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Proliferating brain cells are a target of neurotoxic CSF in systemic autoimmune disease

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Abstract

Brain atrophy, neurologic and psychiatric (NP) manifestations are common complications in the systemic autoimmune disease, lupus erythematosus (SLE). Here we show that the cerebrospinal fluid (CSF) from autoimmune MRL-lpr mice and a deceased NP-SLE patient reduce the viability of brain cells which proliferate in vitro. This detrimental effect was accompanied by periventricular neurodegeneration in the brains of autoimmune mice and profound in vivo neurotoxicity when their CSF was administered to the CNS of a rat. Multiple ionic responses with microfluorometry and protein peaks on electropherograms suggest more than one mechanism of cellular demise. Similar to the CSF from diseased MRL-lpr mice, the CSF from a deceased SLE patient with a history of psychosis, memory impairment, and seizures, reduced viability of the C17.2 neural stem cell line. Proposed mechanisms of cytotoxicity involve binding of intrathecally synthesized IgG autoantibodies to target(s) common to different mammalian species and neuronal populations. More importantly, these results indicate that the viability of proliferative neural cells can be compromised in systemic autoimmune disease. Antibody-mediated lesions of germinal layers may impair the regenerative capacity of the brain in NP-SLE and possibly, brain development and function in some forms of CNS disorders in which autoimmune phenomena have been documented.

1. Introduction

The overall prevalence of the autoimmune disease systemic lupus erythematosus (SLE) is estimated to range from 1 : 245 to 1 : 1000 individuals, and according to recent epidemiological studies, the number of affected people in North America alone may exceed 1 million (Lahita, 1995). Neurologic and psychiatric (NP) manifestations of unknown etiology are common in SLE and have been proposed to represent a more severe form of the disease, often denoting a graver prognosis (Bombardier et al., 1992; Rubin et al., 1985). Contemporary imaging techniques indicate that profound metabolic alterations and neuronal loss accompany neuropsychiatric lupus, or NP-SLE (Colamussi et al., 1995; Sibbitt et al., 1994). Periventricular lesions, cerebral atrophy,

Abbreviations: ACA, anti-cardiolipin antibodies; ANA, anti-nuclear antibodies; CSF, cerebrospinal fluid; FJB, Fluoro Jade B stain; NP-SLE, neuropsychiatric systemic lupus erythematosus; PBS, phosphate buffered saline.

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and ventricular enlargement of unknown etiology occur in up to 50% of patients (Baum et al., 1993; Bosma et al., 2000).

Similar to SLE in humans, an inbred strain of MRL/MpJ-Fas^{lpr} (MRL-lpr) mice develops an accelerated SLE-like condition (Theofilopoulos, 1992) accompanied by various neurological dysfunctions (Hess et al., 1993; Vogelweid et al., 1994), an anxious/depressive-like behavioral state (Sakic et al., 1994), ventricular enlargement (Denenberg et al., 1992), neuronal atrophy, and retarded brain growth (Sakic et al., 2000a; Sakic et al., 1998). Pathological abnormalities are also seen in the choroid plexus, a structure that synthesizes most of the cerebrospinal fluid (CSF) volume (Duprez et al., 2001; Schwartz and Roberts, 1983). Widespread damage of the blood–brain barrier in MRL-lpr mice is accompanied by lymphocyte and monocyte infiltration into the choroid plexus (Alexander et al., 1983; Farrell et al., 1997; Hess et al., 1993), in some cases as early as 8 weeks of age (Vogelweid et al., 1991). Areas around the third and lateral ventricles show enhanced neurodegeneration, as revealed by staining with Fluoro Jade B (Ballok et al., 2003), excessive DNA fragmentation (Sakic et al., 2000b) and expression of cell adhesion molecules (Zameer and Hoffman, 2003). More interestingly, the CSF from diseased MRL-lpr mice reduces the viability of pyramidal neurons in a primary neuron–astrocyte co-culture (Maric et al., 2001). Taken together, periventricular distribution of brain lesions led to the hypothesis that the viability of cells in the subependymal layer of the brain is compromised due to sustained interactions with soluble cytotoxic factors in the CSF. The pathogenic cascade may include drainage of circulating immune factors (e.g. activated cytotoxic lymphocytes and/or autoantibodies) into the CSF and their diffusion into neighboring interstitial tissue, with subsequent detrimental effects on cell function. Given that neural stem cells and progenitors in the periventricular zone constitute the largest pool of proliferative brain cells (Morshead and van der Kooy, 2001), one may expect profound consequences on brain development, repair capacity, and behavior if this cell population is affected by the autoimmune process, either during embryogenesis or during ontogeny.

As a first step in testing the above mechanism, we examine the viability and morphology of murine-derived, multipotent neural stem cells (C17.2) and neural stem cell/progenitor preparations (neurospheres) after incubation with CSF from autoimmune animals. Given that the mammalian retina is a nucleus of the CNS and is composed of clearly defined neuronal layers, we also examined whether the CSF from MRL-lpr mice has neurotoxic effects in vivo by using the posterior rat eye chamber as a target tissue. This technically convenient approach also allowed us to examine whether CSF is indeed cytotoxic across species, as observed with the CSF from an NP-SLE patient administered to the mouse hippocampus (DeGiorgio et al., 2001), and whether previously reported in vitro cytotoxicity of CSF from MRL-lpr mice (Maric et al., 2001) can be observed in vivo. In addition, the death of one of our NP-SLE patients provided us with a unique opportunity to compare the cytotoxic effects of CSF obtained from animal and human forms of SLE using an in vitro system.

2. Materials and methods

2.1. Animals

MRL/MpJ-Fas^{lpr} (MRL-lpr) mice develop an accelerated form of an SLE-like disease, while age-matched congenic MRL/MpJ (MRL+/+) mice show only mild manifestations of systemic autoimmunity and inflammation (Theofilopoulos, 1992). The MRL-lpr males and congenic, age-matched MRL+/+ controls were obtained from The Jackson Laboratory (Bar Harbour, ME) and a local breeding colony. Healthy (non-autoimmune) CD1 males were purchased from Charles River Canada. Mice were housed in groups of four per cage and maintained under standard laboratory conditions. Sampling was performed on old (~4 months of age) and young animals (~5 weeks of age). The experimental protocols were approved by a local animal care committee and carried out in accordance with the rules and regulations of the Canadian Council of Animal Care.

2.2. Sample collection

Mice were anesthetized with an i.p. injection of Somnotol (65 mg/kg, 0.15–0.2 ml). The thoracic cavity was exposed to cut the inferior vena cava and quickly aspirate ~1 ml of blood with blood collecting tubes (Fisher Scientific, Nepean, ON). The animals were perfused intracardially with 40 ml of phosphate buffered saline (PBS) and processed for CSF collection, as described previously (Maric et al., 2001). Upon dissection of neck muscles a custom-made glass micropipette (Drummond Scientific, Broomall, PA; rinsed repeatedly with 70% EtOH) was inserted into the cisterna magna and 15–20 μl of aspirated CSF was transferred into autoclaved 50-μl plastic vials. They were centrifuged at 3000 r.p.m. over 3–5 s and examined under a brightly lit white background. The presence of red blood cell precipitate was used as a marker of contamination and a criterion for disposal (~15%–20% of the samples).

Several classes of leukocytes infiltrate the choroid plexus and meninges of MRL-lpr mice, including T- and B-cells (Zameer and Hoffman, 2004). Given that these cells are occasionally seen in the ventricle lumen (Sakic et al., 2000b), they were estimated in CSF samples from five MRL-lpr mice by immunostaining for T-cells (CD3-PE, Cat No. 550353 and CD8a-PE, Cat No. 553033, BD Biosciences Pharmingen, San Diego, CA) and B-cells (CD45R/B220-FITC, Cat No. 553087), cytospinning (7000 r.p.m. for ~15 s), and counting on a hemocytometer. More B-cells than T-cells were seen, but they were rarely observed under