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# Development of spontaneous optic neuropathy in NF-κBp50-deficient mice

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**ABSTRACT**

*Although the transcription factor, nuclear factor-kappa B (NF-κB) is known to regulate cell death and survival, its precise role in neuronal cell death within the central nervous system remains unknown. The purpose of this study was to investigate the role of NF-κBp50 in the age-related survival of retinal ganglion cells (RGCs). Eyes of mice with a deleted NF-κBp50 gene and its wild-type mice at each of age were studied by histopathological studies. The number of RGCs was counted using retrograde labelling methods. Mice were subjected to intravitreous injection of N-methyl-D aspartate (NMDA) to induce RGC death. In NF-κBp50-deficient mice, the number of RGCs significantly decreased with age in total independence of intraocular pressure measurement. Optic nerves of p50-deficient mice showed hypertrophy astrocytes and enlargement of the axons, together with a decreased number of axons. Immunohistochemistry (IHC) showed a strong expression of glial fibrillary acidic protein. The histological results show obvious excavation of the optic nerve head in NF-κBp50-deficient mice at 10 months of age. Intravitreal injection of NMDA in young p50-deficient mice damaged RGCs more intensively than in control animals. We further noticed that auto-antibodies against RGCs were produced in NF-κBp50-deficient mice. Our results show that p50 deficiency induced age related RGC death, indicating a new insight into the role of p50 in the pathophysiology of neuropathy, and further experiments with NF-κBp50-deficient mice may provide new targets for therapeutic intervention for human glaucoma.*

**KEYWORDS: NF-κBp50, optic neuropathy, retinal ganglion cell**

## Introduction

Retinal ganglion cells (RGCs) are a type of neuron located in the retina. A reduction in the number of these cells is often associated with a decrease in visual acuity and visual field loss. Glaucoma, one of the leading causes of blindness in the world, is a common disease characterized by progressive death of RGCs. Intraocular pressure (IOP) has been regarded as a major risk factor for developing glaucoma1,2; however, several lines of evidence have been gradually accumulating that suggest several risk factors such as ischemia, glutamate and genetic background, which also play important roles in the development of glaucoma.3,4 Furthermore, autoimmunity that directly recognizes retinal proteins has been associated with developing neuropathy in glaucomatous patients.5,6,7

NFKB1(p50) is bound to RelA(p65) to form the NF-κB complex, which is called as classical NF-κB. The p50(NFKB1)/p65(RelA) heterodimer is the most abundant form of NF-κB.8,9 The NF-κB complex is inhibited by IκB proteins, which inactivate NF-κB by trapping it in the cytoplasm. Phosphorylation of serine residues on IκB proteins by IκB kinases (IKKα or IKKγ) marks them for destruction via the ubiquitination-proteasome pathway (UPP), thereby allowing the activation of NF-κB.8,9 Activated NF-κB translocates into the nucleus and binds targeting DNA at NF-κB-binding motifs, and then activates gene transcription.10 On the one hand, p50 is required for binding DNA, and on the other hand, p65 is an essential factor for gene transcriptional activation. The wide functional implications of NF-κB are reflected in the reviews on the relationship to cancer, on the relationship to neuronal plasticity, and on the treatment of neurologic disorders.11 In the central nervous system (CNS), NF-κB is expressed ubiquitously in both neuronal and glial cells, where it plays a key role in various physiological mechanisms, such as synaptic activity, learning, memory, survival and apoptosis.12 Also, NF-κB mediates pathophysiological events associated with acute neurodegeneration, including trauma, seizure and ischemia, and also chronic neurodegeneration such as Alzheimer disease11; however, the precise role of NF-κB in such cell death within the CNS is controversial. On the one hand, NF-κB was reported to prevent neuronal apoptosis,13,14,15 although it also appears to promote apoptosis.16 Thus, in order to clarify the precise roles of NF-κB in neuronal cell death, we analyzed the phenotypes of NF-κBp50-deficient mice, with special reference to the retina, leading us to become aware of the involvement of NF-κBp50 in RGC death. In the present study, we investigated the age-related survival of RGCs in NF-κBp50-deficient mice and found that these mice exhibited many features resembling human glaucoma disease. The findings in the present study seem to provide new insight into the role of NF-κBp50 in the pathophysiology of age-related neurodegeneration.

## Material and Methods

**Purpose:** The transcriptional regulator, NF-κB/Rel family are involved in neuronal cell death and survival. Previously, we reported that NF-κBp50-deficient mice exhibit many features resembling human normal tension glaucoma (NTG). The developmental mechanism of human NTG is not clearly understood, and a radical curative treatment has yet to be established. Our aim is to elucidate the signal cascade which mediates the spontaneous optic neuropathy in p50-deficient mice as a model of human NTG.

To demonstrate the expression and activation of pro-apoptotic factors, which mediate the death of retinal ganglion cells (RGCs) in NF-κBp50-deficient mice, western blot (W.B.) and luciferase reporter assays with retinas derived from p50-deficient and wild type mice, and cultured RGC-5 cells were performed. Furthermore, we tested the neuroprotective effects of chemical reagents (memantine, lomerizine, and tacrolimus) against N-methyl-D-aspartate (NMDA)-susceptible RGCs damage according to in vitro experiments with RGC-5 cells. To elucidate the NF-κB-mediated death signaling, the effects of chemical reagents on spontaneous optic neuropathy were examined by histopathological studies.

## Results and Discussion

W.B. experiments and luciferase reporter assays showed that NF-κB-inducible BCL2-associated X protein (Bax) and a pro-apoptotic factor, activated caspase 3 were expressed in the retina of p50-deficient mice as well as NMDA-treated RGC-5 cells. Further, the constitutively active cleaved forms of calcineurin (CaN), which have been reported to lead to apoptosis, were detected in the retina of NF-κBp50-deficient mice as well as NMDA-treated RGC-5 cells. Pre-treatment with tacrolimus markedly protected RGC-5 cells from NMDA-induced neurotoxicity, and then both spontaneous RGC death and degenerative changes to the optic nerve in NF-κBp50-deficient mice were significantly reduced by the chronic administration of tacrolimus. The experiments with cultured RGC-5 cells supported the results of histological examinations with NF-κBp50-deficient mice, suggesting that CaN activation leads to NF-κB-induced Bax activation and caspase 3 activation, and mediates spontaneous optic neuropathy in NF-κBp50-deficient mice.

Recent studies show the potential role of the immune system in the pathogenesis of glaucoma.5,6,7,17,18 These studies demonstrate that auto-antibodies against ocular antigens are up-regulated in the serum

of patients with NTG, such as heat-shock proteins, rhodopsin, γ-enolase, glutathione-S-transferase, phosphatidylserine, glycosaminoglycans and neurofilaments.5,6,7 Meanwhile, NF-κBp50-deficient mice exhibited multiple immune response defects in a non- specific response to infection and in responses involving B lymphocytes.19,20 In the present study, it is noteworthy that IHC with serum derived from NF-κBp50-deficient mice revealed auto-antibody production in NF-κBp50-deficient mice. Therefore, we surmise that some autoimmune responses may be associated with this interruption of RGCs' long survival in NF-κBp50-deficient mice. This result suggested that the potential auto-antibodies against cells in GCL were produced in NF-κBp50-deficient mice.

NF-κBp50-deficient mice showed many features resembling human NTG: late onset and spontaneously developing RGCs' cell death, axon loss associated with increasing connective tissue, strong expression of GFAP, the characteristic excavation of the optic nerve head and hypertrophic morphological change of astrocytes in the retina and optic nerve, under normal IOP condition. The characteristic optic disc cupping may be attributable to RGC death, as seen clinically in glaucoma patients. We found that NF-κBp50-deficient mice exhibited susceptibility to NMDA-induced neurotoxicity. Furthermore, as shown in glaucoma patients, auto-antibodies to RGCs are produced in NF-κBp50-deficient mice, suggesting the development of autoimmunity as a possible mechanism for the onset of progressive RGCs' death. Although further study remains to be performed, on about the genetics and a etiology of neurological disease, including glaucoma.

## Conclusion

We demonstrated that the number of retinal ganglion cells (RGCs) in NF-κBp50-deficient ($p50^{-/-}$) mice was significantly decreased compared with that in its parental mice, $p50^{+/+}$ mice, suggesting that these animals exhibited features resembling those in human NTG. We provide a new insight into the role of NF-κBp50 in the pathophysiology of optic neuropathy, it shows possible target molecules for the development of therapeutic reagents. Our research findings may provide new targets for therapeutic intervention in human NTG.

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