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Neuroprotection as a treatment for glaucoma: pharmacological and immunological approaches

M. SCHWARTZ

Department of Neurobiology, The Weizmann Institute of Science, Rehovot - Israel

ABSTRACT. *Primary open-angle glaucoma is a chronic, progressive optic neuropathy associated with a gradual decline in visual function, which may lead to blindness. In most cases, the optic neuropathy is associated with increased intraocular pressure. It is now generally accepted, however, that normalization of pressure, although necessary, is often not-sufficient as a remedial measure. This is because of the existence of additional factors, some of which emerge as a consequence of the initial damage. This situation is reminiscent of the response to a traumatic axonal insult, in which some of the damage is immediate and is caused by the insult itself, and some is secondary and is caused by a deficiency of growth-supportive factors as well as by toxic factors derived from the damaged tissue. Accordingly, the author has suggested that glaucoma may be viewed as a neurodegenerative disease and consequently amenable to any therapeutic intervention applicable to neurodegenerative diseases. There is evidence that neuroprotection can be achieved both pharmacologically and immunologically. Pharmacologic intervention neutralizes some of the effects of the nerve-derived toxic factors and possibly increases the ability of the remaining healthy neurons, at any given time, to cope with the stressful conditions. Immunologic intervention boosts the body's repair mechanisms for counteracting the toxicity of physiologic compounds acting as stress signals.* Eur J Ophthalmol 2003; 13 (Suppl. 3): S27-S31

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INTRODUCTION

Glaucoma is a neurodegenerative disease of the optic nerve and is a leading cause of blindness. The pathogenesis of optic nerve neuropathy in glaucoma is a matter of debate. Increased intraocular pressure (IOP) is probably the most frequently encountered risk factor in primary open-angle glaucoma, leading to the widely held view that increased IOP plays a central role in the initiation and development of glaucomatous neuropathy by increasing the mechanical forces on the lamina cribrosa of the optic nerve head. It is common experience, however, that the disease may continue to progress even if the IOP is reduced and kept within the normal range, suggesting that mechanical compression is probably not the only reason for the optic nerve damage (1-5).

Recent experimental and clinical evidence has indicated that glaucomatous neuropathy is also associated with other risk factors known to contribute to neurodegeneration in the acutely or chronically injured central nervous system (CNS). Included among these are optic nerve head ischemia, inhibition of neurotrophic factor transport, and the presence of secondary risk

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factors such as an increase in the excitatory neurotransmitter glutamate or in nitric oxide synthase. These findings led us to suggest that glaucoma should be viewed as a neurodegenerative disease (6), and that its treatment, in addition to lowering the IOP, should therefore include neuroprotective therapy (6-9). Neuroprotection can be achieved by counteracting risk factors, increasing the resistance of cells to the stressful conditions, or both. There is a danger, however, that interference with the physiologic response, although possibly beneficial at the site of pathology, may nevertheless be harmful to the normal tissue, leading to undesirable side effects. A more favorable approach from the clinical point of view is to harness and augment the tissue's own defense machinery.

Among the toxic risk factors triggered by the degenerating nerve itself is an uncontrolled increase in the amounts of certain biochemical compounds, with harmful consequences for the tissue. One such compound is the excitatory amino acid glutamate, which normally acts as a major neurotransmitter but is neurotoxic when its physiologic levels are exceeded. Glutamate was found to be increased in the vitreous of glaucomatous patients, as well as in animal models of glaucoma and of crush-injured optic nerves (10-12). Similarly, the retinas of damaged optic nerves of both human patients and animal models were found to contain increased concentrations of nitric oxide (13), a compound whose toxicity is evident from the fact that inhibition of the enzymes that mediate its increase arrests or at least slows down the degeneration. The presence of these biochemical compounds in abnormally high amounts may cause the death of neighboring neurons that were not destroyed or damaged by high IOP or any other primary insult. Also, as discussed in the next section, it should be noted that even if the environmental toxicity is not severe enough to cause cell death directly, it may nevertheless lead to death because of the toxicity-enhanced susceptibility of any spared neurons to glutamate and other toxic mediators, or the lower ability of these neurons to tolerate even normal IOP.

Attempts to halt the spread of damage have included neutralizing the mediators of toxicity, inhibiting signal transduction associated with death signals, and increasing the resistance of vulnerable neurons to the injurious conditions. None of these approaches, however, makes use of the system whose chief function is to maintain and protect the organism — namely, the immune system. There are several reasons why the very system best qualified for the job has not been called upon. First, in most neurodegenerative diseases (such as Alzheimer's disease, Parkinson's disease, or glaucoma) the active process takes place in the CNS, an immune-privileged site where any immune activity has long been considered harmful. Second, according to common wisdom, intervention by the adaptive immune system is needed only in cases of damage associated with invading microbes (pathogens), and such pathogens are not involved in the spread of damage in neurodegenerative diseases. Third, some beneficial effect on the postinjury spread of damage has been obtained with anti-inflammatory drugs, leading in many cases to oversimplified conclusions about the role of immune activities in the injured CNS (14-18).

For all of the above reasons, exploitation of adaptive immunity was, until recently, not seriously considered as a worthwhile approach in the attempt to stop the spread of damage.

Beneficial autoimmunity in the damaged CNS

Using rat models of partially crush-injured optic nerves and contused spinal cords, in which degeneration progresses both laterally and longitudinally, we recently observed that a well-controlled adaptive immune response is beneficial in slowing down the post-traumatic spread of damage. The immune response was mediated by T cells directed against CNS-associated self-antigens, such as myelin basic protein (MBP) (19-22), myelin oligodendrocyte protein (MOG), or proteolipid protein (PLP), or against peptides (encephalitogenic or non-encephalitogenic) derived from these proteins (23). T cells directed against encephalitogenic cryptic epitopes were as effective as those directed against non-encephalitogenic cryptic epitopes (19, 23) in displaying neuroprotection, indicating that the observed neuroprotection was not related to the virulence of the autoimmune response. The response could be achieved either by active immunization with the proteins (or peptides) or by passive transfer of T cells activated by them (22). On the basis of these findings, we suggested that autoimmune T cells can protect CNS neurons from the postinjury spread of damage. We further showed that the

neuroprotective autoimmunity is not primarily the result of an experimental manipulation but is an endogenous response that is awakened by the damaged neurons, although apparently not strongly enough to be effective (24). It thus appears that this T cell-mediated autoimmune response is a physiologic mechanism whereby the body attempts to cope with trauma-related nerve damage to the nervous system but, presumably because of an evolutionary trade-off, the recruited autoimmune response, in its natural state, is not sufficient (25-28).

The beneficial autoimmunity can, in principle, gain access to the damaged tissue at any time, because even the healthy CNS is receptive to surveillance by T cells, which unlike immunoglobulins or macrophages are not restricted by the blood–brain barrier.

The way in which the T cell-mediated immune response exerts its neuroprotective effect is not yet fully understood. Like most of the activities of adaptive immune cells, the activity is likely to be antigen-dependent. Thus, in order to exert their neuroprotective activity, the T cells need to be reactivated at the site of injury. For example, protection against glutamate toxicity in the eye could be achieved by antigens derived from proteins residing in the retina but not in the optic nerve (29). Our recent demonstration of antigen-dependent production of neurotrophic factors by T cells points to neurotrophin production as a possible facilitator of the protection provided by the T cells (28). As a source of neurotrophins, T cells have certain advantages over neuronal cells: 1) because of their mobility, T cells can be recruited to supply areas that run short of neurotrophins due to damage; 2) the amount of neurotrophin production by T cells is determined by reactivation through signals coming from the tissue, a feature unique to immune cells; and 3) the type of neurotrophin produced may also be affected by the nature and/or intensity of the stress signals. In addition, our studies suggest that T cells, locally activated, activate resident microglia to deal more effectively with the threat (Shaked et al, unpublished data).

Exploitation of T cell-mediated autoimmunity for the treatment of degenerative diseases

The finding of autoimmune neuroprotection of nerve cell bodies and fibers in the hostile environment of the injured rat spinal cord or optic nerve leads us to believe that this beneficial activity might be a feature of other degenerative events as well. In seeking ways to boost autoimmune response, it is important to bear in mind that the response must be well controlled to avoid exceeding the risk threshold and inducing an autoimmune disease.

Any self-protein, as a potential antigen for immunization, has sites that are immunodominant (and therefore encephalitogenic) and sites that are immunosilent. Dominance varies with the nature of the protein and the genetically determined major histocompatibility complex of the species, strain, or individual. Accordingly, the chances of finding a consensus sequence among individuals with respect to a silent (i.e., safe) epitope are very small.

We recently observed that the known copolymer-1 (Cop-1), a synthetic antigen used to treat patients with multiple sclerosis, can serve as a safe antigen (31, 32). Passive and active immunization with this four-amino-acid copolymer reduces the damage caused by mechanical insult to the optic nerve or by intravitreally injected glutamate. The protection from glutamate toxicity has far-reaching implications because glutamate is a common mediator in many CNS disorders, including glaucoma, and thus the above active or passive immunization, by reducing the toxicity, might be of therapeutic value in protecting nerves from further degeneration. The success of immunization with Cop-1 in reducing the neuronal losses resulting from optic nerve insult, whether to the axons or directly to the cell bodies, encouraged us to study the effects of Cop-1 immunization in a rat model of glaucoma. Our recent results, using a rat model of ocular hypertension, showed that active immunization with Cop-1 leads to a significant reduction in retinal ganglion cell loss resulting from an increase in IOP (32-34).

The fact that immunization with Cop-1 protects retinal ganglion cells from death in a rat model of high IOP even under conditions where the pressure is reduced and then kept low is potentially of great advantage from the clinical point of view, because even a pressure reduced to normal is not necessarily safe for patients with glaucoma, in whom the remaining neurons are more vulnerable than normal ones. Moreover, reduction of the IOP to a level that might be considered safe in such patients (i.e., to 12 mmHg)might not be feasible. Thus, under conditions where the pres-

sure is reduced but is still higher than the patient's retinal ganglion cells can tolerate, additional protective long-term therapy is needed.

CONCLUSIONS

Recent findings in our laboratory have shown that CNS insults, whether mechanically or biochemically induced, evoke a T cell-dependent beneficial response, which we interpret as providing a versatile back-up protection when the specific local mechanisms for buffering of potentially toxic physiologic compounds are unequal to the task. Active immunization with myelin-associated self-antigens appears to be a way to enhance this endogenous response, and thus represents a promising strategy for boosting physiologic mechanisms of protection. Self-antigens, however, can induce an autoimmune disease in susceptible individuals, whereas Cop-1, the copolymer recently tested in our laboratory, is known to be a safe antigen. If anything, it will suppress autoimmune disease onset. Thus, vaccination with Cop-1 essentially simulates vaccination with self-antigens, but in a safe way.

It seems reasonable to assume that immunization, if successful, will provide a more global, multifactorial, and long-lasting protection than the local buffering system can supply. This is especially important in the case of chronic CNS disorders such as glaucoma, because at any given time there are neurons at different stages of health, vulnerability, and amenability to neuroprotective intervention. Because Cop-1 is a safe drug, it can be adapted immediately as a therapeutic protocol for glaucoma, wiht the appropriate formulation and regimen.

Reprint requests to:
Michal Schwartz, MD
Department of Neurobiology
The Weizmann Institute of Science
76100 Rehovot, Israel
michal.schwartz@weizmann.ac.il

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