP-27 and HSP-60 specific T cell responses were detected in normally colonised mice but not detected in germ-free mice (7).

HSPs have a cellular protective effect and are upregulated in stressful conditions and can promote recovery from stress. However, HSPs are also highly antigenic, and immune responses to HSPs could contribute to glaucoma progression (14). Intracellular HSP-27 is highly inducible in the retinal glial cells and acts in an anti-apoptotic manner. HSP-27 antibodies result in RGC apoptosis by destabilising actin cytoskeleton (15,16). Chen *et al.* found the frequency of HSP-27 and HSP-60 specific T cells and antibodies were higher in both POAG and normal tension glaucoma (NTG) patients when compared to age-matched healthy individuals (7). The number of peripheral blood samples included in the study was limited so there remains uncertainty regarding the estimate effect size and direction.

Chen *et al.* study (7) should be commended for its methodological robustness in demonstrating intersecting pathways in glaucomatous neurodegeneration in a mouse model. Understanding these mechanisms will help identify upstream factors to exploit in the prevention or treatment of glaucoma. Elevated IOP is unlikely to be the only trigger for glaucomatous neurodegeneration. Metabolic stress due to age-related mitochondrial dysfunction and the subsequent accumulation of reactive oxygen species in neurons can stimulate cytokine release and microglial activation and neuroinflammation (17). RGCs have a high energy demand making them sensitive to oxidative damage. In the context of ageing, this can present as a chronic low-grade pro-inflammatory response. Other stress conditions could also lead to retinal T cell infiltration and immune-mediated neural damage (18).

A likely explanation of the gut-eye axis is the proximity of immune cells along the gastrointestinal tract and dysfunction of the blood-retinal barrier when under stressed conditions. Antigen-presenting innate immune cells embedded in the subepithelial lamina propria tissue of the digestive tract are close to the gut microflora (19). Toll-like and NOD-like receptors on these cells recognise the pathogen-associated molecular patterns, which trigger signalling cascades leading to pro- or anti-inflammatory cytokine expression (20). The blood-retinal barrier can be compromised by stressed conditions such as elevated IOP but also by other factors. Elevated IOP causes local inflammation in the retina and optic nerve inducing T cell infiltration. T cells specific to bacterial HSP can be activated by host HSPs through molecular mimicry under stress conditions such as elevated IOP, leading to immune-mediated neural damage (7). There are many homologies between bacteria and human proteins making HSP unlikely to be the only cross-reacting antigen for retinal T cells.

Elucidating culpable antigens in the gut-eye axis in humans with glaucoma is complex and cannot be underestimated.

Other research groups have identified links between glaucoma and microflora in the oral cavity and gut. Oral bacterial loads (16S rRNA) of patients with glaucoma were found to be higher than healthy controls in a study population that was predominantly African American (21). This study did not use age-matched controls. Low dose subcutaneous bacterial lipopolysaccharide (LPS) was injected into two glaucoma mouse models (spontaneous and induced) to simulate the conditions expected in chronic subclinical bacterial infections. LPS administration resulted in axonal degeneration via microglial activation at the optic nerve and retina, upregulation of toll-like receptor 4 (TLR4) signalling and complement cascade (21).

Studies have shown a link between *Helicobacter pylori* and glaucoma measuring increased titres of antibodies against *Helicobacter pylori* in POAG patients (22-25). When taken together these studies suggest a common factor such as gut dysbiosis that causes susceptibilities to both POAG and *H.pylori* infection. Gut microflora plays a role in the activation of microglial activation in the central nervous system (CNS). Host microflora by-products were found to regulate microglial homeostasis in terms of maturation, differentiation, and function (26). The exact mechanism by which human host microflora may activate the innate or adaptive immunity of retina or optic nerve is not yet well understood.

A variety of host, genetic, environmental and behavioural factors, as well as various pathological states can alter host gut microflora (27). Thus, making microbiome research in humans challenging and difficult to control. Advances in next-generation sequencing and bioinformatics will undoubtedly help generate a greater understanding of the composition and function of gut microflora and its influence on the pathogenesis of glaucoma. More research is needed to determine transcriptional activity, protein expression and metabolic by-products profiles of host microflora to identify potential biomarkers of gut dysbiosis that lead to glaucoma progression. More evidence using from gnotobiotic animal models and functional observational studies are required before an investment into large scale longitudinal clinical trials (28). Human longitudinal studies starting in the earlier disease phases would be preferential to understand the causative relation between microflora and glaucomatous neurodegeneration and to develop treatments aimed at halting the progression of optic nerve damage.

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