

# The Role of Glia, Mitochondria, and the Immune System in Glaucoma

*Gülgün Tezel<sup>1,2</sup> and the Fourth ARVO/Pfizer Ophthalmics Research Institute Conference Working Group<sup>3</sup>*

The fourth annual conference of the ARVO/Pfizer Ophthalmics Research Institute was held on Friday and Saturday, April 25 and 26, 2008, in Fort Lauderdale, Florida. This conference series, funded by the ARVO Foundation for Eye Research through a grant from Pfizer Ophthalmics, provides an opportunity to gather experts from within and outside ophthalmology to develop strategies to improve research into the causes and new treatments of blinding eye diseases. This year's conference focused on glaucoma research. As originally put forward by Robert Ritch, a major goal was to define strategies essential for the improvement of the current understanding of the role of glia, mitochondria, and the immune system in glaucomatous neurodegeneration. The scientific discussion of different opinions was also intended to illuminate promising treatment strategies for neuroprotection.

A working group of 39 scientists from the fields of glaucoma and ocular immunology and working outside the traditional bounds of vision research, discussed the involvement of glia, mitochondria, and the immune system in glaucomatous neurodegeneration. Many observers from ARVO, Pfizer Ophthalmics, and ophthalmic research also attended the conference.

The conference was divided into sessions formatted to evoke discussions focused on four areas of research:

Session I: Dysfunction of the Retina and Optic Nerve Head Glia during Glaucomatous Neurodegeneration

Session II: Mitochondrial Dysfunction Leading to Neurodegenerative Injury in Glaucoma

Session III: Immune System Involvement in Glaucoma

Session IV: Immunomodulatory Treatment Possibilities for Neuroprotection

Each of these sessions was moderated by two experts in the area of discussion and began with a 10-minute overview of the session topic followed by a 30-minute keynote presentation by an outside expert and 10 minutes of discussion. Invited outside experts covered several areas of research, including the role of microglial senescence in autoimmune neurodegeneration (Wolfgang J. Streit, McKnight Brain Institute of University of Florida, Gainesville, FL), the role of mitochondria in neurode-

generative diseases (Gary E. Gibson, Weill Medical College of Cornell University, New York, NY), mechanisms of autoimmune injury in the central nervous system (CNS) (Hartmut Wekerle, Max Planck Institute of Neurobiology, Martinsried, Germany), complement cascade in mediating synapse loss and axonal degeneration (Beth Stevens, Stanford University, Stanford, CA), and immunomodulation by stem cells (Tamir Ben-Hur, Hadassah University Hospital, Jerusalem, Israel). In addition to the keynote speakers, four or five invited speakers for each session presented relevant data for 10 minutes and then led discussions for 25 minutes. Each session ended with a summary discussion of 20 minutes led by the session's moderators. During these discussions, attendees voiced their opinions and worked together to highlight current knowledge and new ideas that are essential to better understand the role of glia, mitochondria, and the immune system in pathogenic mechanisms and to search for new treatment possibilities. Lively discussions were successful in defining the most important questions that are unanswered or need further exploration. This conference report provides a synopsis of discussions and introduces guidelines for future research.

## DYSFUNCTION OF THE RETINA AND OPTIC NERVE HEAD GLIA DURING GLAUCOMATOUS NEURODEGENERATION

Progressive degeneration of retinal ganglion cells (RGCs) and their axons is the primary cause of glaucomatous visual loss. However, many aspects of this blinding disease are unclear, and current treatment options are not sufficient to block neurodegenerative injury in patients with glaucoma. It is for this reason that different laboratories have focused their efforts on better understanding of the precise pathogenic mechanisms of glaucomatous neurodegeneration and development of innovative treatment methods for neuroprotection. Growing evidence now supports that not only the events intrinsic to RGCs, but also environmental signals from other cells are critical to overcome cell death stimuli, and RGC-glia interactions are critically important for different aspects of glaucomatous neurodegeneration.

Glial cells perform specialized functions in support of neurons, and virtually every aspect of the development, homeostasis, and function of the visual system involves neuron-glia partnership. Glial cells insulate neurons, provide physical support, and supplement them with several metabolites and growth factors. These neurosupportive cells also play important roles in axon guidance and control of synaptogenesis. The major glial cell type in the retina and optic nerve head, astrocytes, exhibit significant homeostatic interactions with RGCs and axons and provide energy support. Besides astrocytes, Müller cells play a key role in the maintenance of RGC bodies in the retina. These specialized macroglial cells are critically important for controlling the extracellular environment, main-

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taining the extracellular glutamate and ion balance, and buffering oxidative stress. Microglial cells with myeloid origin constitute another glial cell type and function as resident macrophages of the retina and optic nerve head. Glial cells, particularly the microglia, play fundamental roles in local immune responses and immunosurveillance. Immunoregulatory functions of these cells also exhibit important links to systemic immune response through a glia-T cell dialog. Another important function of glial cells is maintaining perivascular barriers and securing immune privilege to protect neurons from potentially damaging effects of an inflammatory immune response.

Despite their relative protection from glaucomatous injury, glial cells prominently respond to glaucomatous stress, including elevated intraocular pressure (IOP), and exhibit an activated phenotype, both in the retina and optic nerve head. As documented by many studies, this chronic activation response of the glia is best characterized by a hypertrophic morphology and increased expression of glial fibrillary acidic protein.<sup>1,2</sup> In addition to morphologic alterations, glial cell functions exhibit profound alterations in glaucoma as supported by dramatic changes in gene expression involved in signal transduction, cell proliferation, cell-cell interaction, cell adhesion, extracellular matrix synthesis, and immune response.<sup>3</sup> Microglial cells also exhibit remarkable alterations in number, size, and distribution during neurodegenerative injury in glaucomatous eyes.<sup>2</sup>

Discussions in this session were mainly centered around the view that despite a variety of functions in support of RGCs in normal eyes, insufficiency and/or dysfunction of glial neurosupportive abilities in glaucomatous conditions may facilitate the neurodegenerative injury in glaucoma. This view is now more widely accepted with the support of emerging evidence that once activated in response to glaucomatous stress conditions, many major support functions of the glia may be weakened or become insufficient due to increasing risk factors, or glial cells may even be neurodestructive in various ways. One of the harmful consequences of the glial activation response in glaucoma appears to be associated with a major role of glia in extracellular matrix remodeling.<sup>1</sup> Tissue remodeling events, particularly at the optic nerve head, may create biomechanical stress on axons, and glial alterations may contribute to creating an environment that is directly or indirectly neurotoxic and also inhibitory for axonal regeneration. A few examples of glial dysfunction with neurodestructive consequences in glaucoma include diminished ability of glial cells in buffering extracellular glutamate<sup>4</sup> and increased glial production of cell death mediators, such as TNF- $\alpha$  and nitric oxide.<sup>5</sup>

Another potentially important consequence of glial alterations in glaucomatous eyes may be associated with their roles in the maintenance of perivascular barriers. Endothelium-glia interaction maintains the conventional blood-brain barrier for solutes, although cellular transport to the CNS may be possible as part of immunosurveillance. This physiological barrier involves the molecular machinery of endothelial tight junctions, membrane channels, and delicate transport systems.<sup>6</sup> As noted during the discussions, the optic nerve head exhibits features lacking classic blood-brain barrier properties, as the tissue of Elschnig does not totally separate the optic nerve head from fenestrated peripapillary choriocapillaries. In addition, peripapillary chorioretinal atrophy zones commonly detected in glaucomatous eyes exemplify sites in which the outer blood-retina barrier is broken. Evidence supports that the perivascular barriers may be further weakened in glaucomatous eyes. A barrier dysfunction may develop as a consequence of glia-related alterations in the milieu, including an increased expression of endothelin-1,<sup>7</sup> which may reduce endothelial tight junctions, and an increased expression of matrix metalloproteinases,<sup>8,9</sup> which may degrade the basement membrane and glia limitans consisting of astrocytic and microglial end feet.

Consistent with this view, some patients with glaucoma clinically exhibit vascular leakage sites, as detected by fluorescein angiography. Another related clinical finding that is commonly detected in patients with glaucoma and is linked to disease progression is splinter hemorrhages at the border of the optic nerve head.<sup>10</sup> Whether these hemorrhages represent the tip of the iceberg in a perivascular barrier dysfunction in glaucoma is unclear. It was also discussed that whether such a dysfunction is a consequence of or a player in the neurodegenerative injury is unknown. However, it is clear that these are accidental sites of direct contact between neural tissue and systemic circulation.

A primary microglial function in the CNS is to provide continuous surveillance of the parenchyma for tissue cleaning. As in other parts of the CNS, these immunologically active cells promptly respond to all kinds of injuries and provide a first line of defense in the retina and optic nerve head. Complement-mediated processes constitute an important component of glial innate immune functions during glaucomatous neurodegeneration as discussed in Session III. In addition to complement components, other molecules may also serve as targeting molecules for glial removal of stressed or injured RGCs. For example, recent *in vitro* and *in vivo* studies demonstrate the expression and function of toll-like receptors (TLRs) in the retina, including glial cells. TLRs are crucial components of innate immune response to microbial components and also facilitate the removal of stressed cells by binding stress proteins. A related question is whether the presence and/or upregulation of TLRs on glia might increase the susceptibility to an autoimmune injury after encountering microbial antigens. In addition to innate immune response, glial cells are capable of initiating adaptive immunity through antigen presentation. Retinal glial cells, most prominently microglia, express major histocompatibility complex (MHC)-class II molecules,<sup>11</sup> required for antigen presentation to T cells. These support that as resident immunoregulatory cells, glia have the potential to initiate the stimulation of an immune response during glaucomatous neurodegeneration, which may have neurosupportive and neurodestructive consequences. These were further discussed in Session III. However, it should be noted here that the presence of reactive glia is commonly accepted as the hallmark of neuroinflammation in the CNS, persistence of which for extended periods leads to tissue damage through proinflammatory cytokines.<sup>5</sup> The continuous nature of glial activation in the glaucomatous retina and optic nerve head therefore appears to be crucial in determining the outcome of an immune response as being neurodestructive rather than neurosupportive.

An interesting discussion was on aging as another important determinant of the ultimate role of glial cells in neurodegenerative injury. Recent work in the aged human brain has provided evidence for deterioration of microglia, and findings of rodent experiments support that microglia are indeed subject to senescent changes.<sup>12</sup> It has been proposed that old age, along with genetic and epigenetic factors, adversely affect the cellular viability and self-renewal capacity resulting in the generation of dysfunctional microglia. Such an age-related attrition in the brain's immune system may contribute to development of neurodegenerative diseases by diminishing glial neurosupportive functions. Since glaucoma is an aging-related disease, it seems quite possible that a similar age-dependent component of glial dysfunction may amplify the glaucoma-related factors, thereby further facilitating the neurodegenerative injury.

An important concept when considering the progression of neurodegenerative injury in glaucoma is secondary degeneration. As is evident in different models of optic nerve injury,<sup>13</sup> RGC death during chronic neurodegenerative injury in glaucoma is not thought to result only from primary injury (commonly believed to be elevated IOP-induced axonal stretching/

ischemia). In addition to the primary injury, RGCs that are not initially injured may also undergo degeneration over time. After an initial injury at the optic nerve head, an injury signal probably spreads through the optic nerve and retina and initiates a chain of cellular events leading to progressive neurodegeneration. Proposed pathways for this secondary degeneration process have mainly been linked to negative effects from neighboring cells, dying RGCs, and/or surviving and activated glia. The wave of secondary degeneration may involve an increased exposure to glutamate released from damaged RGCs, as well as an insufficiency in buffering extracellular glutamate due to glial dysfunction.<sup>4</sup> Growing evidence also supports that the loss of glial neurosupportive functions and/or initiation of glia-originated neurotoxic effects may facilitate spreading of the neurodegenerative injury in glaucoma. In addition, an autoimmune component may be involved in this secondary neurodegenerative injury, as discussed in Session III. Better understanding of cellular processes associated with a widespread injury signal during glaucomatous neurodegeneration is of great importance to neuroprotection, because of a greater window of intervention. What seems contrary to the concept of secondary degeneration is the intrinsic adaptive response, also referred to as preconditioning-induced tolerance. It is well documented by numerous studies that neuronal cells exposed to a sublethal insult become resistant to a subsequent period of lethal insult, because early upregulation of intrinsic adaptive/protective mechanisms initially provides resistance to cell death.<sup>14</sup> Are intrinsic mechanisms insufficient to provide an adaptation to the initial insult in glaucoma, or does sustained exposure to noxious stimuli potentiate cell death programs? Many additional factors associated with glaucoma, such as chronicity of injurious conditions and age-dependent dysregulation of tissue response mechanisms, may play a role in a cumulative deterioration of the homeostatic balance, thereby promoting the spread of neuronal damage, rather than favoring retained cell survival.

A final discussion topic of the session was neuroprotective treatment possibilities by targeting glial cells. Based on discussions in which glial cells are commonly considered to be participants of the neurodegenerative injury process in glaucoma, modulating the glial response appears to be a promising strategy to reduce axonal damage and RGC soma death, while facilitating axonal repair and regrowth of surviving RGCs. Such a glia-targeting treatment strategy could be directed at blocking the initial glial response, reversing the neurodestructive consequences of glial activation, or regaining glial neurosupportive functions. Different views brought forward a wide variety of potential treatment targets to modulate glia-associated factors. These include TGF- $\beta$ 2, TNF- $\alpha$ , nitric oxide synthetase-2, epidermal growth factor receptor, neurotrophic support, glutamate transporters, endothelin-1, immunoregulation, interleukin-6, Fas/FasL, extracellular matrix remodeling, oxidative stress, and protein modifications (generation of advanced glycation end products, citrullination). However, treatment strategies designed to prevent glial activation may be a double-edged sword, and the type and timing of treatments should be carefully optimized to inhibit neurodegenerative effects of glia while maintaining the neurosupportive and neuroregenerative outcomes. A critical question that arose during discussions was which experimental model(s) accurately mimic various conditions in human glaucoma and are therefore useful to test neuroprotective treatments. Although animal models are useful tools for elucidating pathogenic mechanisms and testing the neuroprotective ability of new treatments,<sup>15</sup> it is very likely that the initiating insults and pathogenic pathways vary between different experimental models.<sup>16</sup> This is also a remaining challenge in generating sufficient and compelling preclin-

ical support to justify testing new agents in well-designed clinical trials with established funding.

## MITOCHONDRIAL DYSFUNCTION LEADING TO NEURODEGENERATIVE INJURY IN GLAUCOMA

Neurons, because of their high energy requirement, are heavily dependent on mitochondria for survival. Mitochondria not only constitute an energy-generating system, but are also critically involved in calcium signaling and apoptosis. Mitochondrial function can be affected by mutations in mitochondrial and nuclear DNA, chemical insults to components of the electron transport chain, and a lack of substrates such as oxygen. The latter is relevant to tissue hypoxia that is believed to be present in the glaucomatous retina and optic nerve head either primarily or secondary to elevated IOP. Any malfunction of the mitochondrial electron transport chain results in an excessive generation of free radicals. When this overwhelms the intrinsic antioxidant capacity, amplified generation of free radicals results in the state of oxidative stress, which is evident in glaucomatous tissues. Are impaired mitochondrial energy metabolism and oxidative stress in glaucoma a consequence of or a cause of neurodegenerative injury? Evidently, oxidative stress leads to oxidative damage to cellular macromolecules such as DNA, proteins, and lipids, along with energy depletion and a local dysregulation of calcium homeostasis, resulting in neuronal degeneration.<sup>17</sup> Free radical injury constitutes a caspase-independent component of the mitochondrial cell death pathway in RGCs.<sup>18</sup> Further supporting the occurrence of oxidative damage during glaucomatous neurodegeneration is that many retinal proteins exhibit oxidative modifications in experimental glaucoma, which may lead to important structural and functional alterations.<sup>19</sup> An example of oxidation end products in human glaucoma is provided by a recent study documenting the amplified generation of advanced glycation end products in association with protein oxidation in the optic nerve head and retina of human donor eyes with glaucoma.<sup>20</sup>

A related discussion was on the view that RGCs in a low energetic state would be more susceptible to any other adverse effect. For example, *in vitro* studies have proposed that by affecting the mitochondria, continuous light exposure may be detrimental to already compromised RGCs in glaucoma.<sup>21</sup>

Mitochondria are morphologically dynamic organelles exhibiting a precise balance of ongoing fission and fusion during development and aging, which can be modified by disease. Mitochondrial fission, characterized by the conversion of tubular fused mitochondria into isolated small organelles, translocation of dynamin-related protein 1, and reduction of cellular ATP, has been shown to be triggered in RGCs with exposure to elevated hydrostatic pressure, *in vitro*. In addition, IOP elevation *in vivo* has been linked to mitochondrial damage in the optic nerve head by the promotion of mitochondrial fission, cristae depletion, and alterations in the expression and distribution of optic atrophy type 1 in DBA/2J mice.<sup>22</sup> Whether the elevated IOP or secondary ischemia leads to mitochondrial alterations in animal models is unclear, as these two insults cannot be fully dissected in human glaucoma. According to an alternative view, primary mitochondrial abnormalities may disturb IOP regulatory mechanisms, thereby leading to the elevation of IOP and initiation of the neurodegenerative injury.

Another exciting focus of discussion was the potential role of reactive oxygen species (ROS) as signaling molecules. It has been proposed that after an initial insult to RGC axons at the optic nerve head, besides neurotrophin insufficiency, increased superoxide generation may also signal apoptosis of the RGC soma. Evidently, there is an increase in mitochondrial superoxide production within RGCs after axonal injury that is

further amplified by oxidative stress.<sup>23</sup> It is also evident that a shift to a reduced intracellular redox state induced by the use of sulfhydryl-reducing agents markedly prolongs RGC survival in *in vitro* and *in vivo* models of axonal injury. RGC mitochondria regulate superoxide production differently from other neuronal cells, most likely as a result of differential expression and function of the mitochondrial electron transport chain components.<sup>24</sup> How ROS act in signaling RGC apoptosis is unknown. An improved understanding of the RGC response to axonal injury in *in vivo* models of chronic pressure-induced glaucoma can provide specific information on ROS-mediated cell death signaling in glaucoma.

Thus, the structure and function of mitochondria are critical determinants of neuronal health, whereas mitochondrial dysfunction leads to RGC death through caspase-dependent and caspase-independent pathways, initiated by the loss of mitochondrial membrane potential, release of cell death mediators, and oxidative stress.<sup>18</sup> These mitochondria-dependent events are precisely controlled by the members of the Bcl2 family of proteins. In glaucoma, the proapoptotic Bcl2 family members Bad, Bax, and Bid have all been implicated in RGC death, with Bax being the principal regulator of the mitochondrial cell death pathway in RGCs.<sup>25</sup> Several upstream modulators of the Bcl2 proteins also exhibit important links to RGC death during glaucomatous neurodegeneration. Death receptor binding can initiate the apoptotic caspase cascade in RGCs, in which caspase-8 is a proximal effector caspase.<sup>5</sup> Besides this extrinsic (receptor-mediated) pathway, after proteolytic cleave by caspase-8, the truncated Bid can also participate in the activation of the intrinsic (mitochondrial) cell death pathway. Other upstream events regulating the Bcl2 family members have also been increasingly recognized as control points within the RGC. For example, the phosphorylation state of Bad is critical in determining whether it is able to translocate to the mitochondrion, where it can promote the release of cytochrome *c*.<sup>26</sup> In its phosphorylated state in the presence of neurotrophic signals, Bad is sequestered in the cytoplasm by binding to the protein 14-3-3.<sup>27</sup> However, in ocular hypertensive eyes Bad is dephosphorylated and 14-3-3 is phosphorylated, thereby resulting in the dissociation of Bad from 14-3-3 and mitochondrial translocation for proapoptotic function. Thus, the phosphorylation state of 14-3-3 also plays an important role in the cytoplasmic sequestration of phosphorylated Bad, thereby adding another level to the regulatory control. Bad can be dephosphorylated by calcineurin, 14-3-3 can be phosphorylated by JNK, and pharmacologic inhibition of calcineurin provides neuroprotection to RGCs in experimental glaucoma as does the inhibition of JNK. Animal models provide a critical experimental tool to further explore cellular mechanisms and specific treatment targets. However, establishing differences between acute and chronic models remains a prerequisite. Particularly concerning the age-dependent component of mitochondrial dysfunction/oxidative stress, it would also be more informative to characterize differences between young and old animals.

The observations just described illustrate the complexity of cellular events that include not only cell death signals, but also intrinsic survival signals triggered in RGCs. Cross-talk between death-promoting signals (such as death-promoting kinase activity, mitochondrial dysfunction, and caspase cascades) and survival-promoting signals (such as neurotrophic signals, antioxidant mechanisms, and expression of proteins that inhibit apoptosis) determines whether an RGC will die or survive the death stimulus. Considerable evidence also supports the involvement of heat shock proteins (HSPs), including HSP27, in intrinsic protection mechanisms of retinal cells in glaucoma. HSP27 is upregulated in experimental models of glaucoma, as well as in human glaucoma.<sup>28</sup> However, it has become apparent that the phosphorylation state of HSP27 is a critical deter-

minant of its ability to act in a protective capacity as detected in glial cells.<sup>29,30</sup> It is unclear whether optic nerve head and retinal astrocytes respond differently to glaucomatous stress, or whether glia supply RGCs with HSPs as a neurosupportive effort in glaucomatous eyes. Additional questions are whether the upregulation of HSPs in glaucomatous RGCs may serve as an antigenic stimulus activating the innate and/or adaptive immune response, or whether they may signal the microglial cleaning of stressed cells after binding TLRs.

As the conclusion of the discussions thus far was that the functional status of mitochondria may threaten neuronal survival, targeting mitochondrial events with a specific chemical inhibitor or genetic manipulation appears to be a logical approach to neuroprotection. The remaining part of the discussions in this session was therefore focused on potential treatment strategies targeting mitochondria-mediated events. There are many similarities described between the neurodegenerative processes in glaucoma and other neurodegenerative diseases.<sup>31</sup> An increasing number of studies over the past couple of decades support that oxidative injury is a common feature of many age-related neurodegenerative diseases. Therefore, data emerging from other neurodegenerative disease models may also benefit glaucoma research. An interesting discussion was on observations related to interactions of oxidants with mitochondrial enzymes that form the  $\alpha$ -ketoglutarate-dehydrogenase complex. Oxidative stress-induced reduction in the activity of this enzyme complex has been linked to numerous age-related neurodegenerative diseases, including Alzheimer's disease. By exhibiting critical links to the pathophysiology, such examples of oxidative injury offer effective targets for anti-oxidant treatments.<sup>32</sup> A criticism was the lack of convincing clinical evidence to support RGC loss in patients with Alzheimer's disease. Despite such controversies, mitochondrial energy deficits and oxidative stress may be central to the neurodegenerative injury in different diseases, but may be expressed differently. Oxidative stress therefore appears to be a common treatment target to provide neuroprotection in different neurodegenerative injuries. Another criticism was also related to the lack of epidemiologic correlations.<sup>33</sup> For example, epidemiologic studies have not detected an association of glaucoma with oxidative stress-causing conditions like light exposure and smoking. Epidemiologic studies have even found diabetes protective against neurodegenerative injury in glaucoma—a likely result of the preconditioning adaptive response, as discussed in Session I. Obviously, susceptibility to neurodegenerative injury is determined by many individual factors and their complex interplay, which is yet to be clarified.

Another relevant discussion topic was the work exploring a treatment paradigm for Leber's hereditary optic neuropathy (LHON), which is known to be associated with mitochondrial DNA mutations. For example, in a recent study, cells with the G11778A mutation in the complex I subunit, which is responsible for most cases of LHON, were infected with a viral vector containing the human mitochondrial superoxide dismutase gene. Augmenting the mitochondrial antioxidant defenses by viral-mediated gene transfer has resulted in increased cell survival.<sup>34</sup> Whether similar treatments directed to mitochondria for enhancing antioxidant defense can be applied in glaucoma remains to be determined. What would be the most appropriate agent(s), who should be treated, and what would be the side effects of various mitochondria-targeted treatment approaches?

Additional discussions on mitochondria-related neuroprotective strategies were focused on the control of mitochondrial function by targeting the Bcl2 family. A caveat in the therapeutic targeting of mitochondria-mediated events is the reversal of the early steps of the cell death cascade. Most important, once the mitochondrial lipid bilayer is compromised after the mito-

chondrial translocation of Bax, cell death is inevitable, because already triggered events, disruption of oxidative phosphorylation, loss in ATP production, increased generation of ROS, and release of cytochrome *c* start the commitment step that represents the point of no return for apoptotic cell death. Consequently, any strategy targeting mitochondria to block cell death will be effective only if the therapeutic intervention occurs before Bax-mediated events.<sup>35</sup> At this point of discussion, targeting the upstream phosphorylation cascades associated with the 14-3-3 scaffolding of proapoptotic Bcl-2 proteins in the cytoplasm appears to be a promising strategy for RGC protection. However, there are other upstream molecular events that have been related to apoptotic cell death independent of mitochondrial changes, which are not yet well understood. These events include a downregulation of normal gene expression in RGCs,<sup>36</sup> and a dramatic shrinkage of RGC somas and dendritic trees before the execution of cell death by apoptosis. Such cellular events, also important in determining the success of neuroprotective treatments, should be further explored.

A very important factor in the neuroprotective treatment of glaucomatous neurodegeneration is that cellular events leading to RGC soma death are independent of the molecular processes leading to axonal self-destruction—most evident in findings that although glaucomatous death of RGC soma can be blocked by genetic mutation of the Bax gene, axonal degeneration proceeds.<sup>25</sup> This evidence emphasizes that neuroprotection may be achieved by parallel strategies targeting RGC somas and axons, which critically need a comprehensive understanding of specific cellular processes in different subcellular compartments using the most representative experimental models. In addition, for the purpose of functional gain, treatment strategies activating RGCs for axonal regeneration and normal function are equally important as strategies inhibiting neurodegenerative signals. Based on these two separate demands, neuroprotective treatment strategies genuinely require a challenging design blocking RGC soma death and axonal degeneration while promoting regenerative events to secure visual function.

## IMMUNE SYSTEM INVOLVEMENT IN GLAUCOMA

The immune system functions in the maintenance of the CNS by providing tissue cleaning and limiting the neurodegenerative consequences of stressful conditions. However, immune system senescence and aging-related risk factors may reduce the ability of the CNS to cope with stressful conditions. A failure in the control of the immune response resulting from increased levels of risk factors may disturb the physiological equilibrium, thereby turning protective immunity into an autoimmune neurodegenerative process.<sup>37</sup> Growing evidence in clinical and experimental studies over the past decade strongly suggests the involvement of the immune system in glaucoma. Serum and tissue findings, including chronic activation of resident immunoregulatory glial cells, altered T-cell repertoires, increased autoantibody production, retinal immunoglobulin deposition, and complement activation, support that both innate and adaptive immune activity accompany glaucomatous neurodegeneration.<sup>38</sup> Are these findings present only in patients with glaucoma with normal IOP? No. Many patients with glaucoma with elevated IOP may also exhibit similar findings of immune activity. Although distinction of such glaucoma subgroups is only arbitrary, it seems quite possible that immunogenic findings are more manifest in patients with normal IOP, while they may easily be disregarded in patients with elevated IOP.

It is unknown whether aberrant immune activity in glaucoma reflects a primary immune deviation. However, recent *in*

*vivo* studies, discussed later, support that glaucomatous tissue stress and neuronal injury may serve as an immunostimulatory signal, thereby eliciting an activated immune response. Although immune response may initially be beneficial for tissue repair, chronic stress-related failure in immunoregulatory mechanisms may lead to neurodegenerative consequences of immune activity facilitating the progression of neurodegeneration at different subcellular compartments of RGCs from the retina to the brain. Different lines of evidence discussed herein suggest that T-cell cytotoxicity, autoantibodies, and the complement cascade contribute to degeneration of RGC bodies, synapses, and axons in glaucoma.

Regarding T-cell cytotoxicity to RGCs, there is *in vitro* evidence that activated T cells may be directly cytotoxic to RGCs and induce RGC apoptosis mainly through death receptor-mediated signaling. Recent *in vivo* studies also support the feasibility of eliciting a T-cell-mediated experimental autoimmune model of glaucomatous neurodegeneration. In rats immunized with HSPs, optic nerve axons are lost and RGCs progressively die by exhibiting a pattern with similarities to human glaucoma, including topographic specificity of cell loss.<sup>39</sup>

It is evident that the glial activation response in glaucomatous eyes involves activation of glial immunoregulatory functions and antigen-presenting ability. The expression of MHC class II molecules on glial cells, required for antigen presentation to T cells, is upregulated in glaucomatous eyes. Not only microglial cells, but also astrocytes exhibit HLA-DR immunolabeling in the glaucomatous human retina and optic nerve head.<sup>2,40</sup> Glial MHC class II molecules are also upregulated in experimental animal models of glaucoma. More interesting, T cells isolated from these ocular hypertensive animals exhibit a stimulated response to retinal proteins as detected by increased proliferation and cytokine secretion of T cells in correlation with neuronal damage. As presented in the conference, initial findings of adoptive transfer experiments in ocular hypertensive rats also support cellular invasion of the retina and increased neuronal damage (Tezel G, et al. *IOVS* 2008;49:ARVO E-Abstract 3699). These preliminary findings support that the immune system is secondarily activated in ocular hypertensive rats and a stimulated T-cell response may facilitate the progression of neurodegeneration. What are the immunostimulatory signals in glaucomatous tissues that can initiate an immune response? Present evidence suggests that the increased expression of HSPs<sup>28</sup> may elicit an immune response, because they are known to be highly antigenic, and the immune system may use changes in the expression of HSPs as a signal to detect and eliminate its own cells that are infected, transformed, or otherwise stressed. As discussed in Session II, amplified generation of ROS is a potent stimulus for RGC death. In addition, recent experimental findings provide evidence that ROS-dependent controlling pathways are also critical for the initiation of an activated immune response. It is evident that ROS regulate immune activity by stimulating the antigen-presenting ability of glial cells, functioning as costimulatory molecules for antigen presentation,<sup>41</sup> and also changing the antigenic repertoire through oxidative protein modifications.<sup>19</sup> Thus, chronic tissue stress at the retina and optic nerve (through HSP upregulation and ROS generation) is an important determinant of the immune response. We do not know whether there are any intrinsic defects of the immune system in patients with glaucoma, which could also contribute to increased susceptibility to immunogenic injury.

Organ-specific autoimmune diseases are triggered commonly by tissue-specific autoaggressive T cells. Despite the privileged immune status and the immunologic self-tolerance to CNS, the immune system is not fully ignorant of nervous tissue. Particularly, during neurodegenerative processes, there

is a shift in the local milieu of the brain from immune-hostile to immune-friendly. In addition to modifications within the CNS, brain-reactive T cells, which are abundant in the healthy immune repertoire but remain innocuous, can be activated and gain access to their target tissues. Before their access to brain tissue, T cells migrating through peripheral lymphoid tissues and blood circulation undergo a profound reprogramming of their gene expression, which renders them fit to enter the nervous system and interact with local cellular elements. These are supported by *in vivo* observations that were made in a rodent model of experimental autoimmune encephalomyelitis (EAE).<sup>42</sup> An amazing presentation of the session included the video-rate, two-photon imaging to trace T cells in CNS lesions of living animals. Fluorescence video microscopy of GFP-tagged myelin basic protein-specific CD4<sup>+</sup> effector T cells documented that activated T cells pass through the blood-brain barrier, invade the CNS tissue, and freely move in the parenchyma. Exogenous autoantigen dramatically changed the motility and function of pathogenic T cells within the EAE lesions, where T cells form contacts with local MHC class II-expressing antigen-presenting cells and produce proinflammatory cytokines, chemokines, and adhesion molecules. Rapid penetration of the CNS parenchyma by autoimmune effector T cells along with multiple autoantigen-presentation events have been found to boost CNS inflammation and aggravate clinical disease. Based on the remarkable density of such immune synapse-like contacts between T cells and target tissue, T-cell-derived proinflammatory mediators have been suggested to act directly on neuronal cells or indirectly by activating local glial cells and attracting and stimulating blood-borne monocytes/macrophages. On the other hand, nonpathogenic, ovalbumin-specific T cells were also found to be recruited to EAE lesions and move through the tissue, but did not contact antigen-presenting cells. However, these cells were similarly arrested and activated after intravenous infusion of ovalbumin and also exacerbated clinical disease. This observation suggests that unrelated antigens such as microbial components may also enter the chronically inflamed CNS and trigger neurodegenerative processes.<sup>42</sup> These findings provide substantial contributions to better understand disease mechanisms in multiple sclerosis and other organ-specific autoimmune diseases. Similar approaches could help improve our understanding of T-cell-mediated immunogenic mechanisms in glaucoma. We currently do not know the organ-specific antigen(s) associated with glaucomatous neurodegeneration. In addition, whether molecular mimicry to microbial components is involved in immunogenic injury is unknown. Despite some supportive preliminary evidence, whether autoimmune T cells pass into the retina and optic nerve parenchyma, and/or interact with resident cells in glaucoma, is also uncertain. However, it should be emphasized that the T-cell invasion is a temporary event, and the lack of observations supporting a parenchymal T-cell invasion in glaucomatous human tissues is not a good argument against an immune pathogenicity and should not exclude a potential immunogenic component of neurodegenerative processes. As noted in Session I, continuous glial activation in the glaucomatous retina and optic nerve head may be sufficient to indicate a neuroinflammatory process in glaucoma.

It is evident that even a small number of T cells can induce a massive cascade of events leading to neurodegenerative injury. It appears that autoimmune reactions are suppressed both systemically and within the target organ and that autoimmune disease results from a breakdown of this suppression.<sup>43</sup> Both neurons and glia in the normal CNS produce immunosuppressive signals that are likely to be attenuated in glaucoma because of neuronal loss and glial dysfunction. To add another degree of complexity, there are bidirectional interactions between T

cells and parenchymal cells. Like many biological responses, there are complex mechanisms regulating T-cell interactions, and the outcome of interactions between invading T cells and resident antigen-presenting cells depends on the nature of T cells and the functional status of parenchymal cells. The type and amount of cytokines and costimulatory molecules induce different effects, either anti-inflammatory or proinflammatory, thereby determining individual differences in susceptibility to autoimmune injury. For example, the expression of modest levels of MHC or costimulatory molecules may inhibit the activation of invading T cells, whereas overexpression of these molecules may promote activation of autoimmune T cells, thereby enhancing the inflammatory cascade leading to tissue damage. If pathogenic T cells are strongly activated, then even lower levels of MHC or costimulatory molecules on parenchymal cells would be sufficient for enhanced activity of invading T cells. In contrast, partially activated T cells appear not to produce pathogenic levels of cytokines, thereby entering a refractory phase of the cell cycle that hinders further activation.<sup>43</sup>

In addition to T-cell-mediated injury, autoantibody-mediated retinal damage has been associated with the pathogenesis of retinal diseases.<sup>44</sup> Increasing serum autoantibodies to different retina and optic nerve proteins have also been identified in patients with glaucoma over the past decade.<sup>38,45</sup> It is unclear whether these serum antibodies are merely an epiphenomenon of the disease process or are pathogenic in nature. There is evidence supporting that exogenously applied antibodies, including HSP antibodies, which exhibit increased serum titers in many patients with glaucoma, can be internalized by retinal neurons. At concentrations similar to those found in the patient sera, these antibodies can facilitate neuronal apoptosis.<sup>46</sup> In support of this experimental finding, there is evidence of retinal immunoglobulin deposition in glaucomatous human donor eyes.<sup>47</sup> On the other hand, some of the autoantibodies detected in the serum are not against RGC proteins and may simply represent a consequence of the disease.

Thus, despite many immunologic associations, a fundamental question remains unaddressed. There is presently no direct evidence to validate that the neurodegenerative injury in glaucoma occurs as the direct result of aberrant cellular or humoral immunity. If there is an immunogenic component of neurodegeneration, what makes this injury organ-specific? Many possibilities must be further evaluated in *in vivo* experimental models. For example, increased autoantibody production may result from normal immunoregulatory processes for host surveillance, and increased serum antibodies may represent the adaptive arm of the immune response to neutralize proteins whose expression or exposure is increased during glaucomatous tissue stress or injury. A more stimulating scenario, however, comes from the evidence of posttranslational protein modifications during the course of glaucomatous neurodegeneration. For example, oxidative protein modifications<sup>19</sup> may change the antigenic repertoire, thereby inducing autoantibody production. Another oxidative stress-related event, generation of advanced glycation end products, may also be associated with the aberrant immune activity in patients with glaucoma. Signaling through a specific receptor for advanced glycation end products has been linked to dysfunction of immunoregulation.<sup>20</sup> It also seems possible that the serum immunoreactivities detected in patients with glaucoma result from molecular mimicry through which an inappropriate host response is mistakenly directed to a self-protein sharing a sequence homology with a specific microbial component.<sup>48</sup>

Analysis of complex antibody profiles in the sera of patients with glaucoma also detects a consistent decrease in some autoantibodies.<sup>45</sup> Importance of such alterations in natural autoimmunity and their relevance to glaucoma also generated

great interest as well as speculation. As noted during the discussions, besides their potential pathogenic importance, serum autoantibodies may serve as biomarkers of the disease. Analysis of complex autoantibody profiles in the serum may therefore have important applications in the future. Additional questions that arose in the discussions included clinical aspects such as whether there is any difference in serum immunoreactivities between newly diagnosed and old disease and whether IOP-lowering treatment results in alterations in the serum autoantibody profile. It was also noted that clinical studies face barriers in studying different aspects of the immune system involvement in glaucoma. Although tissue biopsies facilitate studies of other target organs of an immunogenic injury, this is not the case in glaucoma.

What are the routes for access of the adaptive immune system to the retina? As documented by *in vivo* two-photon imaging, autoreactive T cells pass through the blood-brain barrier and invade the CNS tissue. In addition, peripapillary chorioretinal atrophy zones, optic disc hemorrhages, and alterations in perivascular barriers, all discussed in Session I, may facilitate the access of serum autoantibodies into retina and optic nerve head tissues in glaucoma. Moreover, findings of a recent study provide striking evidence of immunoglobulin production locally by retinal cells, including retinal pigment epithelial cells (Tezel TH, et al. *IOVS* 2008;49:ARVO E-Abstract 208). This exciting observation, when verified further, may change many currently accepted concepts of ocular immunology. Supportive of local antibody production, aqueous humor samples obtained from patients with glaucoma exhibited increased autoantibody titers, even exceeding the serum titers.<sup>49</sup>

Finally, growing evidence supports the involvement of the complement cascade in the neurodegenerative injury in glaucoma. Recent histopathologic studies of human tissues as well as *in vivo* studies using animal models have demonstrated that different complement components are synthesized during glaucomatous neurodegeneration.<sup>50,51</sup> As presented in the conference, ongoing studies using genomics and proteomics also support the activity of the classic complement cascade in experimental glaucoma, including C1q, the initiating protein, and C3, a downstream protein. In addition, terminal complement complex has been shown to be formed in the retina of both human and rat glaucoma (Luo C, et al. *IOVS* 2008;49:ARVO E-Abstract 3284),<sup>51</sup> whereas limited evidence also supports alterations in complement regulatory molecules. These discussions were further motivated by findings of a more recent study of mice deficient in complement components C1q or C3. Findings of this exciting study provide direct evidence that the classic complement cascade is involved in selective elimination of unwanted synapses during development and suggest that complement-mediated synapse elimination may also become aberrantly reactivated in neurodegenerative disease. Regarding glaucoma, neuronal C1q becomes upregulated in a mouse model of glaucoma and is relocalized in retinal synapses early in the disease.<sup>52</sup> These observations reveal that stressed or injured RGCs are targeted and destroyed through complement mediated processes involving reactive glia. Complement activity, as a necessary step, may minimize the activation and/or duration of inflammation by simply serving as a tissue cleaning process for removing debris from dying RGCs in glaucomatous eyes. However, inappropriate activation of the complement cascade can also accelerate the neurodegenerative injury, since complement activation has the potential to exacerbate RGC death through bystander lysis or glial activation. These also bring about many questions. Are there any differences between complement activity during development and disease? What is the first signal for complement activity during glaucomatous neurodegeneration, whether glia-driven signals initiate the complement cascade for tissue cleaning, or

whether signals from stressed or injured neurons initiate complement-mediated neurodegenerative processes for self-destruction? What are the surface markers that allow complement to bind RGCs for cell lysis or activation of apoptosis programs? Is oxidative stress involved in aberrant activity of the complement cascade? Are alterations in the expression or function of complement regulatory molecules important? Interactions between adaptive immune cells and complement components are also unclear. Is immune complex required for complement activity in glaucoma? What are the possible treatment targets to prevent complement-mediated neuronal injury? Most important, does inhibition of the complement cascade delay degeneration of RGCs or axons in human glaucoma?

The conclusion of this session was that, although uncontrolled immune activity may be a potent stimulus for neurodegenerative injury, caution must be applied in the interpretation of data relevant to glaucomatous neurodegeneration, as many observations await further confirmation. Several issues concerning the immunogenic mechanisms are poorly understood and many fundamental questions remain. Ongoing efforts should better illuminate cellular mechanisms involved in the regulation and dysregulation of immune activity, thereby having important implications for glaucomatous neurodegeneration and its neuroprotective treatment.

## IMMUNOMODULATORY TREATMENT POSSIBILITIES FOR NEUROPROTECTION

Efficient control of intrinsic immunoregulatory mechanisms and interactions between adaptive immune cells (lymphocytes) and resident and infiltrating innate immune cells (resident glia and infiltrating macrophages) are critical to determine the outcome of an immune response as being protective or destructive. Advances in understanding of immunogenic mechanisms support that although immunoregulatory mechanisms make neuronal tissue homeostasis possible, dysregulation of normal immune activity may result in autoimmune neurodegenerative injury. Therapeutic manipulation of the immune response for the gain of tissue repair is therefore referred to as immunomodulation. Ongoing studies are testing an increasing number of innovative immunomodulatory strategies as a promising alternative to classic immunosuppressive treatments.<sup>53</sup>

One of the most efficient and specific ways to treat organ-specific autoimmune diseases has been based on systemic administration of native autoantigen or its altered peptide variants. The mechanism underlying the therapeutic success of antigen-based strategies remained unclear for many years, until it was shown that high doses of antigen drive specific autoreactive T cells into activation-induced cell death. Such strategies have been considered to be an important therapeutic option for the treatment of autoimmune diseases, because they selectively target depletion of disease-inducing T cells in blood circulation or trapping these cells in peripheral lymphoid organs, thereby preventing them from invading the target organ.

Antigen-based immunomodulatory treatments aiming to elicit immunogenic tolerance to tissue-specific antigens may also induce regulatory T cells. For example, expression of specific uveitogenic retinal antigens in the periphery by hydrodynamic DNA vaccination has been found to afford protecting from autoimmune injury through induction of regulatory T cells.<sup>54</sup> However, many issues concerning the induction of regulatory versus effective immune responses should be carefully considered, since such approaches are not risk free and may exacerbate rather than protect from disease. Advantages and disadvantages of protein versus DNA vaccines, with or without adjuvant injection, application time, delivery route, vehicle, and duration of effect, should also be carefully weighed.



Antigen-based strategies have also been proposed to modulate T-cell activity, somehow converting them to a protective role. This proposal is based on the view that the immune system is constantly involved in the maintenance of CNS functional integrity, and the adaptive and innate immune cells play a protective role under various neurodegenerative conditions in the brain. It has been suggested that the loss of immunity to certain self-antigens or its insufficiency in the presence of increased levels of risk factors play an important role in neurodegenerative processes. It has been proposed that vaccination could be a means of recruiting the immune system to help eliminate many adverse factors associated with glaucomatous neurodegeneration and perhaps also supporting cell renewal and repair. Such a vaccine is thought to induce a beneficial immune response that recruits immune effector cells to counteract or neutralize some destructive factors, thereby preventing disease progression, although not its onset. Synthetic antigens that weakly cross-react with self-antigens in the retina and optic nerve are proposed for vaccination, whereas the choice of antigen, dose, regimen, and timing remain decisive. Similarly, modulating the innate component of immune activity—namely, the microglial response—may help tissue cleaning and repair, increase local production of growth factors, and promote neuronal survival and neurogenesis; however, the number, site, and activity status of microglia are also critical to determine distinctive effects on neuronal cell fate.<sup>37</sup> To put the view into practice, which states that T-cell/glia/macrophage interactions should be well-controlled and synchronized, precise control mechanisms of the immune activity should be identified.

Alternative immunomodulatory strategies include T-cell degrading antibody treatment or stem cell transplants. Neural stem cell transplantation was originally proposed as a means of replacing cells in neurodegenerative diseases of the CNS. However, recent data regarding their beneficial effects in various animal models of neurologic diseases indicate that transplanted stem cells may also be immunomodulatory by attenuating deleterious inflammation, protecting neural tissue from degeneration, and enhancing endogenous recovery processes. When transplanted into the CNS of rodents with EAE, multipotential neural precursor cells have been attracted by the inflammatory process to migrate exclusively into inflamed white matter. Intraventricular transplantation of neural spheres has significantly reduced the clinical and pathologic signs of EAE, including brain inflammation, axonal damage, and demyelination.<sup>55</sup> Systemic administration of neural precursors has also exerted an immunomodulatory effect and inhibited neuroinflammation by peripheral immunosuppression. These exciting observations suggest that neural stem cells offer a feasible method for immunomodulation, although many details remain unclear. An important question to address is how the therapeutic effect of stem cell therapy in neurodegenerative diseases can be optimized to be able to use both regenerative and immunomodulatory properties of these cells.

A related discussion was focused on the activities of immune system cells that can contribute to neurogenesis. Local immune response has been proposed to play a crucial role in awaking the dormant neurogenesis niche, and immunologic manipulations have been suggested to serve as a therapeutic means for controlled migration of stem/progenitor cells to injured CNS sites.<sup>56</sup> Similar to glia, adult neural stem/progenitor cells also express TLRs, which may have important implications in neurogenesis, as well as in innate immunity as discussed in previous sessions. An improved understanding of TLR signaling and its distinct and opposing functions can help modulate cell fate decisions.

Targeting specific immunomodulators involved in immunogenic injury constitutes another strategy for immunomodula-

tion. As discussed through this session, tissue infiltrating macrophages and resident microglia maintain normal tissue homeostasis. In many immunogenic injuries to the retina, persistent changes in microglia and macrophage behavior, including cytokine secretion, play a critical role in the overall phenotype by driving T-cell responses, contributing to neuronal loss, and facilitating regenerative failure via impaired turnover of retinal progenitor cells.<sup>57</sup> Therefore, targeting specific immunomodulators involved in this sequence of events could also be a logical immunomodulatory strategy. Obviously, identification of molecules involved in disease pathogenesis would be the critical center point of such a treatment option for glaucoma similar to previous applications in other diseases.<sup>58</sup>

What impact will immunomodulatory treatment strategies have in glaucoma? There is no doubt that the wisdom of immunomodulation as a treatment option for glaucomatous neurodegeneration awaits a better understanding of the role of immunogenic mechanisms. Identification of molecules involved in immunoregulation and dysregulation and identification of target autoantigen(s) and neurodestructive mediators would then offer specific immunomodulatory methods of treating glaucoma.

## FINAL REMARKS

Through this stimulating conference, expert scientists reviewed the current understanding of the role of glia, mitochondria, and the immune system in glaucomatous neurodegeneration. Present evidence provides a clearer perspective as to how these seemingly independent events actually intersect and interplay. A critical dynamic balance of cellular interactions and intracellular pathways determines neuronal cell fate in response to stressful conditions. Immune response to a stressful insult may initially be beneficial in limiting neurodegenerative consequences. However, growing evidence supports that a failure to properly control immune activity may subsequently convert protective immunity into an autoimmune neurodegenerative process in glaucoma, resulting as much from neuronal injury and glial dysfunction as from immune system dysregulation (Fig. 1). Are there any intrinsic defects to RGCs and/or glia that could specifically increase the susceptibility of RGCs to an immunogenic injury? This question is the key that can lead us to a better understanding of the immunogenic component of glaucomatous neurodegeneration. Chronic tissue stress and age-dependent factors appear to be critical in the failure of regulation of immune activity as well as the increase of neuronal susceptibility to injury in glaucoma. Mitochondrial dysfunction and the resultant oxidative stress are directly involved in neuronal damage, but may also facilitate dysregulation of immune activity during glaucomatous neurodegeneration. Similarly, chronic activity response and the accompanying dysfunction of neurosupportive glia under glaucomatous stress may initiate potentially neurotoxic influences, as well as affect immunoregulatory functions.

It is evident that glial antigen presentation is stimulated in glaucomatous tissues, along with the loss of normal immunosuppression due to neuronal loss and glial dysfunction. Oxidative stress stimulates the antigen presenting ability of glial cells in glaucoma, whereas ROS also act as costimulatory molecules during antigen presentation. Many other consequences of oxidative stress may also facilitate aberrant immune activity. For example, oxidative protein modifications may change the antigenic repertoire and may also downregulate immunoregulatory molecules, thereby serving as an immunostimulatory signal. Many factors evident in glaucoma, including an increase in antigenicity due to increased protein expression and posttranslational protein modifications, an increase in highly antigenic

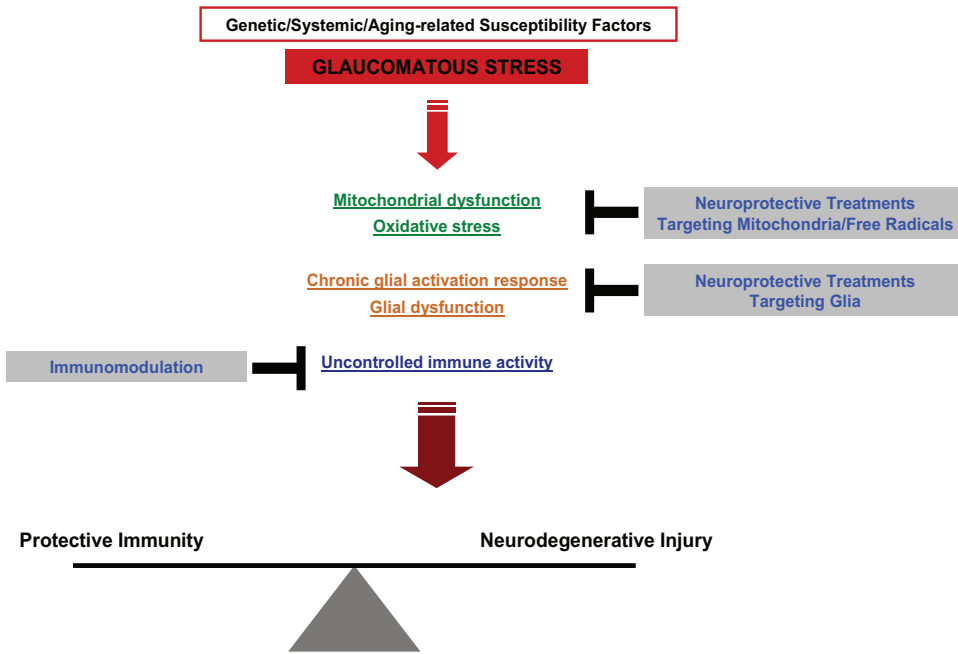


FIGURE 1. There is a critical dynamic balance that determines neuronal cell fate in response to stressful conditions. Immune response may initially be beneficial in limiting the neurodegenerative consequences; however, a failure to properly control immune activity may convert protective immunity into an auto-immune neurodegenerative process in glaucoma.

stress proteins, and an increase in the exposure of proteins due to cell death, may further contribute to failure in the control of immune activity in glaucoma. In addition, chronic tissue stress in glaucomatous eyes may lead to increased contact of the retina and optic nerve head tissues with systemic immune cells due to alterations in perivascular barriers. Thus, oxidative stress appears to be a critical factor placed at the glia/mitochondria/immune system intersection during glaucomatous neurodegeneration (Fig. 2). Oxidative stress resulting from an amplified generation of ROS due to mitochondrial dysfunction in glaucoma, along with the aging-dependent component of oxidative stress, represents a route for the conversion of a protective immune response into a neurodegenerative process over a chronic and possibly cumulative period.

By highlighting the most important questions that are still unanswered or need further exploration, this conference outlined future experimental goals. Based on a broad scientific exchange, ongoing research should be designed to improve the current understanding of the role of glial activation, mitochondrial dysfunction, and the immune response in compartmental events during glaucomatous neurodegeneration, including RGC soma death and axonal degeneration. These studies should validate whether immune activity plays a causative role in the initiation and/or progression of glaucomatous neurodegeneration. An improved understanding of the precise cellular mechanisms of glaucomatous neurodegeneration and necessary validation of its immunogenic component can provide biomarkers and help design neuroprotective treatments to ma-

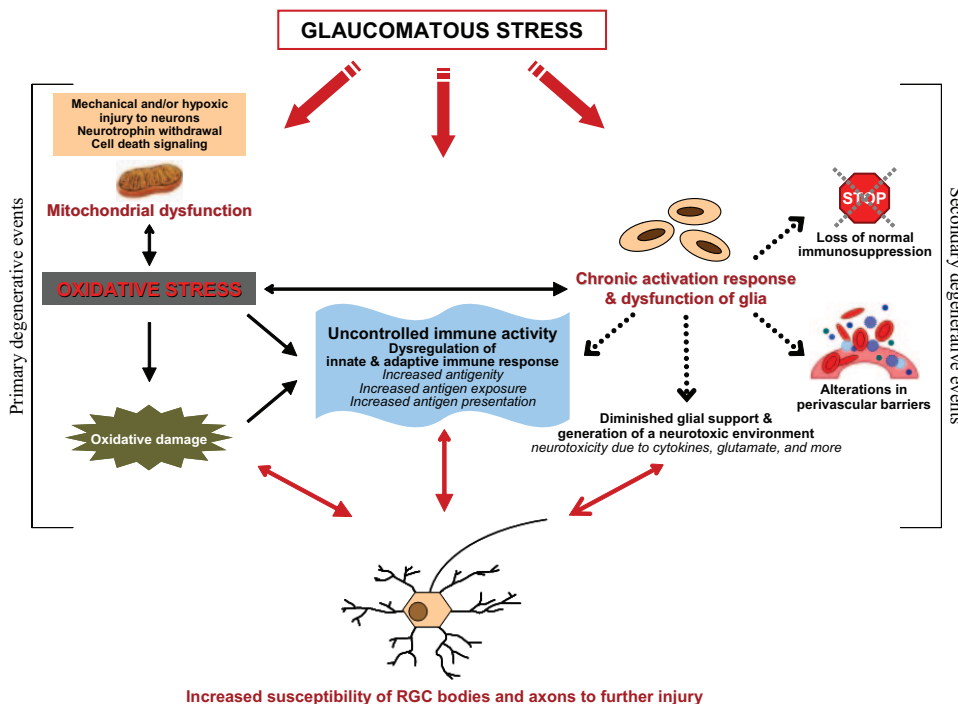


FIGURE 2. Oxidative stress appears to be a critical factor in the neurodegenerative consequences of mitochondrial dysfunction, glial activation response, and uncontrolled activity of the immune system during glaucomatous neurodegeneration.

nipulate the immune response toward tissue repair and enhanced neuronal survival and function, while avoiding autoimmune neurodegenerative injury.

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