ANNALS of Clinical and Translational Neurology
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AMERICAN NEUROLOGICAL ASSOCIATION

BRIEF COMMUNICATION

Estrogen-induced glial IL-1β mediates extrinsic retinal ganglion cell vulnerability in murine *Nf1* optic glioma

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Received: 27 September 2023; Revised: 20 December 2023; Accepted: 27 December 2023

doi: 10.1002/acn3.51995

Abstract

Optic pathway gliomas (OPGs) arising in children with neurofibromatosis type 1 (NF1) can cause retinal ganglion cell (RGC) dysfunction and vision loss, which occurs more frequently in girls. While our previous studies demonstrated that estrogen was partly responsible for this sexually dimorphic visual impairment, herein we elucidate the underlying mechanism. In contrast to their male counterparts, female *Nf1*^{OPG} mice have increased expression of glial interleukin-1β (IL-1β), which is neurotoxic to RGCs *in vitro*. Importantly, both IL-1β neutralization and leuprolide-mediated estrogen suppression decrease IL-1β expression and ameliorate RGC dysfunction, providing preclinical proof-of-concept evidence supporting novel neuroprotective strategies for NF1-OPG-induced vision loss.

Introduction

Vision loss is one of the most significant morbidities associated with brain tumors arising in the neurofibromatosis type 1 (NF1) cancer predisposition syndrome.[1,2] In this respect, 15%–20% of children with NF1 develop low-grade gliomas (pilocytic astrocytomas) of the optic pathway (optic pathway glioma; OPG) that result in axonal injury, retinal ganglion cell (RGC) loss, retinal nerve fiber layer (RNFL) thinning, and subsequent vision loss.[1] Similarly, *Nf1*-OPG preclinical mouse models also exhibit time-dependent axonal injury, RGC loss, and RNFL thinning,[3,4] establishing these avatars as excellent platforms to define the cellular and molecular mechanisms underlying OPG-induced RGC degeneration. Leveraging these resources, prior research suggested both cell-intrinsic and cell-extrinsic causes for RGC death. Impaired cAMP regulation accounts for the RGC-intrinsic vulnerability in *Nf1*-mutant mice, such that cAMP elevation partly ameliorates RGC loss in vivo.[5] However, the mechanism underlying the cell-extrinsic effects remains unelucidated.

Insights into drivers of cell-extrinsic RGC loss derive from studies demonstrating that girls with NF1-OPG more frequently require treatment for vision loss.[6–8] Using *Nf1*^{OPG} mice, only female *Nf1*^{OPG} mice exhibit significant RGC loss and RNFL thinning, despite the fact that both male and female *Nf1*^{OPG} mice develop tumors of equal volumes and proliferation rates.[6,9] This sexually dimorphic effect is caused by estrogen, such that surgical or chemical ovariectomy ameliorates *Nf1*-OPG-induced retinal pathology.[9] In the current study, we demonstrate that estrogen regulates glial IL-1β production, which is sufficient to cause *Nf1*-mutant RGC death in vitro. Moreover, both pharmacologic IL-1β and hypothalamic estrogen inhibition restore RGC numbers and RNFL thickness in female *Nf1*^{OPG} mice to wild type levels in vivo, revealing potential neurorestorative approaches for attenuating vision loss in children with NF1-OPG.

Materials and Methods

Mice

Experiments were performed under an approved Washington University Institutional Animal Care and Use Committee protocol. Mice had *ad libitum* access to water and food under 12-h light/dark cycles. *Nf1*$^{flox/mut}$; GFAP-Cre (*Nf1*$^{+/-}$ mice with somatic *Nf1* gene loss in neuroglial progenitors; *Nf1*^{OPG} mice), *Nf1*$^{+/-}$ (heterozygous

germline *Nf1* gene inactivation), and wild type (WT) mice were maintained on a C57BL/6 background.[10] Surgical ovariectomy was performed in the Washington University Mouse Genetics Core.[9]

Pharmacological treatments

Four- to six-week-old female $Nf1^{OPG}$ mice were treated with 15 mg/kg leuprolide (Lupron) or saline intraperitoneally every day or with 2.5 mg/kg anti-IL-1β or control IgG antibodies intraperitoneally every other day for 8 weeks.

Immunohistochemistry

Mice were perfused with Ringer's solution followed by 4% paraformaldehyde (PFA). Optic nerves and eyeballs were post-fixed in 4% PFA overnight before processing for paraffin (optic nerves) or OCT (eyes) embedding. Immunohistochemistry and immunofluorescence staining were performed on 5-µm-thick optic nerve sections and 10-µm-thick retinal cryosections, respectively, using appropriate primary and secondary antibodies (Supplementary Table S1).[11]

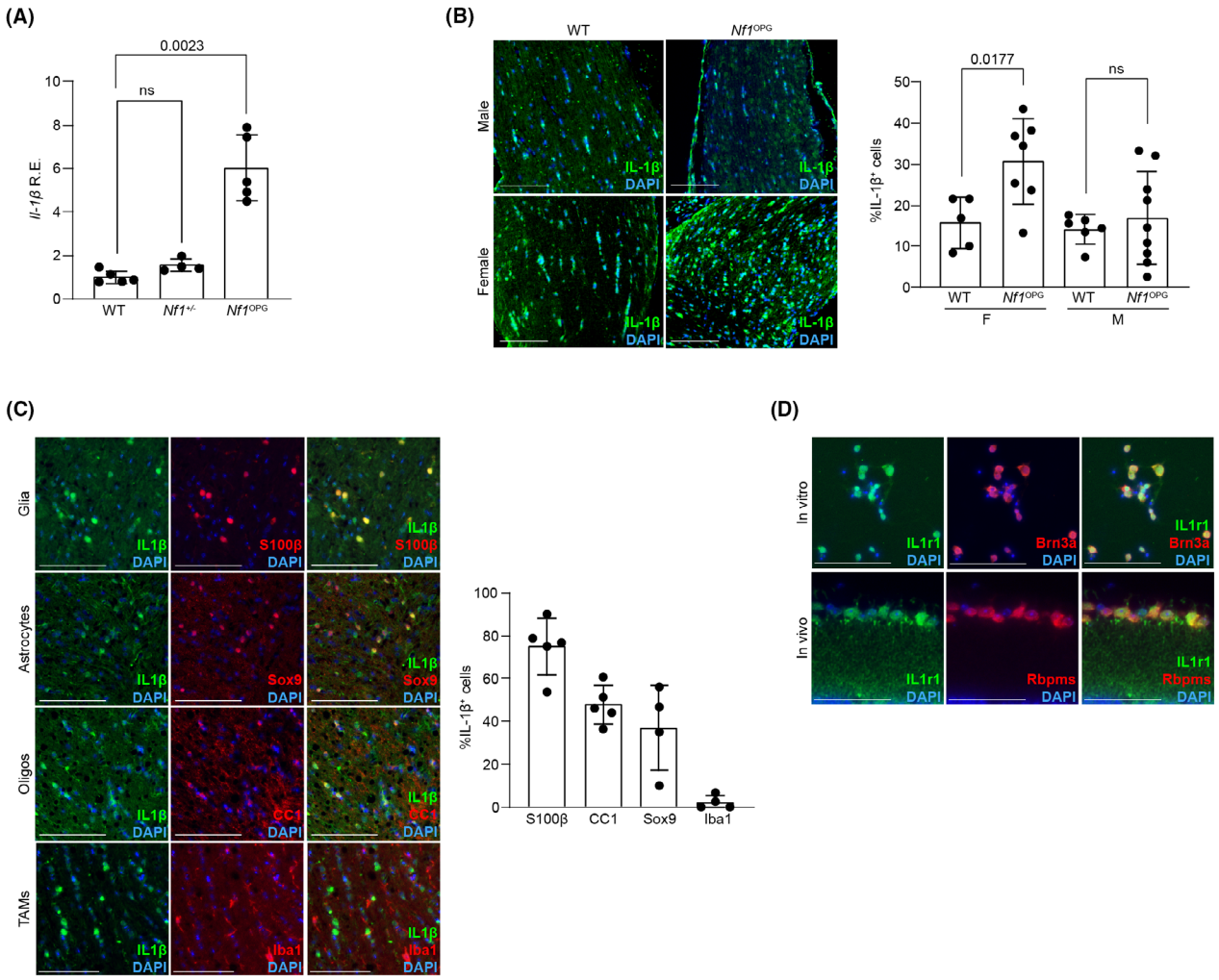


Figure 1. Glial IL-1β expression is increased in the optic nerves of female $Nf1^{OPG}$ mice. (A) Interleukin-1β (*Il-1β*) expression is increased in $Nf1^{OPG}$ mouse optic nerves relative to $Nf1^{+/-}$ and wildtype (WT) mice (WT, $n = 5$; $Nf1^{+/-}$, $n = 4$; $Nf1^{OPG}$, $n = 5$). (B) IL-1β protein expression is increased in female (F WT, $n = 5$; F $Nf1^{OPG}$, $n = 7$), but not male (M WT, $n = 6$; M $Nf1^{OPG}$, $n = 9$), $Nf1^{OPG}$ mouse optic nerves, relative to sex-matched WT controls. (C) The majority of IL-1β-expressing cells (green) are S100β$^+$ glial cells (red, top panel), including Sox9$^+$ astrocytes (red, second panel) and CC1$^+$ oligodendroglia ("oligos," red, third panel), but not Iba1$^+$ tumor-associated monocytes (TAMs, red, bottom panel). (D) Expression of the IL-1β receptor, interleukin-1 receptor 1 (IL1r1, green), in Brn3a$^+$ (red, top panel) primary $Nf1^{+/-}$ retinal ganglion cells (RGCs) in vitro and Rbpms$^+$ (red, bottom panel) RGCs in the retina of $Nf1^{OPG}$ mice in situ. Data are represented as means ± SEM. A, Kruskal–Wallis test with Dunnett's posttest correction. B, Mann–Whitney test. *p* values are indicated within each panel. Scale bars, 100 µm.

Quantitative RT PCR

RNA was isolated from optic nerves using the NucleoSpin RNA Plus kit and cDNA generated using the High-Capacity cDNA Reverse Transcription kit. Real-time quantitative PCR (RT-qPCR) was performed by TaqMan gene expression. ΔΔCT values were calculated using *Gapdh* as a normalization control.

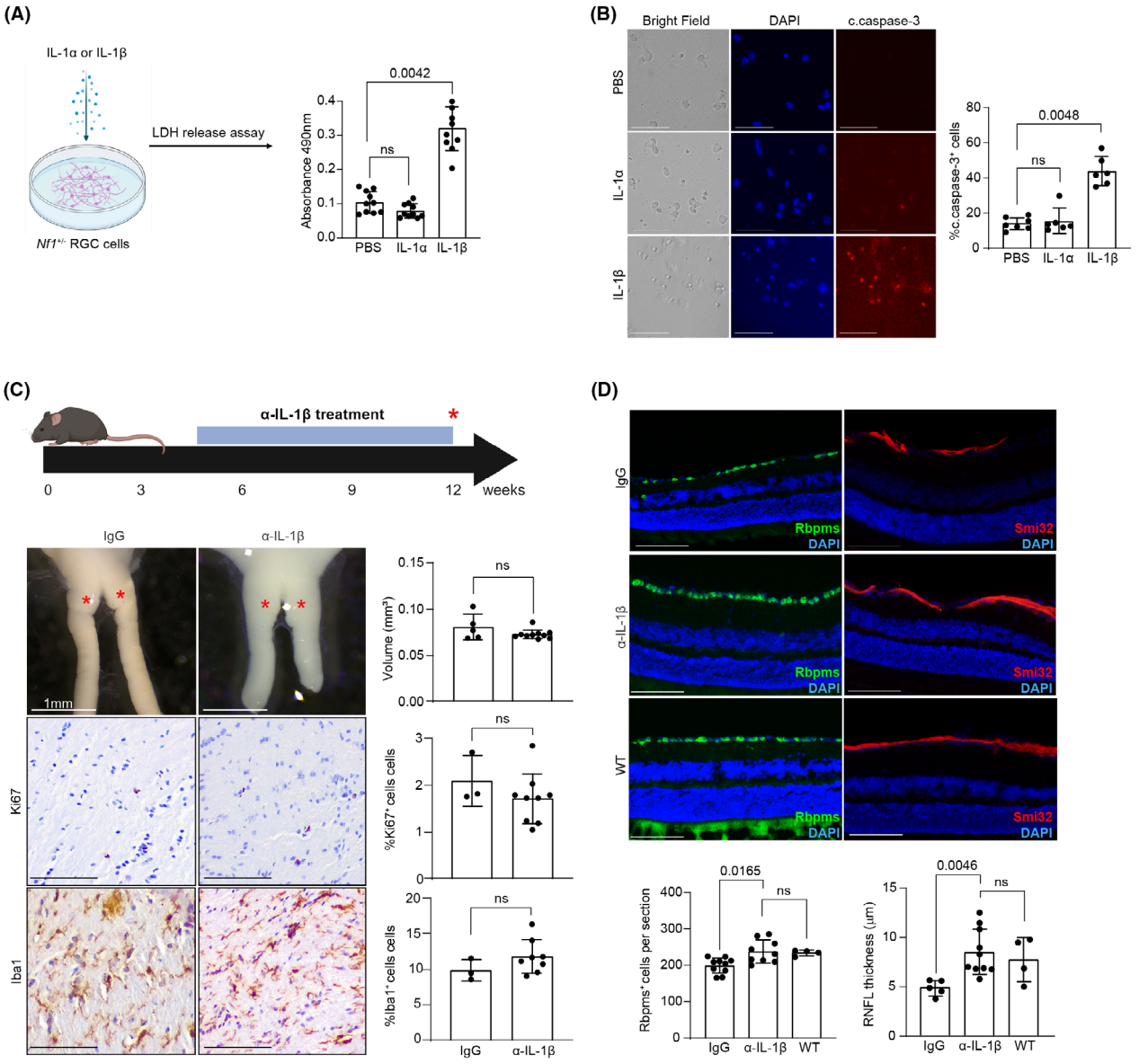


Figure 2. IL-1β is sufficient to induce RGC apoptosis in vitro and necessary for *Nf1*-OPG-induced RGC death in vivo. (A) Primary $Nf1^{+/-}$ RGCs were treated with IL-1α or IL-1β for 24 h before assessing cell death using a lactate dehydrogenase (LDH) assay. IL-1β, but not IL-1α, induced RGC death in vitro (PBS, $n = 10$; IL-1α, $n = 10$; IL-1β, $n = 9$). (B) Cleaved caspase-3 expression in $Nf1^{+/-}$ RGCs following incubation with PBS (top panel), IL-1α (middle panel), or IL-1β (bottom panel). $Nf1^{+/-}$ RGCs exhibit increased apoptosis upon incubation with IL-1β, but not IL-1α (PBS, $n = 7$; IL-1α, $n = 6$; IL-1β, $n = 6$). (C) Female *Nf1*-OPG mice were treated with neutralizing IL-1β (α-IL-1β) or control IgG antibodies from 4 to 12 weeks of age. No change in optic nerve volume (red asterisks mark the location of OPG; IgG, $n = 5$; α-IL-1β, $n = 10$) nor in the percent of $Ki67^+$ (proliferating) or $Iba1^+$ (TAM) cells was observed after α-IL-1β treatment compared to IgG-treated controls (IgG, $n = 3$; α-IL-1β, $n = 8$). (D) Quantification of RGC content ($Rbpms^+$ cells/section, green; IgG, $n = 10$; α-IL-1β, $n = 9$; WT, $n = 4$) and retinal nerve fiber layer (RNFL) thickness ($Smi32^+$ fibers, red; IgG, $n = 5$; α-IL-1β, $n = 10$; WT, $n = 4$) in the eyes of $Nf1^{OPG}$ females treated with α-IL-1β or control IgG antibodies relative to untreated WT female mice. Data are represented as means ± SEM. A, B, and D, Kruskal–Wallis test with Dunnett's posttest correction. C, Mann–Whitney test. *p* values are indicated within each panel. Scale bars are 100 μm unless otherwise noted.

Primary RGC cultures

RGCs were isolated from postnatal day 4–7 $Nf1^{+/-}$ mice.[11] After 5 days in vitro, RGCs were treated with 1 μg/mL IL-1β, 1 μg/mL IL-1α, or saline for 24 h, and lactate dehydrogenase (LDH) release analyzed using the CyQUANT LDH Cytotoxicity Assay kit. Immunocytochemistry was performed on PFA-fixed $Nf1^{+/-}$ RGCs using appropriate primary and secondary antibodies (Table S1).

Statistical analysis

Statistical analysis was performed using Prism 9.0 (GraphPad). Analyses of two groups were performed using a nonparametric Student's t test (Mann–Whitney test), where analyses of three or more groups were performed using a nonparametric one-way ANOVA (Kruskal–Wallis test) with Dunn's multiple comparison correction. Statistical significance was defined as $p < 0.05$.

Results

Female $Nf1^{OPG}$ mice harbor increased optic nerve glial IL-1β expression

IL-1β is a cytokine previously implicated in central nervous system (CNS) injury.[12,13] To examine *Il-1β* RNA expression in the setting of murine *Nf1*-OPG, we performed RT-qPCR on optic nerves from WT, $Nf1^{+/-}$, and $Nf1^{OPG}$ mice. Whereas WT and $Nf1^{+/-}$ optic nerves had comparable *Il-1β* expression, *Il-1β* expression was increased 5.8-fold in $Nf1^{OPG}$ mice (Fig. 1A), which was confirmed at the protein level to be restricted to female $Nf1^{OPG}$ mice (Fig. 1B).

To identify the cell population responsible for IL-1β expression, immunofluorescence co-labeling experiments were performed. The majority of the IL-1β-producing cells express the glial marker S100β, including $Sox9^+$ astrocytes and $CC1^+$ oligodendrocytes. In contrast, tumor-associated monocytes (TAMs, $Iba1^+$ cells) and oligodendrocyte precursor cells ($PDGFR\alpha^+$ cells; not shown) express little IL-1β (Fig. 1C). Consistent with IL-1β as a potential neurotoxic cytokine for RGCs, RGCs express the IL-1 receptor (IL1r1) in vitro and in vivo (Fig. 1D).

IL-1β is sufficient for RGC death in vitro and necessary for RGC death in vivo

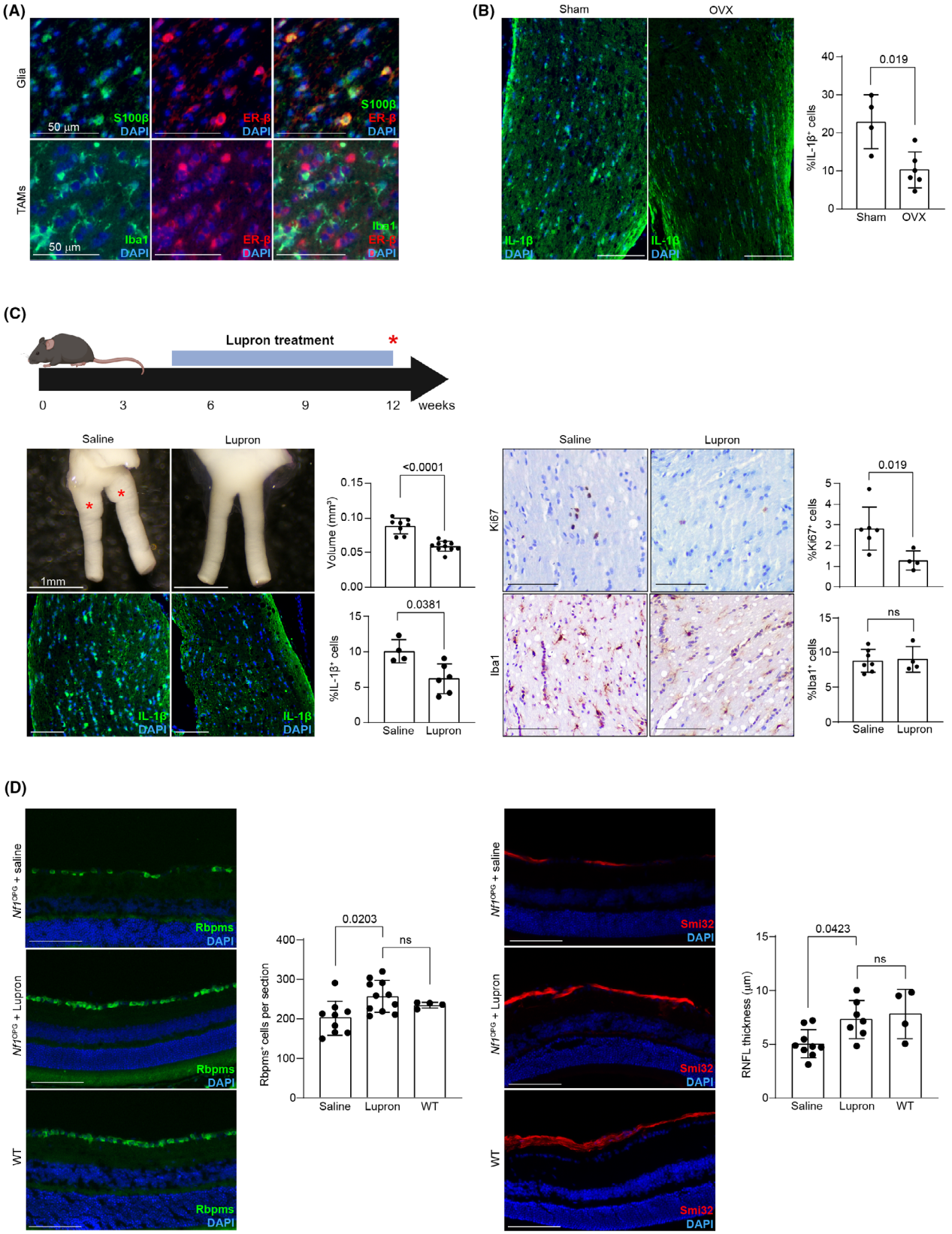
To determine whether IL-1β is *sufficient* to induce $Nf1^{+/-}$ RGC death in vitro, LDH release was measured from $Nf1^{+/-}$ RGCs treated for 24 h with IL-1β, saline, or IL-1α, a closely related cytokine that also activates IL1r1 (Fig. 2A).[14] Treatment with IL-1β, but not IL-1α, caused RGC death (two-fold increase), which reflected an increase in RGC apoptosis as measured by cleaved caspase-3 immunofluorescence (Fig. 2B).

To determine whether IL-1β inhibition could ameliorate RGC death and axonal loss in vivo, female $Nf1^{OPG}$ mice were treated with neutralizing IL-1β (α-IL-1β) or control IgG antibodies for 8 weeks. While IL-1β inhibition had no effect on optic nerve volume, tumor proliferation ($\%Ki67^+$ cells), or TAM content ($\%Iba1^+$ cells) (Fig. 2C), RGC cell number and RNFL thickness per retinal section increased by ~20% and ~ 74%, respectively, to levels indistinguishable from WT mice (Fig. 2D). These results demonstrate that while IL-1β is not required for tumor growth, it is *necessary* for *Nf1*-OPG-induced RGC death and RNFL thinning.

Inhibition of estrogen-induced IL-1β production rescues $Nf1^{OPG}$ retinal pathology

As we previously showed that the sexually dimorphic RGC dysfunction observed in female $Nf1^{OPG}$ mice was partly due to estrogen, rather than a male protective effect,[9] we hypothesized that estrogen might be responsible for IL-1β-induced retinal pathology. Consistent with this idea, $S100\beta^+$ glial cells express estrogen receptor beta (Fig. 3A), such that surgical ovariectomy decreased $IL\text{-}1\beta^+$ cell content in the optic nerves of female $Nf1^{OPG}$ mice

Figure 3. Inhibition of estrogen-induced glial IL-1β production reverses $Nf1^{OPG}$ retinal and optic nerve pathology. (A) Estrogen receptor-β (ER-β, red) is expressed in $S100\beta^+$ ("glia," green, top panel), but not $Iba1^+$ (TAMs, green, bottom panel), cells in the optic nerves of female $Nf1^{OPG}$ mice. (B) Surgical ovariectomy (OVX) reduced the percent of $IL\text{-}1\beta^+$ cells in the optic nerves of female $Nf1^{OPG}$ mice (sham, $n = 4$; OVX, $n = 6$). (C) Female $Nf1^{OPG}$ mice were treated with Lupron or saline from 4 to 12 weeks of age. While the optic nerve volume (red asterisks mark the location of OPG; saline, $n = 8$; Lupron, $n = 10$), percent of $IL\text{-}1\beta^+$ cells (saline, $n = 4$; Lupron, $n = 6$) and percent of proliferating ($Ki67^+$) tumor cells (saline, $n = 6$; Lupron, $n = 4$) were decreased, there was no change in the percent of $Iba1^+$ cells (TAM) in the optic nerves of Lupron-treated mice compared to controls (saline, $n = 6$; Lupron, $n = 4$). (D) Treatment with Lupron increased RGC content ($Rbpms^+$ cells, green) and retinal nerve fiber layer (RNFL; $Smi32^+$ fibers, red) thickness in the eyes of *Nf1*-OPG female mice relative to saline-treated $Nf1^{OPG}$ and untreated WT female mice (saline, $n = 9$; Lupron, $n = 11$; WT, $n = 4$). Data are represented as means ± SEM. B and C, Mann–Whitney test. D, Kruskal–Wallis test with Dunnett's posttest correction. *p* values are indicated within each panel. Scale bars are 100 μm unless otherwise noted.



(Fig. 3B). Leveraging this new finding, we sought to determine whether pharmacological agents that suppress estrogen levels in children with NF1 and precocious puberty[15] could block IL-1β production, RGC death, and RNFL thinning. For these experiments, female *Nf1*^{OPG} mice were treated with the gonadotropin-releasing hormone (GnRH) agonist, leuprolide (Lupron), for 8 weeks. After treatment, the decrease in optic nerve IL-1β^{+} cell content was accompanied by a 33% decrease in optic nerve volume and a 55% decrease in tumor proliferation (%Ki67^{+} cells) (Fig. 3C). Importantly, RGC number and RNFL thickness increased by ~25% and ~45% after Lupron treatment, respectively, to levels indistinguishable from WT controls (Fig. 3D).

Discussion

Approximately 25%–30% of children with NF1-OPG experience some degree of vision loss due to RGC dysfunction. This visual loss can range from minimal to profound, and is not effectively reversed by currently available tumor-focused chemotherapies.[7] Herein, we leverage *Nf1*^{OPG} mice, which form tumors at ages similar to other murine models of pediatric brain tumors[16,17] and recapitulate the sexually dimorphic retinal and optic nerve pathology of children with NF1-OPG, to establish that estrogen-driven glial IL-1β production is responsible for the cell-extrinsic OPG-associated retinal pathology. These findings raise two key points.

First, we demonstrate that *Nf1*^{OPG} mice express the neurotoxic cytokine IL-1β in a sexually dimorphic manner, which is both necessary and sufficient for RGC death and optic nerve injury in female *Nf1*^{OPG} mice. The finding that glial cells, rather than TAMs, as previously reported,[12] produce IL-1β in female *Nf1*^{OPG} mice likely reflects the non-specific effects of minocycline.[18] In contrast, treatment of female *Nf1*^{OPG} mice with the more selective monocyte inhibitor (PLX3397) had no effect on IL-1β production (not shown). Intriguingly, IL-1β inhibition offers neuroprotection without affecting tumor maintenance, suggesting that IL-1β acts independently of the cellular circuits governing *Nf1*-OPG growth.[19] Current studies are focused on elucidating the mechanism underlying estrogen-regulated glial IL-1β production.

Second, we demonstrate that estrogen is responsible for glial IL-1β production, such that Lupron, used to suppress precocious puberty in children with NF1,[15] restores RGC content and RNFL thickness to WT levels in *Nf1*^{OPG} mice. Additionally, anti-estrogen therapy also reduces tumor growth, suggesting that estrogen receptor function in other cells is relevant to *Nf1*-OPG maintenance, independent of IL-1β signaling.[4,9] While further work is required, this finding provides proof-of-concept preclinical support for the use of these agents to attenuate vision loss in children with NF1-OPG, especially for those with prechiasmatic OPGs who also exhibit a female predominance of vision loss.[7,8] Current studies are focused on defining the minimal treatment doses and durations to ameliorate retinal pathology and vision loss in *Nf1*^{OPG} mice using clinically relevant measures, including ocular coherence tomography and visual optomotor system testing.[4,6]

Taken together, these new preclinical discoveries establish that neuroprotective strategies targeting both the *NF1* mutation-induced intrinsic (e.g., intracellular cyclic AMP levels[6]) and estrogen- and IL-1β-mediated extrinsic vulnerabilities might be harnessed to restore or prevent vision loss in children with these common brain tumors.

Acknowledgments

We thank Taylor John-Lewis for technical assistance and Olivia Cobb for assistance with the statistical analysis. This work was partly funded by a Research Training Grant from the National Institute of Neurological Disorders and Stroke (R25-NS090978 to Y.T.), a Research Program Award from the National Institute of Neurological Disorders and Stroke (R35-NS097211 to D.H.G.), and a Gilbert Family Foundation Vision Recovery Initiative grant (to D.H.G). The Washington University Ophthalmology Core facility is supported by funding from the National Eye Institute (P30EY002687).

Author Contributions

Concept and design of the study: Y.T. and D.H.G. Acquisition of data: Y.T., J.C., N.W., and S. B. Analysis of data: Y.T. and J.C. Drafting and editing the paper and figures: Y.T. and D.H.G.

Conflict of Interest

The authors declare no relevant conflicts of interest.

Data Availability Statement

No exome, genome, epigenetic, or microarray data were generated as part of this study.

References

1. de Blank PMK, Fisher MJ, Liu GT, et al. Optic pathway gliomas in Neurofibromatosis type 1: an update: surveillance, treatment indications, and biomarkers of vision. J Neuroophthalmol. 2017;37(Suppl 1):S23-S32.

2. Listernick R, Ferner RE, Liu GT, Gutmann DH. Optic pathway gliomas in neurofibromatosis-1: controversies and recommendations. Ann Neurol. 2007;61(3):189-198.
3. Toonen JA, Anastasaki C, Smithson LJ, et al. NF1 germline mutation differentially dictates optic glioma formation and growth in neurofibromatosis-1. Hum Mol Genet. 2016;25 (9):1703-1713.
4. Toonen JA, Ma Y, Gutmann DH. Defining the temporal course of murine neurofibromatosis-1 optic gliomagenesis reveals a therapeutic window to attenuate retinal dysfunction. Neuro Oncol. 2017;19(6):808-819.
5. Brown JA, Gianino SM, Gutmann DH. Defective cAMP generation underlies the sensitivity of CNS neurons to neurofibromatosis-1 heterozygosity. J Neurosci. 2010;30 (16):5579-5589.
6. Diggs-Andrews KA, Brown JA, Gianino SM, Rubin JB, Wozniak DF, Gutmann DH. Sex is a major determinant of neuronal dysfunction in neurofibromatosis type 1. Ann Neurol. 2014;75(2):309-316.
7. Fisher MJ, Loguidice M, Gutmann DH, et al. Visual outcomes in children with neurofibromatosis type 1-associated optic pathway glioma following chemotherapy: a multicenter retrospective analysis. Neuro Oncol. 2012;14 (6):790-797.
8. Fisher MJ, Loguidice M, Gutmann DH, et al. Gender as a disease modifier in neurofibromatosis type 1 optic pathway glioma. Ann Neurol. 2014;75(5):799-800.
9. Toonen JA, Solga AC, Ma Y, Gutmann DH. Estrogen activation of microglia underlies the sexually dimorphic differences in Nf1 optic glioma-induced retinal pathology. J Exp Med. 2017;214(1):17-25.
10. Bajenaru ML, Hernandez MR, Perry A, et al. Optic nerve glioma in mice requires astrocyte Nf1 gene inactivation and Nf1 brain heterozygosity. Cancer Res. 2003;63 (24):8573-8577.
11. Anastasaki C, Mo J, Chen JK, et al. Neuronal hyperexcitability drives central and peripheral nervous system tumor progression in models of neurofibromatosis-1. Nat Commun. 2022;13(1):2785.
12. Guadagno J, Swan P, Shaikh R, Cregan SP. Microglia-derived IL-1β triggers p53-mediated cell cycle arrest and apoptosis in neural precursor cells. Cell Death Dis. 2015;6 (6):e1779.
13. Ferrari CC, Pott Godoy MC, Tarelli R, Chertoff M, Depino AM, Pitossi FJ. Progressive neurodegeneration and motor disabilities induced by chronic expression of IL-1beta in the substantia nigra. Neurobiol Dis. 2006;24 (1):183-193.
14. Dunn E, Sims JE, Nicklin MJ, O'Neill LA. Annotating genes with potential roles in the immune system: six new members of the IL-1 family. Trends Immunol. 2001;22 (10):533-536.
15. Hannah-Shmouni F, Trivellin G, Beckers P, et al. Neurofibromatosis type 1 has a wide spectrum of growth hormone excess. J Clin Med. 2022;11(8):2168.
16. Cordero FJ, Huang Z, Grenier C, et al. Histone H3.3K27M represses p16 to accelerate gliomagenesis in a murine model of DIPG. Mol Cancer Res. 2017;15 (9):1243-1254.
17. Wetmore C, Eberhart DE, Curran T. Loss of p53 but not ARF accelerates medulloblastoma in mice heterozygous for patched. Cancer Res. 2001;61(2):513-516.
18. Wang X, Zhu S, Drozda M, et al. Minocycline inhibits caspase-independent and -dependent mitochondrial cell death pathways in models of Huntington's disease. Proc Natl Acad Sci U S A. 2003;100(18):10483-10487.
19. Guo X, Pan Y, Xiong M, et al. Midkine activation of CD8+ T cells establishes a neuron-immune-cancer axis responsible for low-grade glioma growth. Nat Commun. 2020;11(1):2177.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1.

