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

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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, this view, patients receiving antihypertensive therapy to control 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 traditionally 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. These myelin-specific T cells were found to be neuroprotective in function, and their beneficial effect on nerve recovery was manifested both morphologically and functionally. 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. The most pronounced effect of the T cells in the injured spinal cord, in addition to the rescue of neurons from otherwise inevitable death, is the reduction of cavity formation. 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

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This was shown by the protective autoimmunity, which is a physiological response to an insult, at least in the CNS.18 This was demonstrated by the ability of T cells to resist the development of an autoimmune disease.19 We further demonstrated that the absence of T cells would wipe out this protective response correlates with the ability, when challenged with a myelin antigen, to resist the development of an 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.24

**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 antigen specificities) might be responsible for both destruction and protection, and the difference in their effects lies in their regulation.20 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 cytokines23 (Fig. 4).

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,19 an altered peptide ligand,26 or a synthetic antigen such as copolymer 1 (Cop-1), all of which can activate low-amounts of self-reactive T cells.21,22 It was recently proposed by David Hafler that Cop-1 be viewed as a weak self-reactive antigen much like an altered peptide ligand.27

**FIGURE 4.** Representation of the cross-talk between T cells and resident microglia, possibly leading to neuroprotection. T cells home to their specific self-antigens residing in the lesion site. Their adhesion there is facilitated by injury-induced changes in the extracellular matrix. On encountering their relevant antigens they become locally activated. The activated T cells, found to be Th1 cells,26 produce cytokines such as interferon-γ, which further activate microglia to become antigen-presenting cells, as well as to buffer glutamate and remove threats. Tmbp, T cells specific to myelin basic protein; ECM, extracellular matrix protein; MG, microglial cell; APC, antigen-presenting cell.

T cells.4 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.9

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?15-17 Our studies demonstrated that the injury evokes a protective autoimmune, which is a physiological response to an insult, at least in the CNS.18 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).18,19

Our working hypothesis was therefore that if protective autoimmune 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 difference between individuals, and tolerance (defined as nonresponsiveness) to myelin would eliminate the ability to resist the consequences of axonal injury. We found that not all animals are equally capable of manifesting this protective response, although all can manifest anti-self (i.e., autoimmune) activity. We showed, moreover, that the ability to manifest a protective response correlates with the ability, when challenged with a myelin antigen, to resist the development of an autoimmune disease.19 We further demonstrated that the absence of T cells indeed wipes out the differences between strains that are “resistant” and those that are “susceptible” to injurious conditions, eliminating the advantage of strains that normally recover better.18,19 Neontal immunization of rats with myelin abolishes the ability of the adult rat to respond to myelin or to withstand injurious conditions imposed by axonal injury.20

Taken together, the above findings led us to formulate the concept of protective autoimmune as a physiological response to a CNS insult and to suggest that individuals differ in their ability to manifest this response. In the case of injury to myelinated axons the response is specific to myelin antigens, and is amenable to boosting by peptides related to derived from myelin. A T cell–dependent protective mechanism was also found to operate in response to glutamate toxicity imposed directly on RGCs.19,21-23 In this case, myelin antigens were unable to boost protection. We therefore reasoned that to be effective, the 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 benefically 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.24

**FIGURE 5.** Effect of chronically increased IOP and Cop-1 immunization on RGC survival in Lewis rats. (A) Laser cauterization, causing occlusion of the episcleral and limbal veins, results in an increase in IOP and subsequent death of RGCs. Three weeks after laser treatment the mean IOP was 30.4 ± 0.42 mmHg (mean ± SE, n = 5) in rats subjected to venous occlusion and 15.8 ± 0.2 mmHg (n = 7) in naive rats. (B) Rats were immunized with Cop-1 in complete Freund’s adjuvant (CFA) (n = 15) or injected with phosphate-buffered saline (PBS) in CFA (n = 15) immediately after laser cauterization. Bars represent RGC loss, calculated as a percentage of the number of RGCs in naive rats (mean ± SEM). The difference in the numbers of RGCs in the two groups was significant (P < 0.002, two-tailed t test). IOP, intraocular pressure; RGC, retinal ganglion cell; CFA, complete Freund’s adjuvant; PBS, phosphate-buffered saline.22
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. 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. In the absence of mature T cells, RGC losses are greater in strains that are better equipped to cope with the stress.

We found that Cop-1 vaccination protects rats and mice from the consequences of optic nerve injury. 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. In a rat model of high IOP, vaccination with Cop-1 significantly reduces the pressure-induced death of RGCs (Fig. 5). An interesting finding was that RGC death in this model was amenable to treatment by anti-TNF-alpha 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. 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. 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.

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References


