CHAPTER

4

The role of commensal microflora-induced T cell responses in glaucoma neurodegeneration

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Abstract

Over the last decade, new evidence has become increasingly more compelling that commensal microflora profoundly influences the maturation and function of resident immune cells in host physiology. The concept of gut-retina axis is actively being explored. Studies have revealed a critical role of commensal microbes linked with neuronal stress, immune responses, and neurodegeneration in the retina. Microbial dysbiosis changes the blood-retina barrier permeability and modulates T cell-mediated autoimmunity to contribute to the pathogenesis of retinal diseases, such as glaucoma. Heat shock proteins (HSPs), which are evolutionarily conserved, are thought to function both as neuroprotectant and pathogenic antigens of T cells contributing to cell protection and tissue damage, respectively. Activated microglia recruit and interact with T cells during this process. Glaucoma, characterized by the progressive loss of retinal ganglion cells, is the leading cause of irreversible blindness. With nearly 70 million people suffering glaucoma worldwide, which doubles the number of patients with Alzheimer's disease, it represents the most frequent neurodegenerative disease of the central nervous system (CNS). Thus, understanding the mechanism of neurodegeneration in glaucoma and its association with the function of commensal microflora may help unveil the secrets of many neurodegenerative disorders in the CNS and develop novel therapeutic interventions.

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Keywords

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1 Introduction

Glaucoma is an age-related multifactorial neurodegenerative disease, characterized by progressive loss of retinal ganglion cells (RGCs) and irreversible visual deficiency. It is predicted that more than 100 million people worldwide will be affected by glaucoma by 2040 (Tham et al., 2014). Elevated intraocular pressure (IOP) is deemed to be the most important risk factor in glaucoma pathology. High IOP triggers microglia activation, immune responses, neurotrophic deprivation, oxidative damage, and mitochondrial dysfunction that eventually lead to RGC degeneration (Gallego et al., 2012; Vu et al., 2012). A currently unresolved issue in glaucoma clinics is that RGC and optic nerve degeneration continues to progress even after the IOP is effectively controlled (Mckinnon et al., 2008).

Recent studies in experimental models of glaucoma revealed that RGCs undergo a prolonged phase of degeneration in glaucoma after IOP returns to a normal level as a result of T cell infiltration and activation; whereas, mice raised in the absence of microflora (germ-free) do not develop neural damage under persistent high IOP (Chen et al., 2018). Heat shock proteins (HSPs) were identified as potential immune-stimulating signals in patients and animal models of glaucoma (Luo et al., 2010). Studies of HSPs, which are highly conserved from bacteria to humans, in systematic autoimmune diseases have demonstrated their ability to serve as pathogenic autoantigens (Barberá et al., 2013; Shoda et al., 2016; Van Eden et al., 2019). In this chapter, we comprehensively summarize the evidence reporting the links between commensal microflora and immune responses in glaucomatous neurodegeneration and further explore the possibility of preventing neuron loss through manipulating the gut-retina axis.

2 Microbiota in neuroinflammation

2.1 Commensal microbiota and immune regulation

There are approximately trillions of microbes in the human gut. Evidence has emerged that commensal microbiota plays a fundamental role in the induction, training and function of the host immune system (Dopkins et al., 2018; Fung et al., 2017). The immune system, vice versa, also contributes to shape and maintain the ecology of the gut microbiota as the microbiota promotes and serves all aspects of the immune system (Arrieta et al., 2014; Belkaid and Harrison, 2017). These commensals benefit the host by taking in excessive and indigestible polysaccharides and fibers and producing short-chain fatty acid and vitamins (Rowland et al., 2018).

Changes in the composition of microbiota are proved to be associated with various autoimmunity and metabolic diseases, including bowel cardiovascular diseases and many others (Clemente et al., 2012).

To determine the effect of microbiota on distant organs, strategies like germ-free mice and rats have been developed as tools for research. Increasing evidence suggests that not only intestinal diseases, but also diseases in tissues distant from the gut, can be impacted by gut commensals (Horai and Caspi, 2019). Alterations in brain physiology and leakage of the blood-brain barrier (BBB) were detected in germ-free mice (Braniste et al., 2014; Erny et al., 2015). It was reported that increased bacterial products including the endotoxin lipopolysaccharides (LPS) and pathogen-associated molecular pattern molecules (PAMP) are produced due to the increased intestinal permeability. Chronic inflammation is thus induced in several tissues through the activation of pattern recognition receptors (PRRs). Although anatomical separation exists, it was shown that the above biological crosstalk occurs in the brain and retina (Ma et al., 2019).

2.2 Microbiota and the autoimmune diseases in the CNS

Gut microbiota has been shown to modulate the development, maintenance and homeostasis of the central nervous system (CNS), including the retina (Tremlett et al., 2017). Germ-free mice and broad spectrum-antibiotic-treated mice exhibited a range of cognitive and neural developmental deficits in the absence of gut microbiota (Mayer et al., 2015). It is so far acknowledged that neurodegenerative diseases, like Alzheimer's disease (AD), Parkinson's disease (PD) and Huntington's disease (HD), are closely associated with alterations in the gut microbiome through the gut-brain axis (Dinan and Cryan, 2017; Ghaisas et al., 2016). Tryptophan metabolites such as serotonin and kynurenine have important functions in both the brain and the gut, which indirectly supports a role of gut microbiota in the brain (O'mahony et al., 2015). A recent paper reported significant deficits in fear extinction learning in antibiotic-treated or germ-free adult mice, revealing significant alterations in gene expression in excitatory neurons and glial cells (Chu et al., 2019). Single-cell RNA sequencing of the brain indicated that the compositions of microbiota not only affect many physiological processes, including development, metabolism and immune cell functions, but also modulate behaviors, such as social activity, stress and anxiety-related responses that are linked to diverse neuropsychiatric disorders (Chu et al., 2019). These results suggest that the disturbance of brain-gut-microbiota axis could result in the pathogenesis and the pathology of neurodegenerative disorders.

Pathological mechanisms like impaired blood-brain barrier function, increased inflammation, and vascular dysfunction, such as that found in AD patients, are commonly shared in retinal neurodegenerative diseases, including age-related macular degeneration (AMD), diabetic retinopathy and glaucoma (Gupta, 2015; Kaarniranta et al., 2011). Gut microbiota metabolites and products may modulate retina-specific immune process and the related cells. Morita et al. reported that intake of a lactic acid bacteria mitigated age-related immune defects by reducing interferon-gamma (IFN-γ)

producing inflammatory CD4-positive T cells in the small intestine and decreasing serum levels of pro-inflammatory cytokines. Interestingly, it also suppressed retinal inflammation by reducing macrophage infiltration thus attenuating RGC loss (Morita et al., 2018). Recently, an obesity-associated gut-microbiota has been shown to drive pathological angiogenesis toward abnormal choroidal neovascularization (CNV) in the retina (Rinninella et al., 2018). Gut commensals were reported to signal directly through the retina-specific T cell receptor to induce autoreactive T cells and trigger uveitis. In contrast, depletion of gut microbiota in animal models of uveitis tempered disease progression (Horai and Caspi, 2019). In patients with primary open angle glaucoma (POAG), increased titers of antibodies against *Helicobacter pylori* was detected, supporting an association of *Helicobacter pylori* to the pathogenesis of glaucoma (Chen et al., 2015; Izzotti et al., 2009). Distinct differences in the compositions of gut microbiota and serum metabolic phenotype between POAG patients and healthy individuals have also been reported, suggesting the potential correlation between gut microbiota and glaucoma (Gong et al., 2020). Together, the evidence supports the existence of a "gut-retina axis" which plays a role in the development and/or progression of chronic ocular neurodegenerative disorders, such as glaucoma (Fig. 1).

2.3 Microbiota and the blood-retina barrier

The CNS is normally shielded behind the blood-brain barrier (BBB) from direct interaction with circulating immune cells, just as the eye is protected by blood-ocular barrier. The blood-ocular barrier is composed of two main barriers: the blood-aqueous barrier and the blood-retinal barrier (BRB), and it is of great importance for the maintenance of visual function (Cunha-Vaz, 1979). The BRB contains the inner and outer compartments; the inner compartment is formed by the tight junctions between retinal capillary endothelial cells, and the outer compartment by the tight junctions between retinal pigment epithelial cells (Cunha-Vaz and Maurice, 1967). The BRB is particularly critical in keeping the eye as an immune privileged site of the body and essential for the homeostatic microenvironment of the retina. Microbial imbalance can result in disruptions of BRB that subsequently can enable the translocation of microflora pathogens and promote the development and progression of ocular disease (Fig. 1). Breakdown of the BRB no longer protects the retina from peripheral leukocytes and induces microglia activation, which release free radicals and inflammatory cytokines. T helper cells, such as Th1 and Th17, may thus be recruited and produce the pro-inflammatory cytokines IFN-γ, TNF-α and IL-17, to participate in chronic neuroinflammation, which may further damage the BRB (Dopkins et al., 2018; Gerber and Nau, 2010). Alterations of the BRB are critically involved in the pathological progression of retinopathy and retinal neurodegeneration.

2.4 Microbiota and microglia development, maturation and function

An important role of microbiota was shown to mediate microglia development and maturation (Tse, 2017). Microglia are originated from the yolk sac and migrate to the brain and retina before the formation of BBB or BRB, and their maturation is

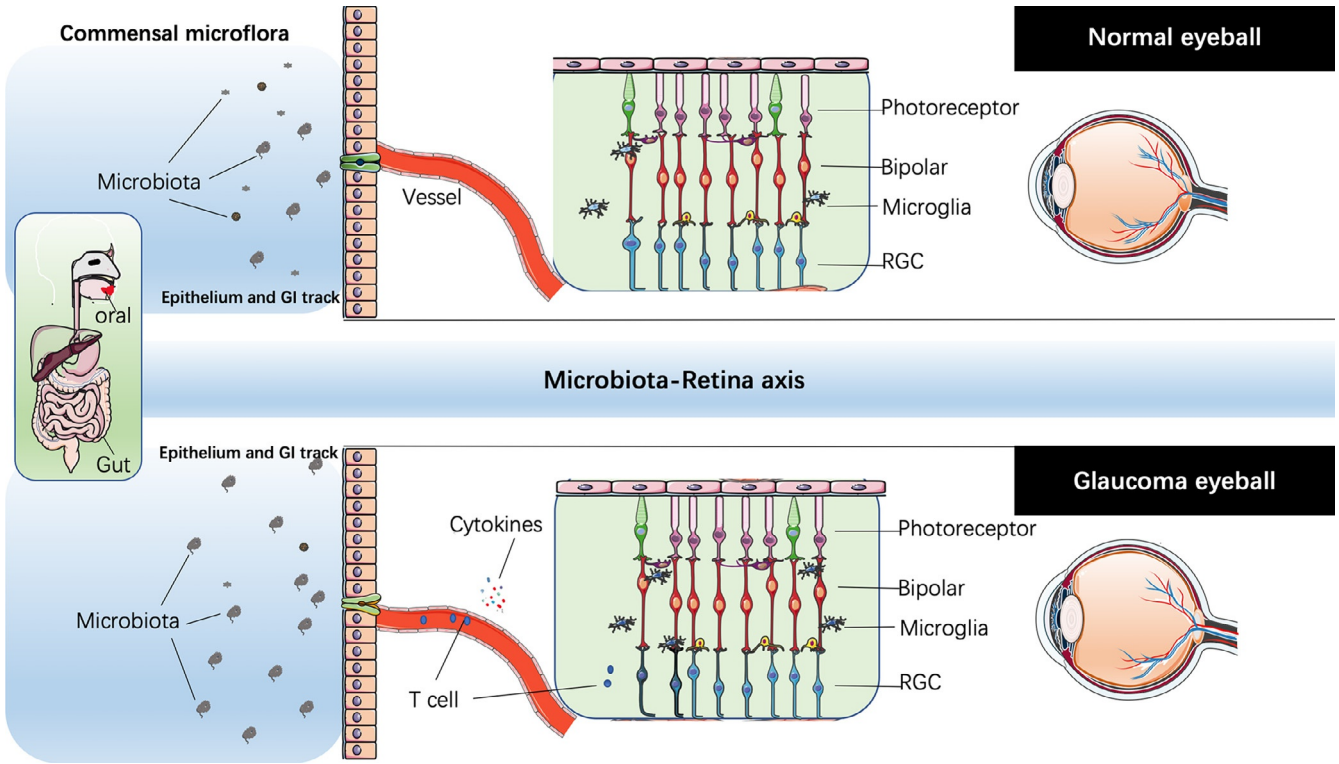

FIG. 1

Schematic illustration of the hypothetic gut-retina axis. The gut microbiota is composed of a large number of bacteria that form a complex ecosystem. The continuous crossing-talk between gut microbiota plays a pivotal role in driving the metabolic pathways. Changes of the species and complexity of gut microbiota were reported in patients with glaucoma and may result in chronic low-grade inflammation that is linked to the increased intestinal permeability, the expression of inflammatory cytokines, and the weakened BRB. Consequently, this may lead to microglial activation and recruitment of mononuclear macrophages and T cells into the retina to contribute to RGC damage in glaucoma. A better understanding of the mechanisms that underlie the "gut-retina axis" may prompt new personalized therapy for glaucoma and other neurodegenerative diseases.

regulated by a precise genetic program and environmental factors (Alliot et al., 1999). Developing microglia are usually associated with cellular development and proliferation, while the adult microglia are often associated with immune responses (Matcovitch-Natan et al., 2016). Mature microglia constantly sense the environment, promote neuronal operation, defend against injury and provide neuroprotection (Hickman et al., 2018).

Germ-free (GF) mice are animals never exposed to bacteria and viruses. Mice were found to develop functional deficiency in innate immunity (Erny et al., 2015). They upregulated microglia transcription and survival factor Sfpi1, c-fms, and colony stimulating factor 1 receptor (CSF-1R)—a key element mediating microglial proliferation, maturation and function (Erny et al., 2017). Microglia from mice tri-colonized by three specific altered Schaedler flora (ASF) bacterial strains also showed different morphology, function and maturation compared to those of normal mice (Erny et al., 2015). Temporal eradication of host microbiota was shown to restore microglia features (Erny et al., 2015).

In the mature brain and retina, invaded pathogens may release PAMPs that trigger microglia secretion of pro-inflammatory cytokines (Fung et al., 2017), thereby changing the inflammatory state in the CNS. Studies show that oral bacterial counts are higher in POAG patients, and chronic peripheral inflammation has also been suggested to contribute to the neurodegeneration in glaucoma by activating microglia (Astafurov et al., 2014). A study demonstrated that high-fat diets accelerate CNV by altering gut microbiota, leading to increased intestinal permeability, chronic inflammation and elevated production of IL-6, IL-1β, TNF-α, and VEGF-A (Andriessen et al., 2016). Short-chain fatty acids and microbiota-derived bacterial fermentation products, in contrast, restores microglia homeostasis (Fung et al., 2017). These observations suggest that not only the quantity, but also the complexity of microbiota, critically affect microglia prosperity. The host bacteria vitally regulated microglia maturation and function, and functional impairment microglia may be rectified, to an extent, by complex microbiota (Erny et al., 2015).

2.5 Microglia and neurodegeneration in glaucoma

Microglia are residential macrophage-like cells in the CNS (Kierdorf and Prinz, 2017; Wei et al., 2019), being responsible for immune regulation. Upon stimulation, microglia undergo phenotypic alteration and contribute to pathological processes in the eye and brain. Microglial activation leads to a robust inflammatory response that includes increased expression of major histocompatibility complex class II (MHCII) on residential microglia and infiltration of monocytes and/or CD4+ T cells in the draining cervical lymph nodes (Williams et al., 2020). Microglia express many pattern recognition receptors. For instance, activated microglia depend on triggering receptors expressed on myeloid cells-2 (TREM2) pathway to initiate phagocytosis activity and cytoskeleton reorganization by binding to various ligands (Wolfe et al., 2019). TREM2 is necessary for microglia to phagocyte amyloid plaque, which is a key point for AD development (Liu et al., 2013). Moreover, microglia are able to

secrete inflammatory cytokines including TNF-α, IL-6, IL-10, IL-12, IL-1β (Lee et al., 2002). A unique subtype of disease-associated microglia (DAM) was found to mediate the pathogenesis of AD. Inhibiting RIPK1, which is highly expressed by microglia, suppressed the transcription of DAM, in turn preventing the accumulation of amyloid plaques in AD (Keren-Shaul et al., 2017). Microglia thus play an extremely important role in the pathogenesis of CNS neurodegeneration.

Similar to what is observed in the brain, homeostatic microglia perceive the environmental signals in the retina (Fig. 1). Active microglia are detected earliest around the optic nerve head (ONH) in DBA/2J mice, before RGC loss (Bosco et al., 2011). Activation of signal-regulating kinase-1 (ASK1), p38 mitogen-activated protein kinase (MAPK) and NF-κB drive microglia in a pro-inflammatory manner, leading to increased production of inflammatory cytokines, such as IL-1β, TNF-α, TGF-β or IFN-γ (Chi et al., 2014; Sappington and Calkins, 2008; Yuan and Neufeld, 2000). They induce the cell apoptotic pathways to cause RGC death and degeneration of the optic nerve (Beynon and Walker, 2012). Suppression of microglia activation has been shown to significantly improve RGC axonal transport and prevent subsequent neurodegeneration (Bosco et al., 2008; Wei et al., 2019).

3 Heat shock proteins in glaucomatous neurodegeneration

3.1 HSPs and their upregulation in glaucomatous neurodegeneration

The molecular signals that link the function of microbiota to microglial activities remain as a central question in neurodegeneration. Heat shock proteins (HSPs) are some of the most abundant proteins in the cell and a series of stress response proteins existing in prokaryotes, including bacteria, or eukaryotes like human cells (Khong and Spencer, 2011). They belong to a superfamily of stress proteins, which is an important part of a complex defense mechanism that can improve cell survival under adverse environmental conditions; however, they have been also associated with multiple autoimmune diseases. For example, HSP60 is involved in the morbidity of rheumatoid arthritis (RA) (Barberá et al., 2013), Crohn's disease (Baca-Estrada et al., 1994), Hashimoto's thyroiditis (Tonello et al., 2015) and Juvenile rheumatoid arthritis (De Graeff-Meeder et al., 1991; Elst et al., 2008; Lorenzo et al., 2015); HSP27 was associated with psoriasis (Besgen et al., 2010); HSP90 contributed to the pathogenesis of systemic lupus erythematosus (Erkeller-Yüksel et al., 1992), whereas HSP70 was related to the incidence of multiple sclerosis (Mansilla et al., 2014) and RA (Shoda et al., 2016). HSPs keep a conservative evolution by the stable species structure and are expressed in all microbes. Some HSPs are induced intracellularly for their housekeeping function, while others are induced by external stimulation for immunoregulation (Liu et al., 2014).

HSPs are typically named according to the molecular size, covering from 10 to 100 kDa. For example, the 60-kD protein is described as HSP60. The classification of

HSP family includes HSP100, HSP90, HSP70, HSP60 and small HSPs. They are located in various sites within cells, with HSP10, HSP60 and HSP75 presented in mitochondria, and others located in the cytoplasmic membrane, cytosol, endoplasmic reticulum or nucleus in physiological conditions (Xu, 2002). The cellular protective effect of HSPs is related to its chaperone function as well as the effect of anti-apoptosis and anti-necrosis; however, their involvement in the activation of inflammatory cells in autoimmunity was also demonstrated (Kregel, 2002; Piri et al., 2016).

Different HSP families are found to have their respective roles in neurodegeneration under stressful stimulation. In a rat model of glaucoma, HSP27 was described to relate to RGC apoptosis and cell damage (Tezel and Wax, 2000); HSP60 participated in the immune responses by elevating the secretion of IL-1β (Swaroop et al., 2016, 2018); HSP70 was the target response protein, protecting cells from apoptosis and necrosis via the suppression of caspase-3 and caspase-9 pathways (Dong et al., 2016; Li et al., 2000; Vasaikar et al., 2015); HSP90 was reported to promote mistranslation in cell stress resulting from the degradation of mutants (Stothert et al., 2017). HSP upregulation is reported in rats and humans with glaucoma (Park et al., 2001; Tezel et al., 2000) as well as in many stressed or injury conditions of the CNS. It is reported to affect the learning and memory formation in AD patients through abnormal calcineurin elevation (Kim et al., 2015). HSP27 and HSP60 were increased in RGCs of microbead induced glaucoma models, and rats inoculated with human HSP27 and HSP60 exhibited glaucomatous optic neuropathy (Chen et al., 2018; Wax et al., 2008). The data suggested that HSPs were involved in the pathogenesis of glaucoma and CNS neurodegeneration.

Microglia, being residential macrophages in the CNS, are thought to be mediated by HSP signaling (Kakimura et al., 2002). HSP-release into the extracellular environment is usually an indication of loss of cell integrity and serves as a "danger signal." HSP-release elicits microglia and innate immune responses through toll like receptor 2 (TLR2) and TLR4 to secrete pro-inflammatory cytokines, such as IL-1β, IL-6 and TNF-α (Jin et al., 2014; Rosenberger et al., 2015; Swaroop et al., 2016). Interestingly, microglia in GF mice is shown to lack TLR2 and TLR4 (Erny et al., 2015) providing an explanation for the lack of microglial activation in these mice. HSP-stimulated microglial activation plays an important role in neuroinflammation via inducing nuclear factor κB (NF-κB) and p38 mitogen-activated protein kinase pathways. HSP60 upregulation enhanced IL-1β secretion and microglial activation (Swaroop et al., 2018). Also, elevated levels of anti-HSP60 antibody in human serum and aqueous humor correlated with microglia activation in glaucoma patients (Bell et al., 2018), supporting the induction of HSP-specific autoimmunity. HSP27 immunization in rats was linked to increased inflammatory cytokine FasL release and microglia activation (Wax et al., 2008). As these studies suggest an association between HSP upregulation and microglial activation, more direct evidence is needed to uncover their complex interactions.

3.2 The role of HSPs in autoimmune conditions and neurodegenerative diseases, including glaucoma

HSPs, especially those presented in the extracellular space, are found to be highly immunogenic and can be processed and presented by antigen presenting cells to stimulate T cell responses. Recent evidence revealed a critical role for adaptive immunity, particularly HSP-specific T cell responses, in mediating glaucomatous neurodegeneration (Chen et al., 2018; Wax et al., 2008). HSPs have been shown to act as pathogenic autoantigens in many autoimmune diseases (Binder, 2014). This was also observed in mouse models of glaucoma (Grotegut et al., 2020). It is reported in mouse models of glaucoma that RGC loss proceeds in two phases: an initial phase that starts soon after IOP elevation and a second prolonged phase that begins at ~2 weeks post IOP elevation but continues after IOP returns to the normal range (Chen et al., 2011, 2018). Elevated IOP induced HSP-responsive IFN-γ-secreting CD4+ T (TH1) cell infiltration into the retina as well as T cell immunity specific to HSPs, leading to the prolonged phase of neurodegeneration. Using T cell deficient mice and adoptive T cell transfer, it was established that T cell-mediate responses are both essential and sufficient for the second phase of glaucomatous neuron loss. In contrast, the initial phase of glaucomatous neurodegeneration was not blocked by T cell deficiency. CD4+IFN-γ+and CD4+IL-4+T cells were detected in the retinas of POAG patients (Guo et al., 2018). Similarly, a marked increase in the frequency of HSP-specific T cells was detected in POAG patients compared to age-matched healthy controls, supporting the human relevance (Chen et al., 2018).

Involvement of HSPs in glaucomatous neurodegeneration is multifaceted. On one hand, HSPs contribute to neuroprotective compensation partly through stimulating cytokine production or enhancing phagocytosis (Kakimura et al., 2002). Upregulation of HSP70 was recognized to protect RGCs from apoptosis and necrosis by reducing caspase-9 and caspase-3 expression (Dong et al., 2016). On the other hand, HSPs act as autoantigens in T cell-mediated glaucoma neurodegeneration (Chen et al., 2018). Bacterial and host HSPs are likely the natural antigens that originally induce HSP-specific memory T cells. In glaucoma, HSP27 and HSP60 are upregulated and stimulate both the innate and adaptive immune response in vivo to cause RGC death (Kalesnykas et al., 2007). Moreover, HSPs also initiate the adaptive immune responses through presenting pathogenic antigens to activate the T cell system. When HSPs bind with antigen peptides, the HSP-antigen complex is generated. After the complex reaches the endoplasmic reticulum, MHC loads and transfers it to the cell membrane, where the antigen is presented to the T cells. This mechanism of antigen presentation changes the prime-T cell to an effective-T cell (Tsai et al., 2019).

Besides T cell responses, increased serum levels of HSP-specific antibodies were detected in human patients and animal models of glaucoma (Tukaj and Kaminski, 2019). Exogenous administration of HSP27 antibodies induced neuronal apoptosis and RGC loss, partly by attenuating the stabilizing effect of native HSP27 on actin

cytoskeleton (Tezel and Wax, 2000). Higher levels of anti-HSP60 antibodies were mainly found in patients with normal tension glaucoma (Guo et al., 2018), which caused RGC loss in animal models due to IgG deposition and microglia activation (Bell et al., 2018).

In line with the critical immune regulation by HSPs, mice raised in the absence of commensal microflora (germ-free mice), where immune cells have not been pre-exposed to bacterial HSPs, do not develop glaucomatous neurodegeneration at all after IOP elevation (Chen et al., 2018). Concomitantly, elevated IOP was unable to initiate HSP-specific T cell responses in germ-free mice (Chen et al., 2018). The results strongly suggest that elevated IOP in glaucoma presents merely a physical stress to RGCs and axons; the subsequent stress-induced events involving retinal inflammation and adaptive immune responses are keys to the pathogenesis of glaucoma. It is thus hypothesized that induction of immune tolerance to HSPs may have a therapeutic potential for preventing neurodegeneration and treating glaucoma. A critical mechanism in the development of self-tolerance involves an immune-regulatory process mediated through specialized regulatory cells, especially regulatory T cells (Van Eden, 2018). These regulatory T cells are active regulators that down-modulate autoreactive immune cells and propagate an active form of dominant tolerance; restoration of tolerance under the diseased conditions usually depend on improving regulatory T cell presence and function. Studies in experimental autoimmune encephalomyelitis model have demonstrated successful induction of tolerance to HSP60 by intranasal administration of HSP60 epitope (Billetta et al., 2012; Zhong et al., 2016). HSP60 peptide treatment in mice led to the induction of HSP60-specific regulatory T cells and attenuated brain and spinal cord neural damage with significant improvement of clinical symptoms. Being microbiota-associated antigens, it is not surprising that HSPs were suggested to be dominant in tolerance induction (Russler-Germain et al., 2017). Possible mechanistic explanations may include their conservative sequences, frequent MHC ligand source and stress-induced upregulation, that act in synergy for the outcome. To date, the initiation and pathways of tolerance induction remain to be further elucidated.

4 Interactions between T cells and microglia

4.1 CNS T cell infiltration and activated microglia

T lymphocytes, which are an important player of the immune system, are categorized into T helper cells, regulatory T cells, and cytotoxic T cells. Once activated, T cells divide rapidly, secrete cytokines and further differentiate into subtypes to assist immune responses (e.g., Th1 secrete IFN-γ, Th2 secrete IL-4, Th17 secrete IL-17, Treg secrete TGF-b, etc.). The roles of pathogenic T cells in CNS neurodegeneration and autoimmunity are attracting increasing investigations. T cell infiltration was detected in the brain of PD patients, specifically reacting to antigenic MHCII derived from α-synuclein (Schetters et al., 2017). In patients with AD, increased T cells were also

found in the brain and located closely to microglia (Rogers et al., 1988). As recent studies showed that retinal ischemia or transient elevation of IOP both induced T cell infiltration into the retina (Chen et al., 2018; Korn and Kallies, 2017; Thi Hong Khanh Vu et al., 2020), the data point toward critical involvement and infiltration of T cells in CNS neurodegenerative diseases. The key question is what has attracted T cells into the CNS, an immune privileged site that is supposed to be shielded from peripheral immune cells.

Virtually in all injury or disease conditions in the CNS, including AD, PD and glaucoma, microglia become activated and migrate to the site of inflammation. This can occur within hours following injury or neuronal insult (Kawabori and Yenari, 2015). Activated microglia undergo phenotypic and functional changes by producing inflammatory cytokines, chemokines, complement and trophic factors. The innate immune responses initiated by microglial activation usually set a foundation for subsequent adaptive immune cells (Nayak et al., 2014). Antigens released from the degenerating neurons within the CNS may recruit antigen-specific T cells into the brain or retina in patients with AD, PD or glaucoma (Chen et al., 2018; Korn and Kallies, 2017). It is acknowledged that the accumulation of reactive microglia and T cells is commonly observed during neurodegeneration (Ellwardt et al., 2016; Korn and Kallies, 2017; Perry et al., 2010; Schetters et al., 2017).

In the brain of PD patients, $CD8^+$ cells were found in close proximity of activated microglia (Schetters et al., 2017). Electron micrograph from gray matter of an AD patient illustrated T cells in apposition to microglia with less dendrites (Togo et al., 2002). Evidence has also been shown that activated microglia and T cells were found to colocalize at the sites of demyelination and oxidative damage in multiple sclerosis (Grebing et al., 2016; Haider et al., 2011; Lucchinetti et al., 2011) and EAE (Greter et al., 2005; Murphy et al., 2010). As parts of the immune response, functional microglia and T cells show some spatial and temporal overlaps, but the interaction of these two cell types at the sites of neurodegeneration remains obscure. Activation of microglia occurs within a day after CNS ischemia/injury (Ahmed et al., 2017; Kawabori and Yenari, 2015), long preceding the detection of infiltrated T cells. In the early stage of EAE, transient disruptions of focal vessels precede microglia activation that is detectable at day 3–6, followed by infiltration of circulating dendritic cells and T cells at day 6–12; these events occur before the clinical onset of disease (Barkauskas et al., 2015). During EAE, the kinetics of myelin uptake by CNS-resident cells suggests that $CX3CR1^+CD11b^+$ microglia are the first cell type to contain myelin antigen, likely modulating T cell responses inside the brain before peripheral APCs arrive (Sosa et al., 2013). In glaucoma and ischemic optic neuropathy, microglial activation as a primary event before RGC death is followed by T cell infiltration into the retina that results in prolonged RGC death (Bosco et al., 2015; Ramirez et al., 2017; Rojas et al., 2014; Thi Hong Khanh Vu et al., 2020; Williams et al., 2017). These observations suggest that activated microglia recruit T cells into the retina and brain under pathological conditions.

4.2 CNS/retina: An immune privileged site

The CNS is an immune privileged site, normally without peripheral immune cells and limited expression of MHC molecules. However, increasing studies reveal that peripheral immune cells, like T cells, could gain access to the CNS and interact with residential microglia and neurons (Korn and Kallies, 2017). Currently, the mechanisms of how T cell infiltrate into the CNS is still unclear.

Effective T cell responses are accompanied by antigen presentation, either with MHCI or MHCII for $CD8^+$ and $CD4^+$ T cells, respectively. While the expression of MHCII in CNS is relatively low in stable condition, it is quickly upregulated in activated microglia (Wyss-Coray and Mucke, 2002). Activated microglia is the primary cell type expressing MHCII in the brain and retina. They were found in close proximity and temporal interaction with T cells, where neurodegeneration and cell death take place. Currently, there is no evidence suggesting that microglia could migrate to the draining lymph nodes of the eye or brain, where antigens are presented to naïve T cells in the periphery (Engelhardt et al., 2017). Theoretically, there should be another mediator in between, bridging the antigen presenting process. Microglia also express PRRs, which bind and internalize foreign or misfolded proteins and are upregulated during neural damage or inflammation (Perry et al., 2010). The same PRRs are used in dendritic cells for antigen uptake and functioning as an APC of T cells. Thus, microglia have the potential for antigen uptake and presentation while they also modulate T cell responses once they enter the brain or the eye (Schetters et al., 2017). These findings provide important insight into the functions of T cells and their involvement in the pathological process of glaucoma and CNS neurodegeneration.

5 Conclusion

Inflammatory response is a pathogenic component underlying neurodegeneration in glaucoma. Microbiota play a fundamental role in the development, maturation, and function of the immune system and in the regulation of BRB and BBB permeability. Microglia are the first-line defending system in the CNS that supervise and participate in the immune response and repairing process, in which development and maturation are mediated by microbiota. HSPs are critical signaling molecules that trigger inflammatory responses and activation of T cells, which also need to be presensitized by commensal microflora in order to propagate progressive neurodegeneration in glaucoma. Research on gut-retina and gut-brain axis has emerged in the past decade to link neurological conditions from glaucoma to AD. These studies may one day lead to the identification of microbiota biomarkers to stratify individuals for personalized therapy.

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