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Retinal synthesis and deposition of complement components induced by ocular hypertension

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Abstract

Inappropriate activity of the complement cascade contributes to the pathophysiology of several neurodegenerative conditions. This study sought to determine if components of the complement cascade are synthesized in the retina following the development of ocular hypertension (OHT) and if complement accumulates in association with retinal ganglion cells. Toward this goal the gene expression levels of complement components 1qb (*C1qb*) and 3 (*C3*) were determined in the retina by quantitative polymerase chain reaction in human eyes with elevated intraocular pressure (IOP) and healthy retinal tissue as well as in a rat model of OHT induced by laser cauterization of the trabecular meshwork and episcleral veins. Immunohistochemical methods were employed to determine the sites of complement deposition in the retina and optic nerve head. Our data demonstrate that transcript levels for *C1q* and *C3* are significantly elevated in retinae subjected to OHT, both in the animal model as well as in human eyes. Immunohistochemical analyses indicate that C1q and C3 accumulate specifically in the retinal ganglion cell layer and the nerve fiber layer. In addition, we demonstrate that the terminal complement complex, or membrane attack complex, is formed both in the human and rat model as a consequence of OHT. Complement activation, particularly formation of membrane attack complexes, has the potential to exacerbate ganglion cell death through bystander lysis or glial cell activation. The results show that complement activation occurs in the retina that has been subjected to elevated IOP, and may have implications in pathophysiology of glaucoma.

Keywords: ocular hypertension; immunohistochemistry; complement system; ganglion cell; glia; animal model; human; gene expression

1. Introduction

Elevated intraocular pressure (IOP) is an important risk factor for glaucoma and to date pharmacological and surgical reduction of IOP remains the only accepted treatment of this disease (Alward, 1998). Glaucoma is a leading cause of vision loss worldwide and, despite many recent improvements in the diagnosis and treatment of this disease, continues to pose a clinical challenge. This group of diseases is characterized by the slow, progressive loss of retinal ganglion cells (RGC) and their axons, and is clinically recognized by progressive excavation of the optic nerve head and resultant visual field loss. Many aspects of the molecular pathogenesis of glaucoma remain unclear and the loss of vision is irreversible. In particular, the biochemical, cellular and molecular events that are initiated by elevated IOP in the retina and in the optic nerve head (ONH) remain active areas of research (Kuehn et al., 2005). A number of studies suggest that RGC die through apoptosis, but the molecular events that precede or initiate this process in the glaucomatous retina remain unclear (Farkas and Grosskreutz, 2001).

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Preliminary microarray data generated by our laboratories indicated that retinal expression of a number of components of the complement cascade is elevated following experimental elevation of IOP in a rat model of ocular hypertension (OHT) induced by laser cauterization of the episcleral veins and trabecular meshwork (Kim et al., 2005). Elevated transcript levels were most consistently observed for the members of complement component 1 complex and complement component 3. The complement system consists of approximately 25 plasma- and membrane-bound proteins that function together to protect the host from bacterial or parasitic infection through T-cell independent mechanisms. Complement can be activated through several pathways, all of which converge to generate multimolecular enzyme complexes that cleave the complement components 3 (C3) and 5 (C5). If left uninhibited, complement activation results in the formation of the terminal complement complex or membrane attack complex (MAC). This macromolecular complex is formed by complement components C5b, C6, C7, C8, and multimers of C9 and forms a transmembrane channel that enables ions and small molecules to diffuse freely through cell membranes via this newly formed pore, resulting in severe disturbance of cellular homeostasis and cell lysis. In addition, complement initiates local inflammatory responses through production of the anaphylatoxins C3a and C5a and promotes phagocytosis of the bacterial cells through opsonization. Recent reports suggest that opsonization is also required for the efficient phagocytosis of an organism's own cellular debris (Taylor et al., 2000). For example, inherited deficiencies of the classical complement pathway components, particularly C1q, are strongly associated with the development of systemic lupus erythematosus or glomerulonephritis presumably by causing autoimmunity through delayed or insufficient clearance of apoptotic cells (Botto et al., 1998).

On the other hand, inappropriate activation of complement appears to be involved in a number of neurodegenerative conditions. Complement plays a crucial role in the pathobiology of many neuroinflammatory conditions, including traumatic or ischemic brain injury, Guillain–Barre syndrome, multiple sclerosis, as well as Alzheimer's and Parkinson's diseases (Cowell et al., 2003; Rancan et al., 2003; Yamada et al., 2004). Recently, genomic variations in the factor H gene, an inhibitor of the complement cascade, have been reported to be associated with the development of age-related macular degeneration (Edwards et al., 2005; Hageman et al., 2005; Haines et al., 2005; Klein et al., 2005). The precise role of complement in the etiology of these conditions is unresolved but it seems clear that local inflammation can exacerbate neuronal loss and that complement inhibition often results in neuroprotection (van Beek and Elward, 2003; Fonseca et al., 2004).

The majority of complement proteins are synthesized in the liver and circulate in the plasma. However, extrahepatic synthesis of complement components has been documented in many cell types including fibroblasts, synovial cells, adipocytes, kidney glomerular mesangial cells, keratinocytes and macrophages (reviewed in (Morgan and Gasque, 1997). In recent years, it has been reported that complement gene transcription and protein synthesis also occurs in glia and neurons, (Nataf et al., 1999) including retinal cells and in the optic nerve following crush (Bora et al., 1993; Sohn et al., 2000; Ohlsson et al., 2003). Endogenous production of complement by neuronal cells may be particularly important in immune privileged sites because it provides a level of protection against invading pathogens, but it introduces the possibility that inappropriate activation or insufficient regulation of complement damages neuronal cells.

The goals of this study were to determine whether complement components are deposited in retinae subjected to OHT, which retinal structures are associated with complement and if the final mediator of complement cell lysis, MAC, is formed. Toward this goal we conducted an immunohistochemical evaluation of the retina and optic nerve head of a rat model of OHT induced by laser cauterization of the episcleral veins and trabecular meshwork (Grozdanic et al., 2004). These data were correlated to those obtained from human donor eyes. Our findings indicate that complement components C1q and C3 are transcribed in the retina and accumulate in the nerve fiber layer (NFL) and ganglion cell layer (GCL) of eyes with OHT. Our data further suggest that MAC is formed in this region, which may further exacerbate RGC damage.

2. Material and methods

2.1. Rat model of ocular hypertension

Elevated intraocular pressure was induced in one eye of adult Brown Norway rats as described in detail previously (Grozdanic et al., 2004). Briefly, animals were anesthetized by isofluorane inhalation and indocyanine green was injected into the anterior chamber of one eye. Twenty minutes post-injection, a diode laser (DioVet, Iridex Corp.) was used to externally deliver 810 nm energy pulses through a 50-μm fiberoptic probe to the region of the trabecular meshwork and episcleral veins in close proximity to the limbal region. Approximately 50 laser spots were delivered through a 300° range of the limbal radius (300 mW energy, 400 ms pulse time). The resulting IOP elevation was measured weekly in isofluorane anesthetized animals with a hand-held tonometer (Tonopen XL, Mentor, Norwell, MA). Only animals that developed elevated IOP were included in this study. Rat eyes were harvested either 14 or 28 days after the induction of elevated IOP.

All research conducted in this study was in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and Iowa State University Committee on Animal Care Regulations.

2.2. Evaluation of optic nerve damage

Thin sections of the rat optic nerve were prepared using standard electron microscopy methods as described by others (Smith et al., 2002). Immediately after death optic nerves were carefully dissected and a 3-mm long portion of optic nerve, located approximately 2 mm posterior to the eye cup, was carefully excised and preserved in 2% glutaraldehyde and 2%