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Regular Article

Progressive multiple sclerosis cerebrospinal fluid induces inflammatory demyelination, axonal loss, and astrogliosis in mice☆

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ABSTRACT

Multiple sclerosis (MS) is an autoimmune disease characterized by inflammatory demyelination and neurodegeneration throughout the CNS, which lead over time to a condition of irreversible functional decline known as progressive MS. Currently, there are no satisfactory treatments for this condition because the mechanisms that underlie disease progression are not well understood. This is partly due to the lack of a specific animal model that represents progressive MS. We investigated the effects of intracerebroventricular injections of cerebrospinal fluid (CSF) derived from untreated primary progressive (PPMS), secondary progressive (SPMS), and relapsing/remitting (RRMS) MS patients into mice. We found discrete inflammatory demyelinating lesions containing macrophages, B cell and T cell infiltrates in the brains of animals injected with CSF from patients with progressive MS. These lesions were rarely found in animals injected with RRMS-CSF and never in those treated with control-CSF. Animals that developed brain lesions also presented extensive inflammation in their spinal cord. However, discrete spinal cord lesions were rare and only seen in animals injected with PPMS-CSF. Axonal loss and astrogliosis were seen within the lesions following the initial demyelination. In addition, Th17 cell activity was enhanced in the CNS and in lymph nodes of progressive MS-CSF injected animals compared to controls. Furthermore, CSF derived from MS patients who were clinically stable following therapy had greatly diminished capacity to induce CNS lesions in mice. Finally, we provided evidence suggesting that differential expression of pro-inflammatory cytokines present in the progressive MS CSF might be involved in the observed mouse pathology. Our data suggests that the agent(s) responsible for the demyelination and neurodegeneration characteristic of progressive MS is present in patient CSF and is amenable to further characterization in experimental models of the disease.

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Introduction

MS is an autoimmune disease of unknown origin, characterized by demyelination and axonal loss throughout the CNS (Gironi et al., 2000; Compston and Coles, 2008). MS often starts out as a clinically isolated syndrome followed by a series of alternating periods of remission and exacerbation, referred to as RRMS (Hafler, 2004; Sadiq, 2005). While patients typically return to near normal neurologic function at the end of each episode, over time, failure of the CNS to remyelinate MS lesions (Franklin, 2002) and regenerate axons (Trapp et al., 1998; Kornek et al., 2000) can lead to an irreversible progression of clinical disability (SPMS) (Ferguson et al., 1997; Lovas et al., 2000). In addition, 10%–15% of MS patients will have clinical progression from onset without remissions (PPMS) (Miller and Leary, 2007).

At present, the therapeutic options for patients with progressive MS are limited, and no regenerative treatment exists for this condition. In addition, although many animal models have been successfully employed to reproduce specific features of the histopathology and neurobiology of multiple sclerosis, there is no single model that fully captures the entire complexity of progressive MS and its heterogeneity (Gold et al., 2006). Moreover, tissue specimens from patients with progressive MS are not generally available, and post-mortem pathology poorly represents the dynamic biological events related to ongoing disease pathogenesis. In contrast, CSF is readily obtainable and can be studied throughout the course of the disease. These proprieties in conjunction with the fact that molecules secreted by resident and infiltrating cells of the CNS drain into the CSF make it a useful tool to monitor CNS biology and disease activity for progressive MS (Awad et al., 2010; Harris et al., 2013; Stangel et al., 2013). Therefore, in vivo studies using CSF obtained from progressive MS patients are likely to yield important and new insights into the mechanisms of progressive disease.

Abbreviations: CSF, cerebrospinal fluid; CTRL, control; EDSS, expanded disability status scale; ITMTX, intrathecal methotrexate; MS, multiple sclerosis; PPMS, primary progressive multiple sclerosis; SPMS, secondary multiple sclerosis progressive; RRMS, relapsing/remitting multiple sclerosis.

☆ Conflict of interest: The authors declare no competing financial interests.

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We recently reported on a study that combined the use of CSF derived from progressive MS patients and cultured human neural progenitors cells, in order to understand the mechanisms of stem cell-driven CNS repair (Cristofanilli et al., 2013). Here, we established a patient-specific model of investigation based on serial injections of CSF derived from MS patients into the mouse brain. Our data show for the first time that characteristic MS lesion pathology can be induced in mouse brain using patient CSF. We hope that this model, which represents a step forward in understanding the cellular and molecular mechanisms underlying disease progression in MS, can help design more effective treatments for this condition.

Methods

Patient selection and CSF collection

CSF was collected with IRB approval and informed consent from 28 patients (20 with clinically definite MS (McDonald et al., 2001) and 8 non-MS controls) (Table 1) seen at the International Multiple Sclerosis Management Practice, the clinical affiliate of the Tisch MS Research Center of New York. Of the 20 MS patients, 8 were secondary progressive, 6 primary progressive, and 6 relapsing remitting. Eight non-MS control (CTRL) CSF samples were obtained for diagnostic purpose from untreated patients with other neurological diseases, including inflammatory (human T lymphotropic virus type-I associated myelopathy and transverse myelitis) and non-inflammatory diseases (spinal cord injury, spinal stenosis, and stroke). All of the MS patients in this study had active disease (see below for definition), and none of them received any immunomodulatory treatment for at least 6 months prior to CSF collection (untreated samples). A second CSF collection was performed in 4 patients who appeared to have stabilized as assessed by the expanded disability status scale (EDSS) after treatment with intrathecal methotrexate (ITMTX) (3 patients) or natalizumab (1 patient). CSF was obtained with sterile techniques either by standard lumbar puncture or by access port aspiration of implanted pumps. CSF samples were processed immediately and kept on ice. Samples were centrifuged at 200 × g for 15 min to remove cells. All samples were confirmed to be free of red blood cell contamination. Aliquots of CSF were stored at −80 °C until use.

Clinical assessment of MS patients

All patients in the study had a complete neurological examination at the onset of the study. In addition, routine brain MRI scans were performed on all study subjects 1–2 weeks before CSF collection. Active disease was defined by the presence of any one of the following criteria in the 6 months preceding CSF sample collection: (1) one or more relapses documented by a neurologist's examination; (2) change in 0.5 point or greater in the EDSS score; and (3) change in MRI, specifically a change in the number or size of lesions or the presence of gadolinium-enhancing lesions.

Cannula implantation

Mice (C57/BL6, males) were purchased from The Jackson Laboratory (Bar Harbor, ME). At 8 weeks of age, animals were anesthetized with a solution of ketamine (100 mg/kg) and xylazine (10 mg/kg). They were positioned in a Kopf Small Animal Stereotaxic Instrument such that their heads were stable and immobile. The skull was exposed and a small hole was drilled at the following stereotactic coordinates in reference to the bregma: −1 mm (anteroposterior axis), 0 mm (medial–lateral axis). A custom made cannula (26 gauge, 5 mm pedestal, cut 3.5 mm below the pedestal; Plastics One, Roanoke, VA) was inserted for its entire length at this position to reach the dorsal third ventricle. The cannula was glued in place using Loctite 454 Instant Adhesive and then with dental cement. Two to three sutures were used to hold the skin around the incision together. A dummy (Plastics One), made to fit the length and gauge of the cannula, was screwed into the guide to keep the passage unobstructed. All animal experiments were approved by the IACUC committee of St Luke's Roosevelt Hospital Center of New York and conformed to NIH guidelines.

CSF injections

Mice were allowed to recover for one week before injections to ensure proper healing of the wound and stability of the cannula. Prior to the injection, the dummy was unscrewed from the guide. An injection needle (Plastics One) was attached to polyethylene tubing (Becton Dickinson, Sparks, MD) and rinsed first with 100% ethanol and then with sterile saline. 22 μl of sterile CSF was loaded into the tubing using a Hamilton Syringe. CSF or saline was injected over the course of 10 min. The needle was kept in place for an additional 5 min to avoid liquid backflow. Injections were given twice a week, for 1, 2, or 4 weeks. Mice were injected daily with BrdU (10 mg/mL) 5 μl/g of body weight from the day of the first CSF or saline injection until sacrifice.

Rota-Rod testing

An accelerating speed Rota-Rod (Panlab/Harvard Apparatus, Holliston, MA, USA) was used to assess locomotive impairment in the mice. Mice were trained twice a day for a week prior to cannula implantation, and then a baseline was measured prior to the first CSF injection for each mouse. Time until fall was measured as the average of three trials the day after each twice weekly injection for four weeks and then normalized against individual baselines.

Mouse sample collection

CSF collection

Samples were collected from all the mice injected with either saline, PPMS-, SPMS-, or CTRL-CSF that survived 8 injections (Table 2A). Anesthetized mice were positioned in a Kopf Small Animal Stereotaxic Instrument so that their heads were stable. The skin and muscle covering the cisterna magna were cut and pulled to the side to reveal

Table 1
Patient demographics.

	PP	SP	RR	CTRL
# of patients (CSF)	6	8	6	8
Age at sample collection, mean (SD), years	50.3 (12.0)	48.1 (9.3)	48.0 (12.4)	45.5 (6.46)
EDSS at sample collection, mean (SD)	6.6 (0.98)	7.5 (0.86)	0.88 (0.74)	NA
Disease duration at sample collection, mean (SD), years	13.83 (9.8)	21.7 (7.8)	2.50 (3.7)	NA
CSF total protein, mean (SD), μg/ml	653.2 (237.3)	657.6 (242.6)	786.2 (162.1)	499.7 (236.3)
CSF total albumin, mean (SD), μg/ml	399.4 (203.9)	294.7 (95.85)	380.0 (120.0)	302.9 (141.2)
CSF cell count, mean (SD), /ml	2541 (2979)	3652 (7844)	4669 (4919)	2795 (3987)

CSF = cerebrospinal fluid. EDSS = expanded disability status scale. PP = primary progressive. SP = secondary progressive. RR = relapsing, remitting. CTRL = controls.

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