Neuroscience Letters 628 (2016) 73–77

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research article

Cellular reactions of the choroid plexus induced by peripheral nerve injury

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HIGHLIGHTS

- Numbers of ED1+ and ED2+ epiplexus macrophages increased with duration of CCI.
- Sham-operated CP also displayed an increase in the number of epiplexus macrophages.
- No significant CP cell proliferation was found after either sham or CCI operation.
- Inflammatory reaction of CP was observed after both nerve injury as well as surgery.

ARTICLE INFO

Article history:
Received 11 April 2016
Received in revised form 7 June 2016
Accepted 8 June 2016
Available online 9 June 2016

Keywords:
Blood–cerebrospinal fluid barrier
Kolmer cells
Macrophages
Chronic constriction injury

ABSTRACT

The choroid plexus (CP) of brain ventricles forms the blood–cerebrospinal fluid (blood–CSF) barrier that is involved in many diseases affecting the central nervous system (CNS). We used ED1 and ED2 immunostaining to investigate epiplexus cell changes in rat CP after chronic constriction injury (CCI). In contrast to naïve CP, the CP of sham-operated rats showed an increase in the number of ED1+ cells of a similar magnitude during all periods of survival up to 3 weeks, while the number of ED2+ increased only at 3 days from operation. In comparison to naïve and sham-operated animals, the number of ED1+ and ED2+ cells in the epiplexus position increased with the duration of nerve compression. We detected no or negligible cell proliferation in the CP after sham- or CCI-operation. This suggests that increased number of ED1+ and ED2+ cells in the epiplexus position of the CP is derived from peripheral monocytes passing through altered blood–CSF barrier. The changes in epiplexus cells indicate that the CP reacts to tissue injury after the surgical approach itself and that the response to peripheral nerve lesion is greater. This suggests a role for an altered blood-CSF barrier allowing for propagation of signal molecules from damaged tissue and nerve to the CNS.

1. Introduction

The choroid plexus (CP) in the brain ventricles consists of vascularized stroma and epithelial cells that constitute the blood–cerebrospinal fluid (blood–CSF) barrier. The vascularized stroma contains loose connective tissue, fenestrated capillaries, and macrophages. The ventricular side of the stroma is covered by epithelial cells that are responsible for secretion of cerebrospinal fluid (CSF). Choroidal epithelial cells are connected by tight junctions that are the main component of the blood-CSF barrier. The epiplexus or Kolmer cells adhere on the ventricular side of the CP epithelial cells and are regarded as part of the CP. These epiplexus cells display diverse morphologies ranging from round to polar and stellate. They also have the typical cytological features of activated macrophages including vacuoles and lysosomes [1–3]. There is a growing body of evidence for a key CP role in many disorders including inflammatory, neurodegenerative, infectious, traumatic, neoplastic, as well as systemic diseases [1,2]. It has been found that peripheral inflammation caused by lipopolysaccharide induces molecular and cellular changes in the CP [4]. Wallerian degeneration of damaged nerve is considered to be aseptic inflammation [5] and produces damage associated molecular patterns (DAMPs) [6]. Moreover, Apkarian et al. [7] found an increased level of IL-1 beta in supraspinal structures after peripheral nerve injury. We hypothesize that Wallerian degeneration molecules, such as DAMPs, can affect the CP via the bloodstream. The aim of our study was to investigate whether a peripheral nerve injury can alter the epiplexus cell composition of the CP. Changes in the number of epiplexus cells may indicate a disruption of blood–CSF barrier and its role in the propagation of molecules from damaged peripheral nerve to CNS.

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http://dx.doi.org/10.1016/j.neulet.2016.06.019
0304-3940/

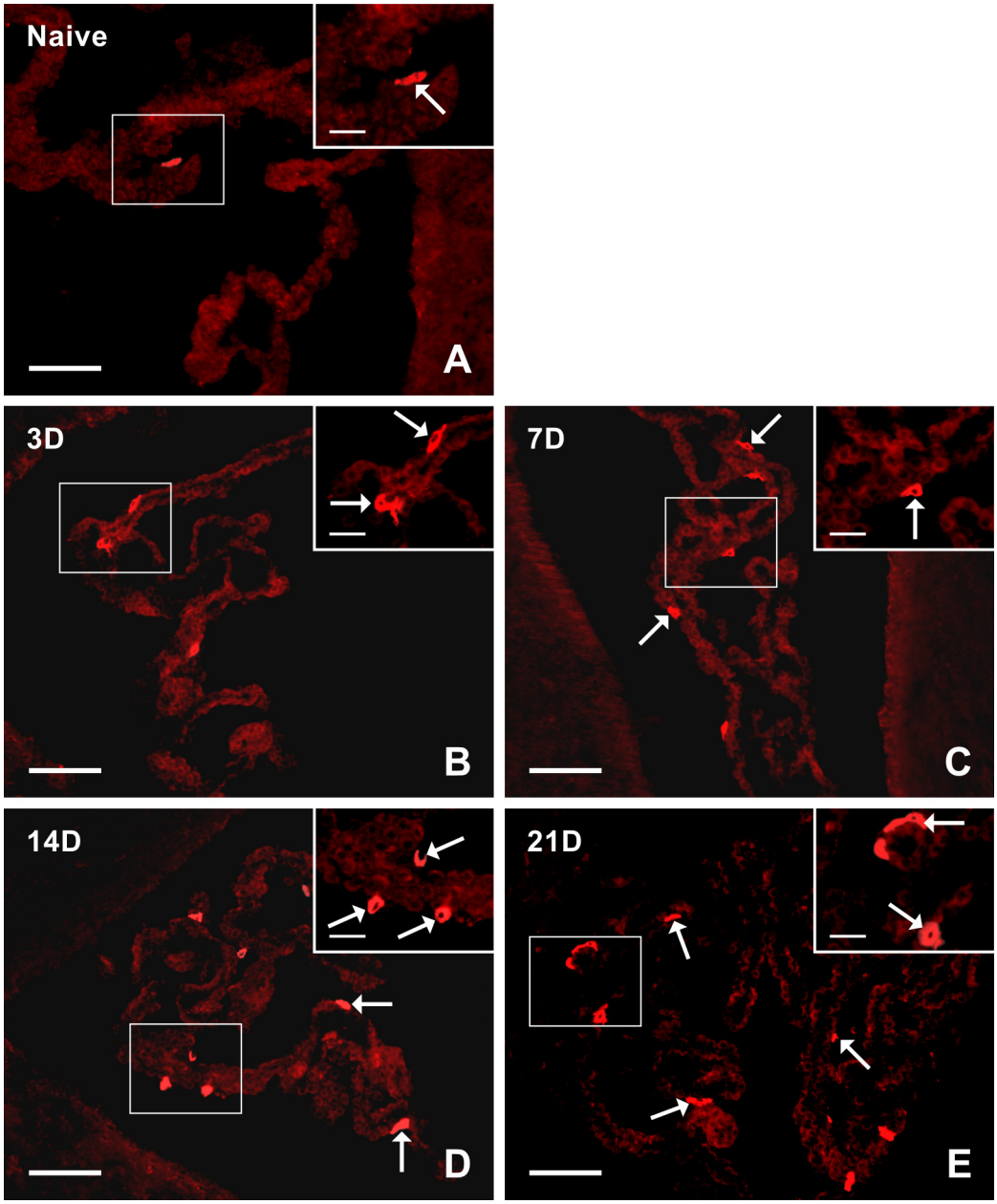

Fig. 1. Representative images showing immunostaining with ED1 antibody in the CP from naïve rats and operated rats at 3, 7, 14 and 21 days (3D, 7D, 14D and 21D) after CCI. Arrows indicate ED1+ epiplexus cells. The insets on the upper right show, at higher magnification, the regions indicated by the box in the main part of the image. Scale bars = 80 μm (main image); 20 μm (insets).

2. Material and methods

2.1. Animals and surgical procedures

The experiments were performed on 52 adult male rats (Wistar, 200–250 g; Animal Breeding Facility, Masaryk University, Czech Republic). All experimental procedures were carried out aseptically and according to protocols approved by the Ethical Committee of Masaryk University, Brno and the Departmental Committee of the Ministry of Education, Youth and Sports, Czech Republic.

Rats were anesthetized with a mixture of 5% ketamine (100 mg/kg) and 2% xylazine (10 mg/kg) administered intraperitoneally. The chronic constriction injury (CCI) of the left sciatic nerve was created using three ligatures (3-0; Ethicon, Somerville, NJ) that reduced the nerve diameter by approximately one-third. The retracted muscles and skin incision were closed with 3-0 silk sutures and the animals were exposed to CCI for 3 (n = 8), 7 (n = 8), 14 (n = 8), and 21 (n = 8) days. The left sciatic nerve was merely exposed but left without any lesion in sham-operated rats surviving for 3 (n = 4), 7 (n = 4), 14 (n = 4), and 21 (n = 4) days.

The CCI- and sham-operated rats as well as naïve rats (n = 4) were sacrificed using CO_2, then perfused transcardially with 500 ml heparinized (1000 units/500 ml) phosphate-buffered saline (pH 7.4) followed by 500 ml of Zamboni's fixative [8]. Brains were

Table 1
Primary antibodies used, their dilutions, time of incubation, and suppliers.

Name	Type of antibody	Dilution	Incubation time	Supplier
ED1	Mouse monoclonal	1:200	240 min	Serotec
ED2	Mouse monoclonal	1:200	16 h	Serotec
Ki-67	Rabbit polyclonal	1:500	240 min	Vector Laboratories