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Pro-Inflammatory Molecules Implicated in Multiple Sclerosis Divert the Development of Human Oligodendrocyte Lineage Cells

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Abstract

Background and Objectives

Oligodendrocytes (OLs) and their myelin-forming processes are lost during the disease course of multiple sclerosis (MS), targeted by infiltrating leukocytes and their effector cytokines. Myelin repair is considered to be dependent on recruitment and differentiation of oligodendrocyte progenitor cells (OPCs). The basis of remyelination failure during the disease course of MS remains to be defined. The aim of this study was to determine the impact of the proinflammatory molecules tumor necrosis factor-α (TNFα) and interferon-γ (IFNγ) on the differentiation of human OPCs.

Methods

We generated human OPCs from induced pluripotent stem cells with a reporter gene under the OL-specific transcription factor SOX10. We treated the cells in vitro with TNFα or IFNγ and evaluated effects regarding cell viability, expression of OL lineage markers, and coexpression of astrocyte markers. To relate our findings to the molecular properties of OPCs as found in the MS brain, we reanalyzed publicly available single-nuclear RNA sequencing (RNAseq) datasets.

Results

Our analysis indicated that both TNFα and IFNγ decreased the proportion of cells differentiating into the OL lineage, consistent with previous reports. Uniquely, we now observe that the TNFα effect is linked to aberrant OPC differentiation in that a subset of O4+, reporter-positive cells coexpressing the astrocytic marker aquaporin-4. At the transcriptomic level, the cells acquire an astrocyte-like signature alongside a conserved reactive phenotype while downregulating OL lineage genes. Analysis of single-nuclear RNAseq datasets from the human MS brain revealed a subset of OPCs expressing an astrocytic signature.

Discussion

In the context of MS, these results imply that OPCs are present but inhibited from differentiating along the OL lineage, with a subset acquiring a reactive and stem cell–like phenotype, reducing their capacity to contribute toward repair. These findings help define a potential basis for the impaired myelin repair in MS and provide a prospective route for regenerative treatment.

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Supplementary Material

Introduction

Multiple sclerosis (MS) is a neuroinflammatory disease of the CNS, characterized by multifocal demyelinating lesions underlying disease relapses. Extent of neurologic recovery and subsequent development of a progressive disease course are linked at least in part to extent of tissue

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Glossary

AQP4 = aquaporin-4; **IFNγ** = interferon-γ; **iPSC** = induced pluripotent stem cell; **MHC** = major histocompatibility complex; **MS** = multiple sclerosis; **NG2** = neural/glial antigen 2; **NPC** = neural stem cell; **OL** = oligodendrocyte; **OPC** = oligodendrocyte progenitor cell; **RNAseq** = RNA sequencing; **ssGSEA** = single-sample Gene Set Enrichment Analysis; **TNFα** = tumor necrosis factor-α.

repair[1] and have yet to be addressed with currently available therapeutics. Initial lesion formation reflects injury of oligodendrocytes (OLs) and their myelin membranes by direct contact with CNS-infiltrating immune cells[2] and their effector molecules.[3] Progressive disease is linked to ongoing loss of OLs. Myelin repair mechanisms are dependent on recruitment and differentiation of oligodendrocyte (OL) progenitor cells (OPCs) shown to be present in the CNS parenchyma. Block of OPC differentiation ability has been observed in situ.[1,4] However, the exact cause remains unknown.

Soluble mediators of tissue injury released by CNS-infiltrating leukocytes include proinflammatory molecules such as tumor necrosis factor-α (TNFα) and interferon-γ (IFNγ). These have been shown to directly affect myelinating OLs and activate other resident glial cells.[5] Both TNFα and IFNγ have been found to be cytotoxic to rodent-derived primary OPCs in vitro.[6-8] However, functional studies of primary human OPCs are limited by access because such cells arise only in later second trimester of development.[9] Previous studies using human-induced pluripotent stem cells (iPSCs) generated by genetic reprogramming[10] have shown that TNFα and IFNγ inhibited the terminal differentiation of OL lineage cells. These cytokines reduced process extension from primary mature human OLs in vitro without dedifferentiation.[11]

Aside from their canonical roles in generating myelinating OLs, OPCs can mediate a number of immune functions. In addition to their motility, it has been noted that OPCs have phagocytic capacity,[12] release proinflammatory cytokines,[13] and express major histocompatibility complex (MHC) molecules,[14,15] which are involved with leukocyte activation and recruitment. The term "reactive" OPC[16] can be used in the instance where OPCs upregulate neural/glial antigen 2 ([NG2], *CSPG4* gene) and increased proliferation in response to tissue injury. Reactive OPCs have been observed to upregulate the same set of genes as astrocytes and microglia after stab wound injury,[17] hinting at a conserved response among glial cells.

In this study, we used a growth factor–based protocol for deriving human OL lineage cells from iPSCs,[18] including the use of a reporter gene under the OL-specific transcription factor SOX10. This approach allowed us to assess the effects of TNFα and IFNγ on early differentiation stages and lineage commitment of these cells. Although both decrease differentiation, we observed that TNFα exposure induces the upregulation of aquaporin-4 (AQP4) in vitro. Bulk RNA sequencing (RNA-seq) analysis of reporter-positive OPCs indicated upregulation of astrocytic genes alongside an acquired reactive OPC signature after TNFα exposure. Furthermore, reanalysis of previously published MS single-nuclear RNAseq datasets confirmed the presence of *AQP4*+ OPCs in situ, preferentially localizing to active lesions in the brain.

Methods

Standard Protocol Approvals, Registrations, and Patient Consents

The use of iPSCs and stem cells in this research was approved by the McGill University Health Centre Research Ethics Board (DURCAN_IPSC/2019–5374).

Human iPSC Culture and OPC Differentiation

Previously characterized healthy control iPSC lines (3,450, 3450-*SOX10*^{mO}) generated in-house at the Early Drug Discovery Unit were used for this study.[18,19] iPSC-derived OL lineage cells were differentiated according to a recently published protocol.[18] Cells were treated with TNFα (100 ng/mL, ThermoFisher Scientific, Mississauga, ON) or IFNγ (100 ng/mL, ThermoFisher Scientific, Mississauga, ON) for 4–6 days before fixation (days 18–21 of differentiation).

Immunofluorescence Staining and Analysis

Cell viability (propidium iodide, PI, Invitrogen, Waltham, MA) and differentiation (O4, R&D systems, Oakville, ON) were determined by immunofluorescence as previously described in detail.[20] In brief, cells were stained pre-fixation with anti-O4 antibody (1:200) and PI (1:200) for 15 minutes at 37°. After fixation, cells were stained with corresponding secondary antibodies (1:400) conjugated with Alexa Fluor 647 (Thermo Fisher Scientific, Mississauga ON). Plates were imaged with a 10X objective using a Zeiss Axio Observer fluorescence microscope (Carl Zeiss Canada, Toronto, ON) or the ImageXpress (Molecular Devices, San Jose, CA) high-content imaging platform after staining for O4 and PI. Cells were counted by a blinded individual using ImageJ software or automatically using ImageXpress software.

Fluorescence-Activated Cell Sorting and Flow Cytometry

For the induced pluripotent stem cell (iPSC) reporter line, sorting was based on reporter intensity using a previously described protocol,[18] with the Fluoresence Activated Cell Sorter (FACS) Aria Fusion (BD Biosciences, San Jose, CA). For phenotyping, cells were stained as previously described, using the LIVE/DEAD cell viability kit (Invitrogen, Waltham

MA) and preconjugated antibodies (O4-APC, Miltenyi, Auburn, CA; AQP4-AF488, Bioss USA, Woburn, MA). Samples were acquired on the Attune flow cytometer (Thermo-Fisher Scientific, Mississauga, ON). Data were analyzed using FlowJo software (BD Biosciences, San Jose, CA).

Bulk RNAseq Preparation and Analysis

FACS-sorted 3450-*SOX10*^{mO} cells were collected, after treatment with TNFα for 4 days. RNA was extracted using the Norgen (Thorold, ON) single-cell RNA purification kit.

Library preparation, sequencing, quality check, alignment, quantification of raw read counts, and normalization of read counts were performed using the same methods as described previously.[21] Because these samples did not exhibit significant heterogeneity, we used DESeq2[22] for differential gene expression analysis following the methodology used previously in our group.[23] Significantly differentially expressed genes were identified using a threshold of log2 fold change >1 and adjusted p value cutoff of <0.05. Hierarchical clustering was performed using the Seaborn "clustermap" function[24] with an average linkage method and correlation-based distances on log2-transformed normalized read counts. We applied custom colormaps to highlight expression differences across conditions with row normalization, and heatmaps were generated with matplotlib[25] and Seaborn to visualize.

Using the gseapy package, we performed single-sample Gene Set Enrichment Analysis (ssGSEA) on the normalized read counts.[26] The ssGSEA was run with rank-based normalization, and gene sets were defined from a GMT file of biological pathways (c5.go.bp.v2024.1.Hs.symbols.gmt).

All single-cell RNA-seq analyses were performed using the same pipeline used in our previous publication[23] on the same publicly available datasets.[27,28]

Statistical Analysis

Statistical analyses were performed using Excel or GraphPad Prism software. Cell culture studies were performed with at least 3 individual replicates per experiment (details in figure legends). The Student t test was used for comparisons between 2 groups or a Dunnett multiple comparisons test was used. p Values < 0.05 were considered statistically significant.

Data Availability

Sequencing data (differentially expressed genes) have been provided in eTable 1.

Results

Proinflammatory Molecules Do Not Induce Cytotoxicity in Human OPCs but Decrease the Proportion of Cells in the OL Lineage

We generated OL lineage cells from human iPSCs,[18] using our previously characterized iPSC reporter line expressing the fluorophore mOrange under the control of the OL-specific transcription factor SOX10 (*SOX10*^{mO}). We sorted *SOX10*^{mO} cells as previously described[18] (eFigure 1), where the fluorescence intensity of the reporter is directly proportional to the developmental status of OL lineage cells. Under basal conditions (Figure 1A), around 45% of cells are mOrange medium, whereas 17% are mOrange high, indicating most early OL lineage cells. To study the effects of the selected molecules on our human OPCs, we simultaneously treated cells with our selected proinflammatory molecules while inducing differentiation. Exposure of the cells to TNFα and IFNγ resulted in a 50% reduction in the proportion of O4+ cells (TNFα $p = 0.0355$, IFNγ $p = 0.0040$) (Figure 1B, eFigure 2). We used our previously published[20] glucose deprivation condition (NG) as a positive control for detection of cell death ($p = 0.0007$). In comparison, we did not observe significant cell death after exposure to TNFα or IFNγ (Figure 1C, eFigure 2). This then prompted us to assess whether another glial cell type was compensating for this decrease in the proportion of OPCs, given how this protocol generates mixed glial cultures.[18] Of interest, we observed that the percentage of AQP4+ astrocytes increased ($p < 0.0001$) after treatment with TNFα, but not with IFNγ (Figure 1D). Building on these findings, we next sought to elucidate the source of the increased proportion of AQP4+ cells in culture.

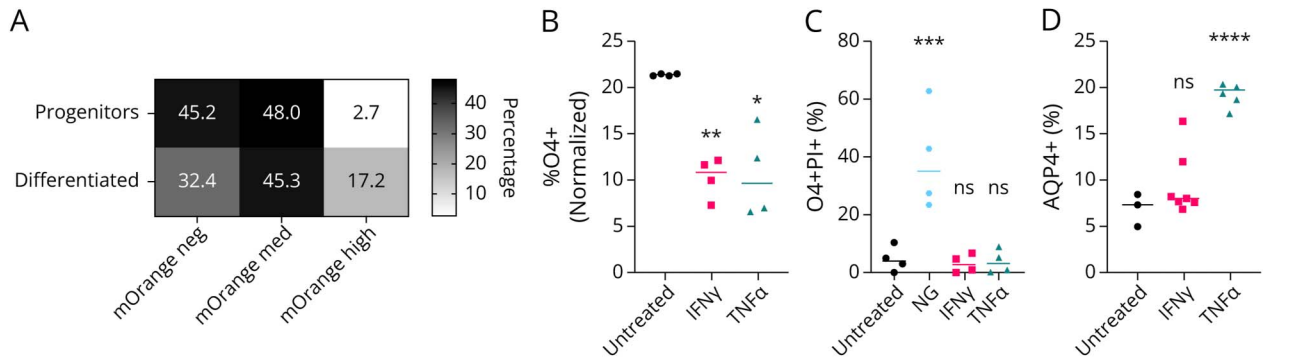
Increase in Astrocyte Proportions Under Inflammatory Conditions In Vitro Derived From an Initial OPC Population

It has been suggested that OPCs may retain or acquire multilineage potential under certain conditions,[29-31] although this has not yet been demonstrated in human cultures. For example, rodent OPCs exposed to fetal bovine serum have been shown to generate astrocytes.[32] With fate mapping techniques in murine models, studies have shown that in both normal development[31] and stress conditions,[33] OPCs are directed into astrocytes. To explore this possibility, we analyzed the O4+ population in our iPSC-derived cell lines for coexpression of other surface markers through flow cytometry. In response to cytokine exposure (Figure 2, A and B), a proportion of the cells coexpressed the astrocyte marker AQP4 with TNFα ($p = 0.0046$) treatment but not with IFNγ ($p = 0.7678$). To establish that this population originated from the OL lineage, we used our *SOX10*^{mO} reporter line.[18] We observed that the AQP4+ O4+ subpopulation of cells expressed *SOX10*^{mO} (Figure 2C), suggestive of an OL lineage origin. These results indicate that a proportion of astrocytes in our cultures after TNFα exposure originated from our *SOX10*^{mO} reporter-positive OPCs.

SOX10^{mO} OPCs Acquire a Reactive and Astrocyte-Like Gene Signature

To determine whether the observed phenotypic responses of our TNFα-treated cells could also be seen at the transcriptomic level, we performed bulk RNAseq after cell sorting of our reporter line.[18] Most of the O4+AQP4+ cells presented with an intermediate *SOX10*^{mO} intensity based on previously published criteria[18] (eFigure 3). After sequencing of our *SOX10*^{mO}-sorted cells, principal component analysis revealed

Figure 1 Proinflammatory Molecules Block Human OPC Differentiation



(A) iPSC OPCs generated from our SOX10^{mO} reporter line, subset based on fluorescence intensity before and after 21-day differentiation, showing an increase in SOX10^{mO} with not all cells being terminally mature (mOrange high). Median percentage value of n = 4 passages displayed in heatmap. (B) Percentage of O4+ OPCs (normalized) as counted after immunofluorescence staining and treatment for 4 days, n = 4 passages. *$p < 0.05$, **$p < 0.01$. (C) Proportion of O4+ PI+ dead OPCs as measured by immunofluorescence staining after treatment for 4 days, n = 4 passages. ***$p < 0.001$. NG = no glucose, positive control. (D) Proportion of AQP4+ astrocytes in culture as measured by flow cytometry after treatment for 4–6 days, n = 3 passages. ****$p < 0.0001$. OPC = oligodendrocyte progenitor cell.

that the primary source of variance is reflected in PC1 (Figure 3A). TNFα treatment led to changes in the gene expression profile in comparison with the control group (Figure 3B, eTable 1), many of the top genes related to immune processes. Our *SOX10^{mO}* cells had an enrichment for terms related to glial cell proliferation and activation (Figure 3C, eTable 2). A previous study[17] observed a common glial response to injury among microglia, astrocytes, and OPCs in response to a stab wound injury, a model relevant to multiple aspects of MS: glial activation, repair, secondary neurodegeneration, and neuroinflammation. To verify whether this is true in our model, we performed a ssGSEA of this conserved gene signature and found most (87%) of the genes upregulated (Figure 3D, eFigure 4). We observed a significant enrichment score of neural progenitor cell (NPC) proliferation signature (Figure 4A), further contributing to the notion of our TNFα-treated OPCs exhibiting the previously described reactive OPC phenotype.[16] To confirm our in vitro findings of OPCs acquiring astrocytic characteristics, we compared our TNFα-treated OPCs with OL differentiation and astrocyte gene signatures (Figures 4, B and C). We observed downregulation of mature OL genes (*MBP, PLP1*) and OPC identity (*PDGFRA, GPR17*) (Figure 4B). Furthermore, we observed a significant enrichment score of astrocytic differentiation (Figure 4C, left panel) and upregulation of key genes (*HES5, BMP2, BMP4, ID2*)[34] (Figure 4C, right panel, eFigure 5), confirming our flow cytometry findings. This suggests that in addition to a reactive OPC gene signature, OPCs exposed to TNFα acquire gene signatures associated with NPC proliferation and astrocytic differentiation, highlighting a complex response to inflammation.

OPCs in the MS Brain Exhibit an Astrocyte-Like Gene Signature

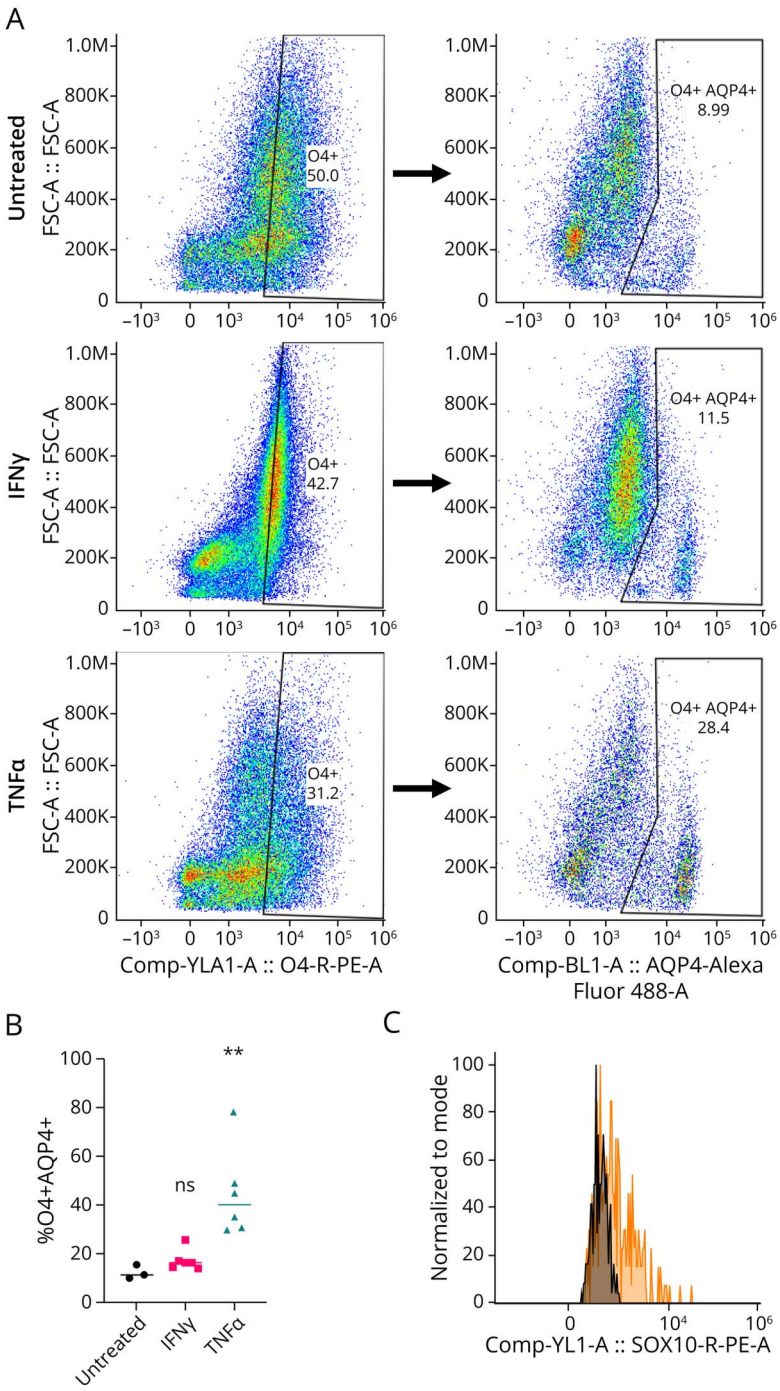
To address the hypothesis that a mechanism altering the balance of resident OPC populations in favor of astrocytes could limit repair in the MS brain, we reanalyzed publicly available single-nuclear RNAseq datasets.[27,28] We investigated the OPC populations marked by *PDGFRA* and *CSPG4* expression (Figure 5A). In this population of OPCs, 5% coexpressed *AQP4* (Figure 5B). Comparative analysis of *AQP4*+ OPCs with top 100 genes of an astrocytic gene signature revealed that *AQP4*+ OPCs are upregulating genes that include *SOX9* and *GFAP* (Figure 5D). We found this subpopulation to preferentially localize to the core of an MS lesion (Figure 5C, eFigure 6). The identification of this OPC population with an astrocytic gene signature localized within human MS brain lesions may prove a novel mechanism preventing adequate remyelination in an inflammatory context.

Discussion

On CNS infiltration during MS, leukocytes and glial cells (astrocytes and microglia) will release cytokines, which have a direct effect on OLs and surrounding glia,[5,35] contributing to an overall proinflammatory microenvironment. These cytokines, among others, are found to be increased in the spinal fluid and peripheral blood of people with MS.[35-37] Microarray analysis of MS lesions has shown an increase in cytokine expression and downstream regulatory pathways.[38] Furthermore, spinal fluid lymphocyte gene expression of both TNFα and IFNγ has been found to be correlated with lesion load as seen by MRI,[36] highlighting the relevance of these cytokines to MS disease pathogenesis.

Maturity state is a significant determinant in the response of glial cells to inflammatory mediators. Previous studies observed TNFα-mediated apoptosis of early A2B5+ glial precursor cells and PDGFRα+ OPCs isolated from 14–20-week fetal human brain samples.[39] Additional studies showed that the myelination potential of these cells was more limited when derived from tissue earlier in gestation.[40] On the other hand, these same mediators induced process retraction in mature

Figure 2 TNFα Increases the Proportion of OPCs Coexpressing AQP4

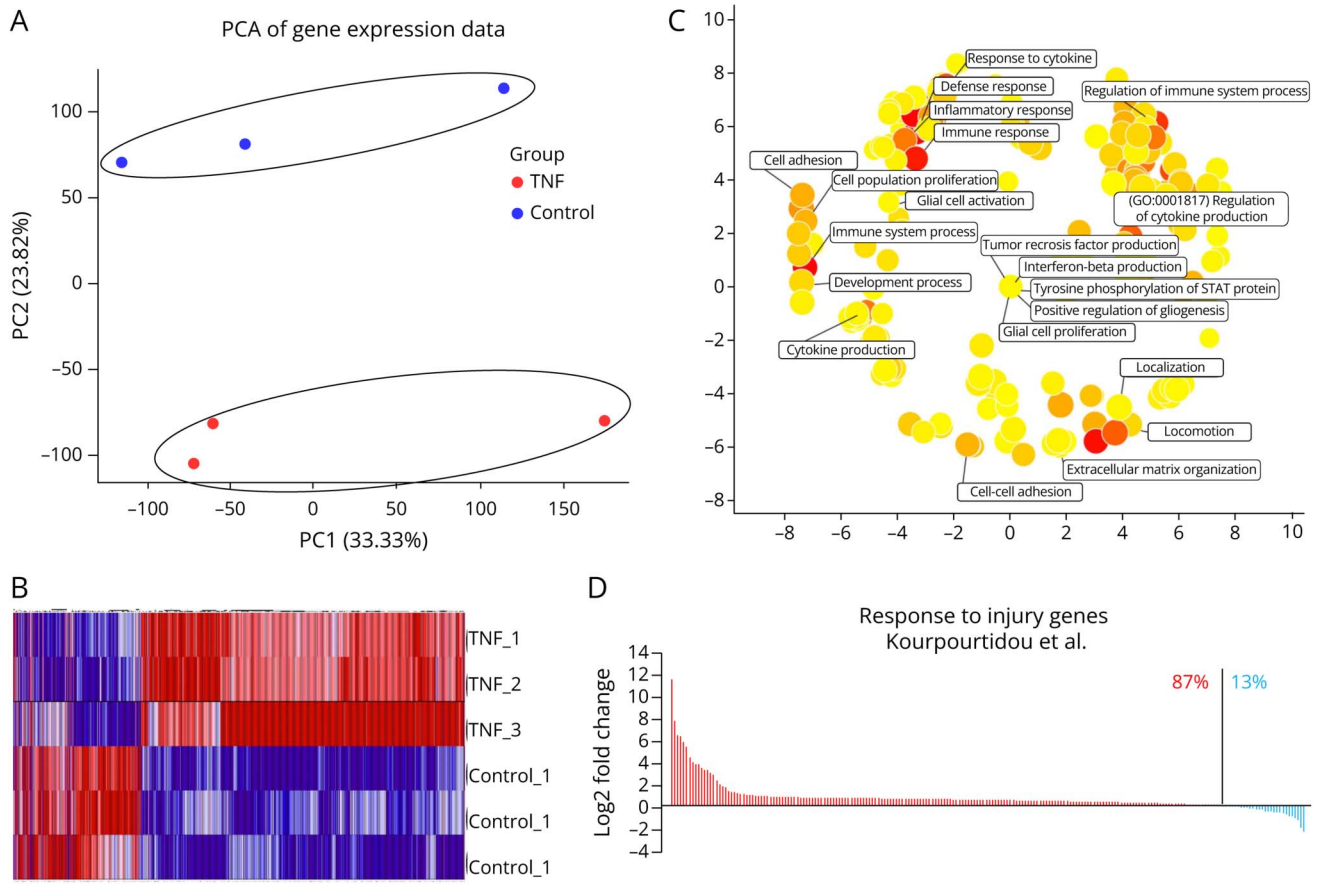


(A) Representative gating scheme of OPC marker O4 (PE) coexpressing astrocyte marker AQP4 (AF488). (B) Percentage of O4+ OPCs also expressing AQP4 as measured by flow cytometry. n = 3, treatment between 4–6 days, $^{**}p < 0.01$. (C) Histogram of $SOX10^{mO}$ fluorescence intensity of O4+AQP4+ cells (orange) against a nonreporter control (black). Fluorescence intensity values normalized to mode. AQP-4 = aquaporin-4; OPC = oligodendrocyte progenitor cell; TNFα = tumor necrosis factor-α.

OLs rather than cytotoxicity.[41] Our use of a growth factor–based iPSC model recapitulates early OL commitment and early remyelination. We found reduced OPCs (O4+) in response to TNFα and IFNγ individually without the induction of cytotoxicity (as seen in rodent models and early human glial precursors). Uniquely, with TNFα but not IFNγ, we observed an increase in AQP4+ astrocytes (Figure 1). These findings demonstrate that proinflammatory contexts in our model may alter the balance of other glial populations, in parallel to reducing OPC differentiation rather than promoting OPC death.

It has been suggested that OPCs may retain the capacity to generate astrocytes in adulthood as a response to pathologic insults.[33,42,43] After neurogenesis in the developing brain, gliogenesis begins with the generation of astrocytes followed by oligodendrocytes, as reviewed previously.[44] Thus, early OL lineage cells may retain the capacity for astrocytic generation.

Figure 3 Transcriptome Changes in iPSC-Derived SOX10-Med Cells on Treatment With TNFα

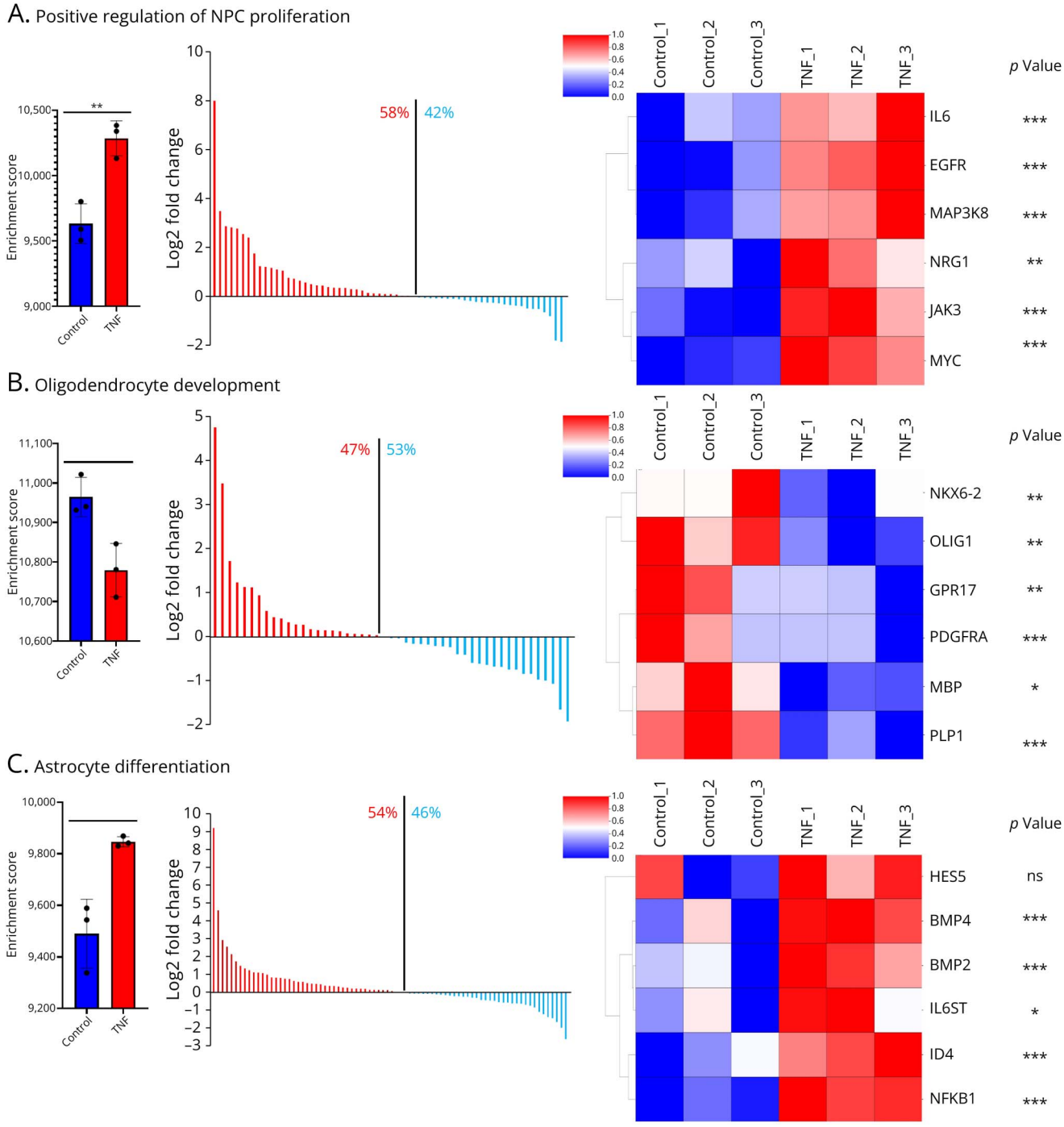


(A) Principal component analysis (PCA) using all detected genes reveals a clear effect of TNFα treatment in PC2. Control samples are shown in blue and treated samples in red. Normalized read counts from all detected genes were used. (B) Hierarchical clustering was performed on significantly differentially regulated genes (adjusted *p* value <0.05 and log2 fold change >1). Normalized read counts were used for clustering, and row normalization was applied to visualize the heatmap. Top genes (log2FC) include *EXOC3L4*, *CSF2RB*, *CXCL10*, *ANO9*, and *CXCL13*. (C) Gene Ontology (GO) analysis of significantly upregulated genes was conducted using g:Profiler, and GO terms were summarized and visualized with the Revigo tool. (D) 214 of 241 genes related to injury response in glial cells as published by Kopourtidou et al. (2024) were detected in our bulk RNAseq dataset. These genes were more enriched in TNFα-treated cells compared with control. 214 genes are visualized using log2 fold change after single-sample Gene Set Enrichment Analysis. TNFα = tumor necrosis factor-α.

It has been shown that certain in vitro conditions may induce the developmental trajectory of OPCs toward an astrocytic fate.[32] Several subsequent studies of spinal cord injury,[33] brain ischemia,[42] and glioma[43] have suggested that this is possible across other disease models as well. In this study, we consider whether a similar phenomenon is being induced by proinflammatory molecules. Using our previously characterized iPSC reporter line (*SOX10*^{mO}) and analyzing the expression of cell surface markers (O4, AQP4), we have observed an increase in an astroglial marker (AQP4) in our cultures after TNFα exposure (Figure 1D) and an emergence of OPC subpopulation coexpressing AQP4 (Figure 2), but not with IFNγ. In addition to reinforcing previous findings regarding the retention of multilineage potential in OPCs,[29-31] this could unveil an alternative mechanism by which the endogenous OPC-mediated repair pathway in the CNS is compromised in MS conditions. Rather than differentiating into myelinating OLs, OPCs may instead give rise to astrocytes, contributing to the failure of proper myelin repair.

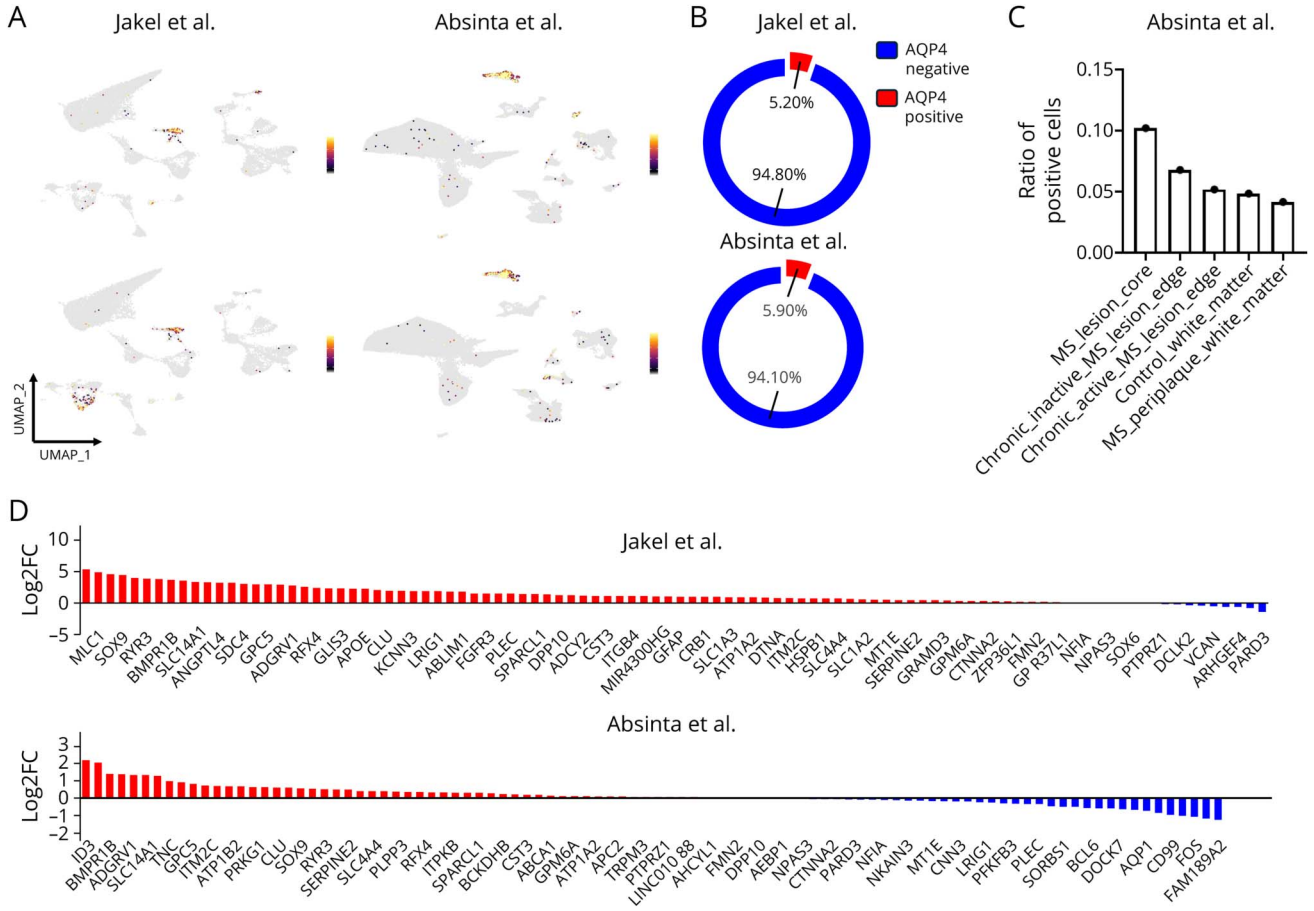
OPCs are immune competent, which is demonstrated by the expression of MHC molecules,[15] for example. Rodent OPC exposure to IFNγ and TNFα has shown to increase phagocytic activity,[45] and OPCs have been shown to engulf axons/synapses in the developing brain.[12] A reactive OPC can be characterized by the increase of NG2 and proliferation,[16] and was first noted in rodents as a hallmark after brain injury. We further observed that gene signatures related to neural stem cells (NPCs) and cell cycle were significantly upregulated (Figure 4A). *CSPG4* (NG2) was moderately upregulated although not significant (data not shown). However, the underlying mechanisms driving this response and its potential consequences for the broader OL population remain unclear. Our findings suggest that inflammatory stimuli can shift OPCs toward an immune-like function at the expense of their oligodendroglial precursor role, thereby potentially impairing their capacity for remyelination (Figures 3 and 4), expanding on previous observations in rodents.[13] By acquiring stem-like signatures and skewing the differentiation response of OPCs after injury (Figure 4), the balance of glial cells becomes

Figure 4 TNFα Treatment of iPSC-Derived SOX10-Med Cells Deviates Glial Differentiation Program and Includes a Neural Progenitor Cell Proliferation Signature



(A) Single-sample Gene Set Enrichment Analysis (ssGSEA) of the NPC proliferation signature shows significant enrichment in TNFα-treated cells compared with control cells (left panel). Log2 fold changes indicate a higher number of genes with increased expression in this signature on TNFα treatment (middle panel). Examples of significantly upregulated NPC proliferation genes are visualized in a heatmap, with hierarchical clustering performed using normalized read counts, and DESeq2-calculated *p* values are provided for each gene (right panel). (B) ssGSEA of oligodendrocyte development genes using normalized read counts shows reduced enrichment of this signature in TNFα-treated cells (left panel). Log2 fold change analysis reveals a higher proportion of genes with elevated expression in control cells compared with TNFα-treated cells (middle panel). (C) ssGSEA of astrocyte differentiation genes indicates greater enrichment of this signature under TNFα treatment conditions (left panel) and a higher proportion of genes with increased expression in this signature (middle panel). Examples of significantly upregulated genes involved in astrocyte differentiation are shown in the heatmap. Statistical significance is denoted as follows: ns = not significant, * = $p < 0.01$, ** = $p < 0.001$, and *** = $p < 0.0001$ (DESeq2 results). NPC = neural progenitor cell; TNFα = tumor necrosis factor-α; ssGSEA = single-sample Gene Set Enrichment Analysis.

Figure 5 Identification and Characterization of Oligodendrocyte Progenitor Cells With Astrocytic Signatures in Human Single-Nuclear RNA Sequencing DataSets



(A) Analysis, clustering, and annotation of human snRNA-seq datasets derived from the studies by Jakel et al. (left panel) and Absinta et al. (right panel). OPC populations were identified using specific marker genes, notably PDGFRA and CSPG4/NG2. (B) A subset of OPCs was found to express AQP4, a marker typically associated with astrocytes. AQP4-positive cells are indicated in red, whereas cells lacking AQP4 expression are depicted in blue. (C) Bar graph depicting ratio of AQP4+ to AQP4- OPCs in different regions of the MS brain from the dataset by Absinta et al. Highest ratio of AQP4+ OPCs found within the core of an MS lesion. (D) Comparative analysis of AQP4-positive OPCs vs a gene signature based on top 100 genes in the astrocyte cluster by Jakel et al. (2019), with the log2 fold change (log2FC) depicted in bar plots. Top panel presents data from the study by Jakel et al., 2019, and bottom panel from the study by Absinta et al., 2021, with upregulated genes shown in red and downregulated genes in blue. AQP-4 = aquaporin-4; OPC = oligodendrocyte progenitor cell; snRNA-Seq = single-nuclear RNA sequencing.

affected with the increased generation of astrocytes as seen in our human OPC cultures (Figure 1).

This study demonstrates that early human OL lineage cells retain multilineage potential alongside their reactive capacity, likely as an adaptive response to stressors such as the proinflammatory conditions characteristic of MS. We observed a decrease in differentiation (Figure 1), in line with previous studies in different models, alongside the emergence of a population identified by the surface markers AQP4, O4, and our reporter (Figure 2). Furthermore, we observed an enrichment in proliferative and astrocytic gene signatures (Figure 4), which may indicate a skewed differentiation response. Relating back to the MS brain, we found a subpopulation of cells expressing *AQP4* within the OPC cluster (Figure 5) with an astrocytic gene signature, with the highest ratio found within MS lesions, suggesting that this is a biological response with disease relevance. Further work describing such lineage changes in vivo would be of importance, specifically with identifying a marker that can be stained for in situ in the MS brain. These findings highlight a potential therapeutic avenue—strategies aimed at promoting OPC differentiation along the OL lineage while inhibiting astrocytic conversion could enhance remyelination and mitigate disease progression.

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the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data. V.E.C. Piscopo: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data. J. Sirois: major role in the acquisition of data; analysis or interpretation of data. Q.L. Cui: study concept or design; analysis or interpretation of data. M. Yaqubi: major role in the acquisition of data. T.M. Durcan: drafting/revision of the manuscript for content, including medical writing for content. R. Schneider: drafting/revision of the manuscript for content, including medical writing for content. J.P. Antel: drafting/revision of the manuscript for content, including medical writing for content; study concept or design.

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Disclosure

The authors report no relevant disclosures. Full disclosure form information provided by the authors is available with the full text of this article at Neurology.org/NN.

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