CEREBROSPINAL FLUID DERIVED FROM PROGRESSIVE MULTIPLE SCLEROSIS PATIENTS PROMOTES NEURONAL AND OLIGODENDROGLIAL DIFFERENTIATION OF HUMAN NEURAL PRECURSOR CELLS IN VITRO

Barbara Cymring, Massimiliano Cristofanilli,* Amy Lu, Hannah Rosenthal, Saud A. Sadiq
Multiple Sclerosis Research Center of New York

INTRODUCTION

➢ Adult multipotent neural precursor cells (NPCs) have the capacity for self-renewal and differentiation into functional brain cells (e.g. neurons, astrocytes or oligodendrocytes) within discrete tissue-specific germinal niches.

➢Due to their intrinsic plasticity, NPCs can be considered an essential part of the cellular mechanism(s) by which the central nervous system (CNS) tries to repair itself after an injury.

➢Although developing evidence indicates that endogenous neurogenesis and gliogenesis occur as part of an 'intrinsic' self-repair process during the course of inflammatory CNS disorders, such as multiple sclerosis (MS), there are no convincing explanations about the overall incapacity of the endogenous stem cells to promote full and long-lasting CNS repair in progressive forms of MS.

➢Many reports suggest that endogenous NPCs, while contributing to CNS repair in MS, may also become the target of the disease itself.

➢ In this study, we investigated the effect of applications of cerebrospinal fluid (CSF) derived from progressive MS patients on the survival, proliferation, and differentiation properties of human embryonic-derived neural stem cells (ES-NSCs) in vitro.

METHODS

➢CSF from primary progressive (PPMS) and secondary progressive (SPMS) MS patients or control CSF was diluted in the culture media (5%) and administered on cultured ES-NSCs.

➢CSF-media was replaced every other day and samples were collected at 14 days post-treatment and analyzed by FACS, q-RT-PCR, immunocytochemistry (ICC), and western blot (WB).

	Controls (n = 19)[a]	SPMS (n = 18)	PPMS (n = 18)
Median age (range)	50 (25–66)	48 (40–69)	55 (30–77)
Gender (M:F)	7 : 12	7 : 11	8 : 10
Median EDSS (range)	n/a	7.25 (3.5–9.0)	6.5 (2–9.0)

Table 1. Patient demographics
SPMS, secondary progressive multiple sclerosis; PPMS, primary progressive multiple sclerosis; EDSS, expanded disability status scale. [a] Control CSF samples from patients with other neurological diseases (Leigh's disease, migraine, post-transverse myelitis, cervical spondylosis, spastic dystonia, parkinsonism, post-CVA, Sjogren's disease, spinal cord injury, encephalopathy, neurosarcoidoisis, andtraumatic brain injury).

RESULTS

A highly pure population of sox2+/Nestin+ NSCs with the ability to differentiate in the mature brain cells (neurons, astrocytes, and oligodendrocytes) was used in this work (Figure1).

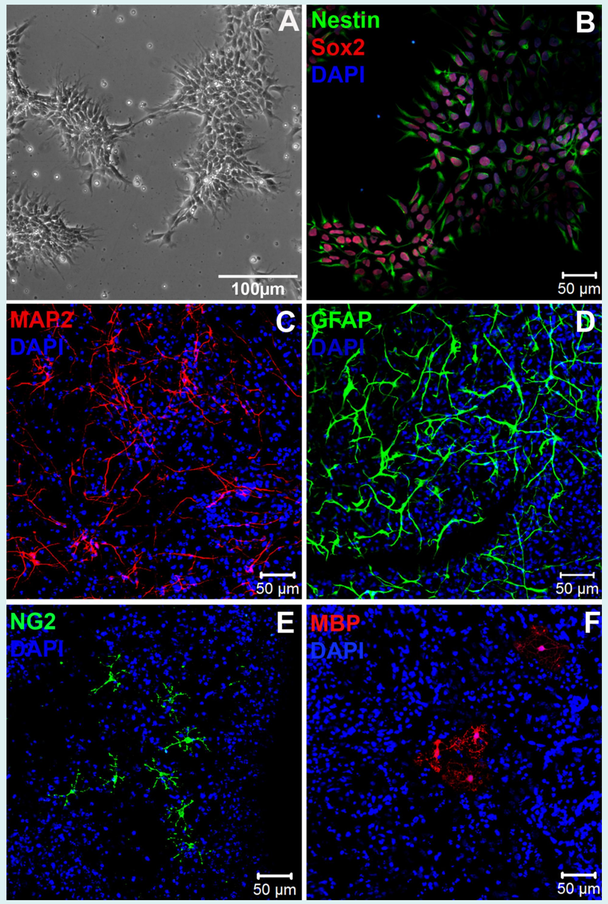

Figure 1. Characterization of the embryonic-derived neural stem cells ENStem-A and their neuroepithelial differentiation potential. Cells seeded in adherent condition, with proliferation media, form rosette like structures visible in phase contrast **(A)** and are sox2+/nestin+ in immunofluorescent staining **(B)**. Two weeks after growth factor removal **(C-F)**, MAP2+ neurons **(C)**, GFAP+ astrocytes **(D)**, NG2+ OPCs (E), and MBP+ myelinating oligodendrocytes **(F)** are present.)

To study the effect of a progressive MS-like milieu on the survival, proliferation, and differentiation properties of NSCs in vitro, CSF from untreated PPMS and SPMS patients as well as from control individuals (Table1) was applied as described in the methods section.

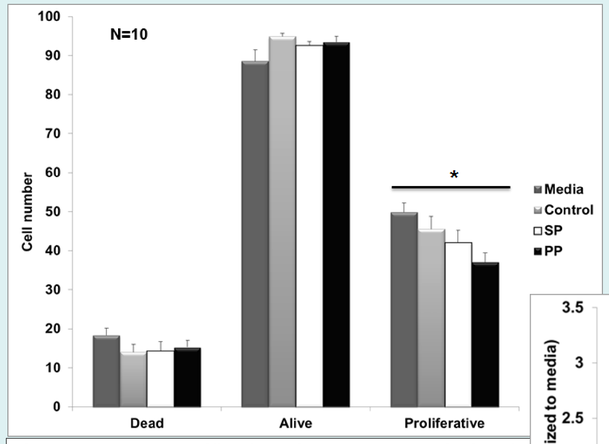

Figure 2. FACS quantification of ENStem-A survival and proliferation after CSF treatment. CSF from progressive MS patients reduced cell proliferation but did not affect cell survival. Error bars represent SEM. *p=0.023, one-way ANOVA [post-hoc analysis: *Tukey HSD Test* (**Proliferative:** P<.005 for: PP vs. Media or C
N= number of patients.

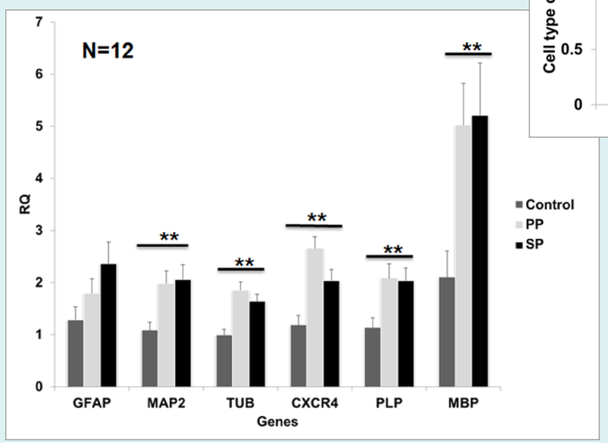

Figure 2. FACS quantification of ENStem-A survival and proliferation after CSF treatment. CSF from progressive MS patients reduced cell proliferation but did not affect cell survival. Error bars represent SEM. *p=0.023, one-way ANOVA [post-hoc analysis: *Tukey HSD Test* (**Proliferative:** P<.005 for: PP vs. Media or CTRL]. N= number of patients.

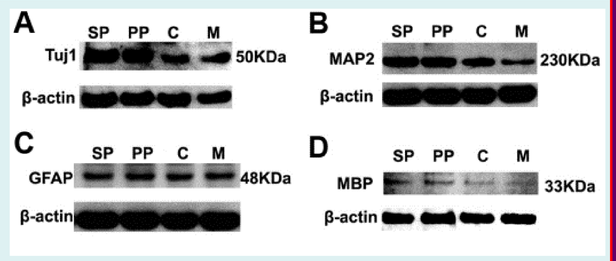

Figure 4. Western blot characterization of ENStem-A after CSF treatment and growth factor removal. Compared to control-CSF (C), CSF from progressive MS patients increased the amount of neuronal (Tuj1 and MAP2) and oligodendritic (MBP) proteins but had no effect on the astrocytic protein GFAP. Each band represents pooled samples treated with CSF from 3 different individuals. The experiment was repeated two more times using different samples, with similar results.

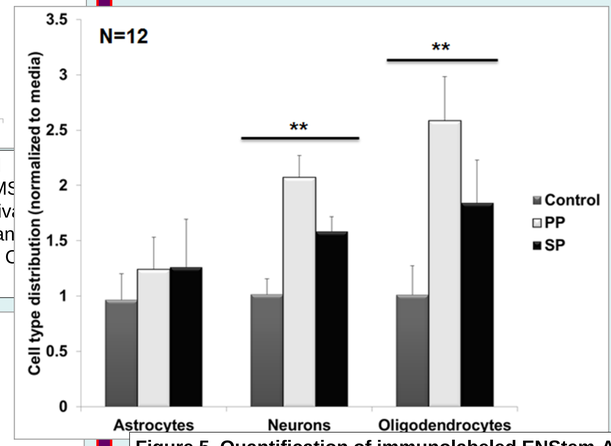

Figure 5. Quantification of immunolabeled ENStem-A after CSF treatment and growth factor removal. CSF from progressive MS patients increased the numbers of ENStem-A derived neurons and oligodendrocytes. For each cell type, values were expressed as percentage of total cell count and normalized to media treated samples. Error bars represent SEM. **p<0.01, one-way ANOVA [post-hoc analysis: *Tukey HSD Test* (**Neurons, Oligodendrocytes:** P<.001 for: SP vs. Control or Media; P<.005 for PP vs. Control or Media]. N= number of patients.

CONCLUSIONS

CSF-MS reduced the proliferation of ES-NSC and increased their differentiation toward neuronal and oligodendroglial cells compared to control CSF.