Neurodegeneration and Neuroprotection in Glaucoma: Development of a Therapeutic Neuroprotective Vaccine
The Friedenwald Lecture

Michal Schwartz

Glaucoma can be viewed as a neurodegenerative disease that is often associated with high intraocular pressure (IOP), and in which at any time there are fibers (optic nerve axons) and cell bodies (retinal ganglion cells) that are vulnerable to degeneration and amenable to protection. According to this view, patients receiving antihypertensive therapy to control an increase in intraocular pressure should also receive neuroprotective treatment that will circumvent, bypass, or reduce the threat to the neurons imposed by the degenerating neurons. Vaccination, proposed herein as a therapeutic measure, slows down disease propagation, in much the same way as it reduces secondary degeneration after acute insult to the rat optic nerve. The proposed vaccination is based on the unexpected discovery of "protective autoimmunity," according to which a mechanical injury (optic nerve crush) or biochemical insult (glutamate toxicity in retinal ganglion cells) evokes a physiological autoimmune response which is specific to self-antigens residing in the site of damage and protects the nerve against the degenerative effects of glutamate and other destructive self-compounds. Protection was found to be boosted, without risk of autoimmune disease development, by vaccination with Cop-1, a synthetic antigenic copolymer which weakly cross-reacts with a broad spectrum of self-reactive (autoimmune) T cells, thus safely activating them for self-repair. The proposed vaccination can therefore be viewed as a way of boosting the body's physiological defense and repair mechanisms. Once its regimen and formulation are optimized for protection of retinal ganglion cells against death induced by an increase in IOP, Cop-1 can be immediately developed as a therapy for glaucoma.

BACKGROUND

Regeneration and Neuroprotection of the Injured Central Nervous System

Injury to the central nervous system (CNS) causes irreversible functional loss, as there is little or no neurogenesis (because adult neurons cannot proliferate and repopulate the site of injury), little or no regeneration (because neurons with axonal damage have limited capacity for spontaneous regrowth), and ongoing secondary degeneration (because the primary neuronal loss creates an environment hostile to neurons that escaped direct injury, causing them to degenerate).

Research in my laboratory over the past two decades has been aimed at finding ways to promote the recovery of damaged nerve fibers. In the course of these studies, it became clear that only part of the functional loss after an injury is due to neuronal losses caused by primary transection of nerve fibers and for which the appropriate therapy would be nerve regeneration. A significant part—and sometimes the major part—of the loss of function is due to delayed degeneration of fibers that escaped the primary injury. This secondary loss is a consequence of numerous injury-related processes, which were found to be common to many acute and chronic neurodegenerative disorders (Fig. 1). The appropriate therapy for preventing or minimizing the degeneration of neurons that escaped direct injury is neuroprotection. Neuroprotective therapy is a general term referring to any therapeutic approach that neutralizes, circumvents, and prevents neuronal losses caused by self-destructive processes. Our research focuses on achieving recovery by both neuroprotection and neuroregeneration.

The devastating processes triggered by the injury affect and are affected by events associated with cells that support the neurons, and not only with the neurons themselves. Thus, for example, if astrocytes die or malfunction as a result of the primary injury, the normal capacity of the neuronal environment to buffer neurotoxic agents is reduced or destroyed. This contributes to an increase in toxicity and further affects both neurons and astrocytes, leading to their death (Fig. 2).

Research groups in many parts of the world have been seeking ways to stop or at least slow down the process of damage propagation as a therapeutic strategy after acute nerve injury. Recently, it became clear that such neuroprotective therapies would also be applicable to chronic neurodegenerative disorders. Common strategies include pharmacological intervention (for example by glutamate-receptor antagonists, α2-adrenoreceptor agonists, Ca2+ blockers, scavengers of free radicals) and molecular intervention (for example, the use of anti-apoptotic or survival genes to increase neuronal resistance to injurious conditions). The neuroprotective strategy developed by my research group, and presented in this lecture, is based on the assumption (borne out by experimental evidence) that the body harnesses the immune system to help cope with the stressful conditions imposed by an injury. In practical terms, this requires reinforcement of the immune response by boosting the body's own mechanisms of defense and repair, while avoiding the risk of autoimmune disease.

Partial-Crush Injury of the Rat Optic Nerve: Relevance for Glaucoma

At an early stage of our research, we developed a model of a well-controlled partial injury of the rat optic nerve as a way to...
Immune Activity in the CNS after Injury

The primary role of the immune system is to protect the body against destructive elements, clear it of threatening material, and facilitate tissue repair. The CNS, however, partly because of its status as a site of “immune privilege,” partly because of its high incidence of autoimmune diseases (such as multiple sclerosis), and partly because inflammation is often seen in conjunction with acute and chronic degenerative conditions, has therefore been considered off-limits to these immune activities. As a result, immune activity in general, and autoimmunity in particular, have been viewed as harmful in the CNS, and the therapeutic strategy for acute CNS injuries and chronic degenerative conditions has therefore often been one of immune suppression.

Our studies suggested, however, that an inflammatory response in the injured CNS, provided that it is well-regulated, is essential in helping the damaged tissue cope with the injurious conditions. Our early work in this connection showed that traumatic injury to the optic nerve is followed by an accumulation of T cells at the site of the lesion. According to the accepted view, such an accumulation would be interpreted as having a negative effect on nerve recovery. In light of our earlier experience with macrophages, however, we thought it possible that the accumulated T cells in the damaged optic nerve might have a beneficial function, but that their activity is not strong enough to have a perceptible effect. This suspicion was borne out when we discovered that increasing the number of T cells that home to the lesion site has a positive impact on nerve recovery (i.e., it reduces the neuronal loss resulting from secondary degeneration), provided that the systemically injected T cells include at least some that are specifically directed to myelin-associated antigens. Subsequent studies showed that this T-cell-mediated protection against neuronal death is not restricted to injury of optic nerve axons but is also evident after spinal cord injury. Thus, passive transfer of myelin-specific T cells into the contused spinal cord of rats protected viable axons from secondary degeneration.

It is important to note that in the injured optic nerve or spinal cord, the beneficial effect of the T cells is discernible in spite of the transient appearance of symptoms of a monophasic experimental autoimmune disease caused by the transferred...
T cells. Thus, the benefit apparently outweighs the cost. We subsequently discovered that similar benefits can be obtained if we use T cells that recognize a nondominant epitope within the myelin antigens. In this way we can obtain the protective benefit unaccompanied by symptoms of the disease.

These and other findings raised an important question: Is the T-cell–mediated neuroprotective activity a physiological response or merely the result of an experimental manipulation? Our studies demonstrated that the injury evokes a protective autoimmune response, which is a physiological response to an insult, at least in the CNS. This was shown by the finding that in the absence of T cells, fewer neurons survive a crush injury to the optic nerve or exposure of RGCs to glutamate toxicity, and that recovery in the optic nerve is better if the insult is preceded by another CNS injury (e.g., spinal cord contusion).

Our working hypothesis was that if protective autoimmunity is a physiological mechanism designed to cope with stressful conditions in the CNS, individuals might differ in their ability to withstand the degenerative consequences of a CNS insult. Also, the absence of T cells would wipe out this protective response, although all can manifest anti-self (i.e., autoimmune) reactions in the eye. We further postulated that these antigens are especially affected by antigens that reside in the eye. We therefore reasoned that the activated T cells must be activated by their specific antigens presented to them at the site where protection is needed—that is, at the site of the lesion. Our experiments indeed showed that RGCs damaged by direct exposure to toxic amounts of glutamate are beneficially affected by antigens that reside in the eye. We further postulated that these antigens are identical with the immunodominant proteins causing the ocular autoimmune disease that develops in strains susceptible to autoimmune disease development. This hypothesis is in line with our perception of an autoimmune disease as a failure in the mechanism controlling the purposeful autoimmune response needed for defense against self-destructive compounds.

Boosting of Protective Autoimmunity as a Therapeutic Approach for Glaucoma

Based on the results summarized above, we suggest that T cells orchestrate the local immune response to destructive self-compounds. We further suggest that: antigenic specificity is needed both for homing of T cells and for their local activation. The activated T cells augment and regulate the local cellular immune response needed to clear the lesioned site of cell debris and other potentially destructive materials. The same cells (i.e., T cells with identical phenotypes and antigenic specificities) might be responsible for both destruction and protection, and the difference in their effects lies in their regulation. Thus, the essence of the beneficial response is a dialogue between T cells and resident microglia. Once activated, the microglia are better equipped to buffer toxicity (e.g., glutamate) in a receptor-dependent fashion (Shaked et al, unpublished observations, 2003), to function more efficiently as phagocytic cells capable of eliminating toxic self-compounds (Shaked et al., unpublished observations, 2003), and to act as a source of neurotrophic factors and cytokines to the activated T cells.

In boosting the beneficial autoimmune response it is important, for reasons of safety, to use a weak antigen (i.e., one that will not induce an autoimmune disease). This can be a cryptic epitope, an altered peptide ligand, or a synthetic antigen such as copolymer 1 (Cop-1), all of which can activate low-affinity self-reactive T cells. It was recently proposed by David Hafler that Cop-1 be viewed as a weak self-reactive antigen much like an altered peptide ligand.
Cop-1, a random copolymer of four amino acids, was originally synthesized to mimic myelin basic protein, and was subsequently approved by the FDA as an effective treatment for multiple sclerosis. We discovered that this copolymer can activate a wide range of low-affinity self-reactive T cells. In this way it can circumvent the tissue specificity barrier needed for T cell–mediated neuroprotection.28 Using a rat model of raised IOP, we recently discovered that the ability of these animals to resist a pressure-induced loss of RGCs is immune-dependent and varies among strains.29 In the absence of mature T cells, RGC losses are greater in strains that are better equipped to cope with the stress.29

We found that Cop-1 vaccination protects rats and mice from the consequences of optic nerve injury.21 We further found that vaccination with Cop-1, unlike immunization with myelin antigens, is neuroprotective in a model of glutamate toxicity in the eye, a myelin-free site.22 In a rat model of high IOP, vaccination with Cop-1 significantly reduces the pressure-induced death of RGCs22 (Fig. 5). An interesting finding was that RGC death in this model was amenable to treatment by antigen–residing in the retina but not in the myelin (Bakalash et al., unpublished data).

**Concluding Remarks**

On the basis of the experiments outlined herein, we conclude that glaucoma, like other neurodegenerative diseases, may be amenable to neuroprotective therapy, possibly administered in combination with anti-hypertensive medication. It should be emphasized that therapeutic vaccination will not prevent the onset of glaucoma, but it may provide a way to prevent or at least slow down its propagation. Vaccination with self- or self-like antigens may be viewed as boosting the physiological mechanism of neuroprotection. Accordingly, patients with glaucoma are likely to benefit from a safely boosted immune response, which is mediated by weak self-reacting T cells, and can be viewed as helping the body to protect itself against destructive self-compounds emerging as a result of an increase in IOP and causing tissue loss. We suggest that the controlled immune response does not exacerbate the outcome of increased IOP directly, but prevents local self-destructive compounds and processes from exacerbating the outcome of the IOP increase. Pathogenic autoimmunity (leading to autoimmune disease) may be a result of breakdown in the regulation of a physiological autoimmune process.30 Cop-1, an FDA-approved drug for the treatment of multiple sclerosis, is a promising candidate as a safe therapeutic vaccine for patients with glaucoma. It is important to note that the proposed vaccine will incorporate Cop-1 in a different formulation and according to a different regimen from those used for treating an autoimmune disease, as the same compound activates different mechanisms in different regimens.21,32 Since degeneration is a highly complex process, in which the players are affected by a multiplicity of factors (some of them mutually contradictory), therapeutic intervention by a single factor is likely to prove only partially effective. Activation of the patient’s own immune cells may provide comprehensive protection that is both self-regulating and self-limiting.

**Author’s Note**

This paper covers part of the material presented in the Friedenwald Award lecture, and is not intended as a comprehensive review. The list of cited publications, therefore, refers mainly to work done in the author’s laboratory.

**Acknowledgments**

The author wishes to thank Shirley Smith for scientific editing.

**References**


