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Inflammatory status of transmigrating primary rat monocytes in a novel perfusion model simulating blood flow

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ABSTRACT

It remains unclear whether monocyte infiltration plays a protective or detrimental role in neurodegenerative disease. The present study characterizes the inflammatory status of primary monocytes in a novel in vitro perfusion model. Monocytes under perfusion do not undergo elevated cell death. However, perfusion does lead to altered morphology, which can be counteracted by anti-inflammatory drugs. Functional studies indicate that cytokine levels are significantly reduced in perfusion compared to stationary conditions and enhanced with brain slices or capillary endothelial cells. Understanding monocyte properties could lead to refined treatment and new ways to interfere with inflammation in diseased brains.

1. Introduction

Monocytes are bone marrow-derived hematopoietic cells that circulate throughout the bloodstream and eventually give rise to tissue macrophages and dendritic cells (Ziegler-Heitbrock, 2007). Peripheral monocytes consist of a wide variety of phenotypically and functionally distinct subpopulations varying in maturation, differentiation, and activation states. These heterogeneous subpopulations are characterized by their differential expression of cell surface markers (Auffray et al., 2009; Buckner et al., 2011). Inflammation and the recruitment of monocytes and monocyte-derived cells into the central nervous system (CNS) have been implicated in a number of neurological disorders including: Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and multiple sclerosis (MS) (Akiyama et al., 2000; Glass et al., 2010; Khandelwal et al., 2011). Upon pathological stimulation (including neurodegeneration or inflammation) or during normal immune patrol, monocytes adhere and transmigrate across the blood–brain barrier (BBB) into the brain where they execute effector functions and differentiate into cells with a microglia-like phenotype. This migration is mainly regulated by cell adhesion molecules (CAMs) and chemokines (Auffray et al., 2007; D'Mello et al., 2009; Prinz and Priller, 2010).

Extensive evidence indicates that inflammation can contribute to further exacerbation of neurodegenerative diseases. In the healthy brain and CNS tissue, inflammatory mediators (cytokines and their receptors) are expressed constitutively at low or undetectable levels. However, upon insult or infection, proinflammatory cytokines and mediators lead to the expression of chemokines and adhesion molecules, recruitment of monocytes and other immune cells to the lesion site, and activation of resident microglia and/or astrocytes. Thus far, the role of monocytes and monocyte-derived cells in disease propagation has been under intense debate. Monocyte-derived macrophages may induce beneficial effects by their secretion of neuroprotective factors, phagocytosis of debris/apoptotic cells and initiation of repair processes (Schwartz and Shechter, 2010). Previous investigations indicate that microglia cause further disease aggravation through uncontrolled inflammation via release proinflammatory mediators and other neurotoxic factors (Lucas et al., 2006). Therefore, further studies are needed in order to better understand the phenotype and migratory dynamics of monocytes during inflammation of the CNS.

However, studies involving primary monocytes have proven difficult. In vitro static culturing methods using glass or plastic vessels quickly lead to monocyte adherence, activation, and differentiation into macrophages (Steiniger et al., 2001). Few studies have shown the successful maintenance of monocyte cultures in the absence of growth factors (M-CSF, GM-CSF) or differentiation (Wirth et al., 1982; Maoz et al., 1986). Thus, we sought to establish an in vitro culturing system that provides more physiological conditions.

The aim of the present study is to characterize the inflammatory status of primary rat monocytes in a novel in vitro perfusion model. This model consists of two peristaltic pumps providing constant flow of medium and cell circulation into a glass chamber applying shear stress (Fig. 1).

Here, we compare the functional properties of monocytes after isolation, exposure to constant flow, and incubation under static conditions. In addition, we evaluate the interaction between monocytes

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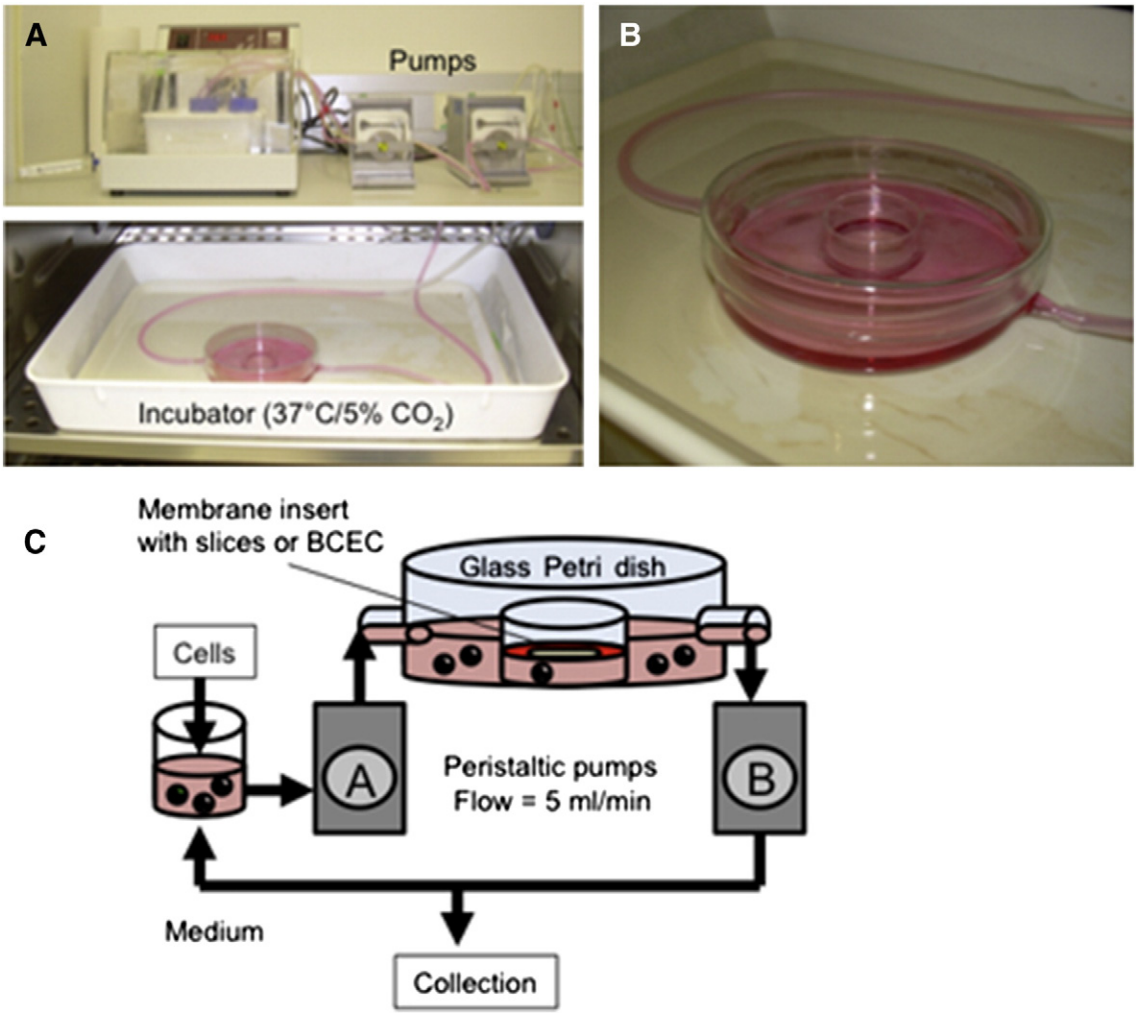

Fig. 1. Schematic diagram of a novel in vitro perfusion system. The system consists of a peristaltic pump (A) which pumps pre-warmed (37 °C) medium with a flow rate of 5 ml/min into a glass Petri dish. A second peristaltic pump (B) pumps the medium back into the medium container allowing recirculation. The glass chamber (glass Petri dish) is located in an incubator at 37 °C and 5% CO_2. The system is connected via plastic tubes (arrows). An inlet in the medium container allows the addition of different substances and cells under sterile conditions. In the glass chamber small membrane inserts containing brain slices or brain capillary endothelial cells can be incubated under constant flow of culture medium. The system also contains an outlet for cell and media collection following the experiment.

and brain capillary endothelial cells (BCEC)/cortex brain slices when under the influence of constant flow including their release of inflammatory markers. Finally, we examine the effects of anti-inflammatory drugs (i.e. minocycline, indomethacin) on counteracting release of proinflammatory cytokines in cortical organotypic brain slices.

2. Methods

2.1. Isolation of primary rat monocytes

Primary rat monocytes were freshly isolated as previously described by us with some modifications (Humpel, 2008; Böttger et al., 2010; Hohsfield and Humpel, 2010). In brief, Sprague–Dawley rats (250 g, Himberg, Austria) were anesthetized by an intraperitoneal injection of 40 mg/kg body weight thiopental (Sandoz, Kundl, Austria) and perfused with 500 ml of 4 °C pre-chilled 10 mM phosphate-buffer saline (PBS)/2.7 mM EDTA/25 mg/ml heparin, pH 7.3 through the left ventricle. The collected effluent was centrifuged at 550 ×*g* for 10 min at 4 °C. The perfusate pellet was resuspended in 50 ml of 10 mM PBS/1% bovine serum albumin (BSA; SERVA Electroporesis, Heidelberg, Germany)/2.7 mM EDTA, pH 7.3 and carefully overlaid on a Percoll working solution (Scriba et al., 1996). After centrifugation at 500 ×*g* for 30 min at 4 °C, peripheral blood mononuclear cells (PBMC) were harvested from the interphase. PBMC were then washed once with 50 ml of PBS and ~20 × 10^6 PBMC were resuspended in 100 µl of PBS/BSA/EDTA. Monocytes were purified from PBMC by negative magnetic selection: PBMC were incubated in a cocktail consisting of four different purified anti-rat monoclonal antibodies (20 µg of each: CD8a (clone OX-8), CD5 (clone OX-19), CD45RA (clone OX-33), PAN T (clone OX-52); all from Cedarlane Laboratories, Szabo, Austria) for 10 min at 4 °C shaking. PBMC were washed once with PBS and resuspended in 100 µl of PBS/BSA/EDTA and 40 µl of MACS Goat Anti-Mouse-IgG Microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany). PBMC were incubated for 15 min at 4 °C on a shaker and following incubation, were washed once with PBS. The cells were resuspended in 1000 µl of PBS/BSA/EDTA and then applied to a MS-MACS column fixed to a strong magnet. The purified monocytes were centrifuged and pooled for further experiments. Approximately 10 × 10^6 cells were isolated from one adult rat. The described isolation procedure yields approximately 90–95% CD68-positive monocytes (Moser and Humpel, 2007; Böttger et al., 2010). During this preparation monocytes were counted using the Cell Coulter Counter (COULTER®Z™ Series, Fischerlehner & Kucera, Innsbruck, Austria) in a range from 5.5 to 10 µm. All animal experiments were approved by the Austrian Ministry of Science and conformed to the