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Fibronectin changes in eosinophilic meningitis with blood–CSF barrier disruption

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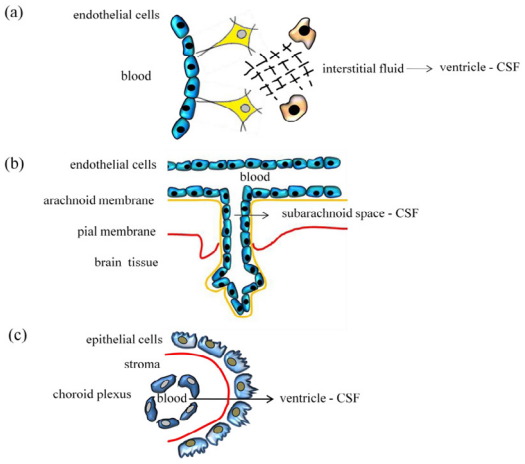
RESEARCH HIGHLIGHTS

- Fibronectin was correlated with the increased permeability of the brain barrier.
- Matrix metalloproteinase-9 was correlated with fibronectin processing.
- Increased fibronectin processing may be associated with barrier disruption.

GRAPHICAL ABSTRACT



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ABSTRACT

Fibronectin, which is present at relatively low levels in healthy central nervous systems (CNS), shows increased levels in meningitis. In this study, fibronectin processing was correlated with the increased permeability of the blood–cerebrospinal fluid (CSF) barrier as well as with the formation of eosinophil infiltrates in angiostrongyliasis meningitis. The immunohistochemistry results show matrix metalloproteinase-9 (MMP-9) is localized in the choroid plexus epithelium. Coimmunoprecipitation demonstrated fibronectin strongly binds MMP-9. Furthermore, treatment with the MMP-9 inhibitor GM6001 significantly inhibited fibronectin processing, reduced the blood–CSF barrier permeability, and decreased the eosinophil counts. The decreased fibronectin processing in CSF implies decreased cellular invasion of the subarachnoid space across the blood–CSF barrier. Therefore, increased fibronectin processing may be associated with barrier disruption and participate in the extravasation and migration of eosinophils into the CNS during experimental parasitic infection.

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1. Introduction

Mature adults of the zoonotic parasitic nematode *Angiostrongylus cantonensis* reside in the pulmonary arteries of permissive hosts (rats) (Alicata and Jindrak, 1970). However, in nonpermissive hosts (humans and mice), the immature adults remain in the central nervous system (CNS) of the host and become the main cause of eosinophilic meningitis and eosinophilic meningoencephalitis (Ismail and Arsura, 1993). In mice infected with *A. cantonensis*, cerebrospinal fluid (CSF) eosinophilia reaches its peak at approximately three weeks and has the same pathogenesis as eosinophilic meningitis (Sugaya and Yoshimura, 1988). CSF protein levels are elevated in patients with angiostrongyliasis (Wan and Weng, 2004). Meanwhile, matrix metalloproteinase (MMP)-9 activity is associated with disruption of the blood–CNS barrier in mice infected with angiostrongyliasis meningitis; this enzyme also promotes cellular infiltration of the subarachnoid space (Chen et al., 2006). Increased blood–CSF barrier permeability is associated with disruption of tight junction proteins as a result of MMP-9 activation in angiostrongyliasis meningoencephalitis (Chiu and Lai, 2013).

Fibronectin is a multifunctional, high molecular-weight glycoprotein found in plasma, the extracellular matrix (ECM), and CSF. Fibronectin is involved in cellular adhesion, cellular migration, and phagocytosis (Ouaissi and Capron, 1985). Fibronectin is also present in the ECM of the meninges, the choroid plexus, and all blood vessels (Silva et al., 1999). Cerebral endothelial cells are surrounded by a basal lamina containing fibronectin (Yang and Rosenberg, 2011). Changes in the levels of this protein in the CNS are associated with various pathological conditions (Knott et al., 1998; Nasu-Tada et al., 2006; Pasinetti et al., 1993). Under normal conditions, fibronectins are present in the CSF at low concentrations. However, when the permeabilities of the blood–CSF barrier and blood–brain barrier (BBB) increase, or when CSF circulation decreases, the fibronectin concentrations in CSF can increase (Goos et al., 2007; Weller, 1992; Weller et al., 1990). The assessment of blood–CSF barrier breakdown by CSF is based on the direct or indirect determination of protein permeability across the barrier. The appearance of plasma proteins in CSF implicates numerous CNS disorders associated with presumed or overt disruption of the blood–CSF barrier (Marchi et al., 2003).

The ECM, which is essential in CNS homeostasis, includes fibronectin as part of its structure. Thus, fibronectin changes in the CSF may be an indicator of neurological disease. Whether fibronectin degradation by MMP-9 occurs and contributes to barrier disruption in parasitic meningitis remains unclear. This study aims to investigate whether fibronectin levels increase during angiostrongyliasis and to determine the correlation between blood–CSF barrier disruption and fibronectin processing.

2. Materials and methods

2.1. Experimental animals

Male BALB/c mice (specific pathogen-free grade and 5 weeks old) were purchased from the National Laboratory Animal Center, Taipei, Taiwan. The mice were maintained in a 12 h alternating light-and-dark-cycle photoperiod and were provided with Purina Laboratory Chow and water ad libitum. The mice were kept in our laboratory for more than one week prior to experimental infection. All procedures that involved animal use and care were approved by the Institutional Animal Care and Use Committee of Chung-Shan Medical University, Taiwan, and were performed in accordance with the institutional guidelines for animal experiments.

2.2. Larval preparation

The infective larvae (L_3) of *A. cantonensis* were originally obtained from wild giant African snails (*A. fulica*) and were propagated for several months in the Wufeng experimental farm (Taichung, Taiwan) by cycling through rats and *A. fulica*. Tissue larvae were recovered using a modified Parsons and Grieve method (1990). In brief, snail shells were crushed, and the tissues were homogenized, digested in a pepsin–HCl solution (pH 1 to 2, 500 IU pepsin/g tissue), and incubated with agitation in a 37 °C water bath for 2 h. Larvae in the sediment were collected by serial washes in double-distilled water and then counted under a microscope. The third larval stage of *A. cantonensis* was confirmed based on the description provided by Hou et al. (2004).

2.3. Animal infection

A total of 90 mice were randomly divided into six groups: D_0, D_5, D_{10}, D_{15}, D_{20}, and D_{25}. Mice were prohibited food and water for 12 h prior to infection. The mice in the experimental groups (D_5, D_{10}, D_{15}, D_{20}, and D_{25}) were infected with 50 *A. cantonensis* larvae via oral inoculation and then sacrificed on days 5, 10, 15, 20, and 25 post-inoculation (PI), respectively. The negative-control mice received only distilled water and were sacrificed on day 25 PI.

2.4. CSF collection

The mice were anesthetized by intraperitoneal urethane (1.25 g/kg) injection. The animals were then placed in a stationary instrument at 135° from the head and body. The neck skin was shaved and swabbed thrice with 70% ethanol. Subcutaneous tissue and muscles were then separated. A capillary tube was inserted through the dura mater into the cisterna magna and, CSF with the capillary tube inpouring. The CSF was injected into a 0.5 mL Eppendorf tube and centrifuged at $3000 \times g$ at 4 °C for 5 min. The supernatant was then collected in a 0.5 mL Eppendorf tube and kept at –80 °C in a freezer.

2.5. Antibodies

Mouse monoclonal anti-fibronectin antibodies and goat anti-mouse polyclonal β-actin antibodies were purchased from Sigma (St. Louis, MO, USA). Goat anti-mouse MMP-9 polyclonal antibodies were obtained from R&D Systems (Minneapolis, MN, USA). Horseradish peroxidase (HRP)-conjugated rabbit anti-goat IgG and HRP-conjugated rabbit anti-mouse IgG were purchased from Jackson ImmunoResearch Laboratories (West Grove, PA, USA).

2.6. Western blotting

The protein content of the CSF samples was determined using commercial protein assays (Bio-Rad, Hercules, CA, USA) with bovine serum albumin (BSA) as the standard. A subsample of each serum was mixed with an equal volume of loading buffer that contained the following: 62.5 mM Tris-HCl (pH 6.8), 10% glycerol (v/v), 2% SDS (w/v), 5% 2-mercaptoethanol (v/v), and 0.05% bromophenol blue (v/v). The mixture was boiled for 5 min prior to electrophoresis on a 10% (w/v) polyacrylamide gel. The separated proteins were then electrotransferred overnight to a nitrocellulose membrane at a constant current of 30 mA. The membrane was washed three times for 30 min each in a 0.15 M phosphate-buffered saline (PBS) containing 0.1% (v/v) Tween 20 (PBS-T) at room temperature. Afterward, the membrane was soaked in a 1:1000 dilution of primary antibodies at 37 °C for 1 h. After another three washes in PBS-T, the membranes were incubated at 37 °C for 1 h with a 1:5000 dilution of the relevant HRP-conjugated secondary antibody. The reactive