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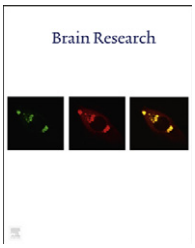

Research Report

Potential role of fibronectin in microglia/macrophage activation following cryoinjury in the rat brain: An immunohistochemical study ☆

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ABSTRACT

To investigate whether fibronectin, a high-molecular weight glycoprotein of the extracellular matrix (ECM), plays a role in the activation of microglia/macrophages after brain injury, we examined the changes in fibronectin and arginase-1, a marker for alternatively activated macrophages, in a rat cryoinjury model using Western blot analysis, real-time reverse transcription PCR and immunohistochemistry. The protein and mRNA level of fibronectin and arginase-1 significantly increased in the injury site of the ipsilateral cerebral cortex at days 4 and 7 after cryoinjury but was decreased at day 14. The immunohistochemical analysis revealed fibronectin expression in ED1-positive microglia/macrophages and reactive astrocytes, in the lesion core and periphery, respectively. Fibronectin immunoreactivity in the lesion was similar to arginase-1 except that fibronectin was detected in the ECM after cryoinjury. The present results suggest that fibronectin was extravasated into injured brain lesions via an impaired blood–brain barrier and stimulated glial cells including microglia and infiltrating macrophages in the lesion core and periphery to become alternatively activated microglia/macrophages, which modulated CNS inflammation after brain injury.

☆ *Sections:* Development, Degeneration and Regeneration, and Aging.

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1. Introduction

Brain injury is associated with various disabilities depending on the location and severity of the lesion (Sato et al., 2001). Animal models of ischemic (Shin et al., 2011) and traumatic (McIntosh et al., 1998; Soares et al., 1995) brain injury have been used to study the mechanisms underlying neurodegeneration and subsequent remodeling. Although these models differ, their initial pathogenic mechanism is similar: hemorrhage, edema, neuronal cell death, macrophage phagocytosis, and reactive gliosis (Moon et al., 2005; Shin et al., 2005). The neuropathology of brain injury hemorrhage is associated with microglial activation *in vivo* (Del Zoppo et al., 2012; Shin et al., 2005) and leakage of plasma fibronectin into the surrounding tissue (Del Zoppo et al., 2012). Plasma fibronectin has been shown to increase after brain injury (Tate et al., 2007a) and to play a role in the regulation of neuroprotection (Tate et al., 2007b). Furthermore, fibronectin's extravasation from blood plasma may stimulate microglia after central nervous system (CNS) injury (Milner et al., 2007; Summers et al., 2009). In this case, microglia stimulated by fibronectin after brain injury are likely further activated into arginase-1-positive alternatively activated macrophages, which modulate CNS inflammation, as shown in a previous study (Aihara et al., 1995).

We previously reported a significant increase in ED1-positive activated microglia/macrophages that remove cell debris after cryoinjury to the cerebral cortex (Moon et al., 2004a) and contribute to remodeling of the injured area through activation of gliosis (Babikian et al., 2010; McIntosh et al., 1998; Moon et al., 2004a). Furthermore, in the spinal cord injury model, macrophages and/or activated microglia have been shown to express proliferating cell nuclear antigen and osteopontin (Moon et al., 2004b) and embryonic intermediate filaments (Kim et al., 2003). However, those cells are positive for arginase-1, a competitive enzyme for inducible nitric oxide synthase (NOS) in spinal cord injury (Ahn et al., 2012).

When considering fibronectin in the head injury model, previous research has not clearly demonstrated whether fibronectin levels are changed in the *in vivo* injured cerebral cortex rat model or whether fibronectin plays a role in the activation of microglia/macrophages *in vivo*. Fibronectin, an extracellular matrix (ECM) glycoprotein, is known to be involved in the activation of microglia (Milner and Campbell, 2003; Milner et al., 2007; Summers et al., 2009) and neuroprotection (Tate et al., 2007b). Furthermore, fibronectin is transiently expressed in astrocytes (Stenzel et al., 2011), and contributes to the formation of glial scars after CNS injury with astrocyte-derived tissue transglutaminase (van Strien et al., 2011).

To better understand the role of fibronectin and arginase-1 in brain injury and the response of glial cells at the periphery of the lesion, we examined the expression pattern and tissue localization of fibronectin and arginase-1 in the rat cerebral cortex after cryoinjury.

2. Results

2.1. *Lesion-induced histological changes*

No lesions were observed in the sham-operated control rats at day 4 or day 7 post-injury (Fig. 1A). In cryoinjured rats, a significant loss of neurons, accompanied by hemorrhage as well as a large number of inflammatory cells infiltrating the subpial gray matter and parenchyma were observed at day 4 post-injury (Fig. 1B). At day 7 post-injury, hemorrhage was reduced, and inflammatory cells were replaced by a glial scar tissue in the lesion core (Fig. 1C, arrows). At day 14 post-injury, the glial scar formation and inflammatory cells were reduced in the ipsilateral cerebral cortex (data not shown).

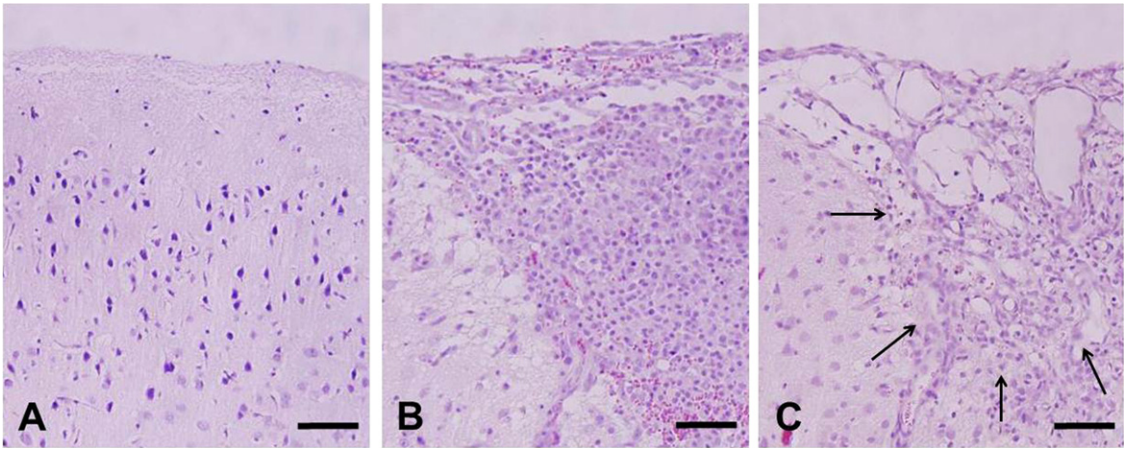

Fig. 1 – Histopathological findings in the cerebral cortex of sham-operated controls (A) and rats at post-injury day 4 (B) and post-injury day 7 (C). At day 4 post-injury, a significant loss of neurons accompanied by hemorrhage and a large number of inflammatory cells infiltrating the subpial white matter and parenchyma was observed (B). At day 7 post-injury, the formation of glial scar tissue was visible ((C), arrows), and inflammatory cells were reduced (C). Hematoxylin and eosin staining. Scale bar = 50 μm.