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Alerted microglia and the sympathetic nervous system: A novel form of microglia in the development of hypertension

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ABSTRACT

Microglia, commonly known as the tissue resident macrophages of the central nervous system (CNS), are ubiquitously expressed in the CNS. Microglia, in their resting, or surveilling, stage, play a critical role in the maintenance of normal neuronal physiology and homeostasis. On activation, microglia can acquire either a neurotoxic (M1) or a neuroprotective (M2) phenotype. Prior to development of the M1 or M2 phenotype, little was known about changes in microglial activity, when subjected to stimuli. It is postulated, that an inability of microglia to maintain neuronal physiology within a normal working range can contribute to the development of cardiovascular disorders (CVDs) such as hypertension, but clear evidence supporting this hypothesis is missing. Even though our understanding of microglial function in a state of CNS injury/inflammation is extensive, the literature concerning role of microglia in the healthy CNS, is limited. Involvement of microglia in the pathophysiology of CVDs, in a neuroprotective/neurotoxic manner, is a key area that requires further investigation.

1. Microglia

Until recently, microglia were considered to play a role solely as immune cells of the CNS, where they were responsible for a wide range of functions during CNS damage (Kettenmann et al., 2011). Recent developments in the field, since 2005 (Nimmerjahn et al., 2005), suggest that microglia are more than just immune cells of the CNS. In fact, it seems that microglia are guardians of the CNS at all times; constantly working to maintain the dynamic neuronal physiology, within its homeostatic levels (Hughes, 2012).

1.1. Origin of microglia

Microglia are cells of mesodermal origin that populate the CNS tissue during early stages of development. Erythroid/myeloid progenitor cells in the yolk sac, during early development, differentiate into tissue resident macrophage progenitor cells. These tissue resident macrophage progenitor cells, with amoeboid morphology, migrate into the brain and invade the brain tissue as microglial cells (Cronk and Kipnis, 2013). These microglial cells are self-renewing (Ajami et al., 2007), in case of depletion, during inflammatory conditions. However, under special defined circumstances, when microglial cells in the CNS are depleted and are unable to replenish their pool, monocytes from bone marrow are capable of replenishing tissue resident macrophages of the CNS (Cronk and Kipnis, 2013).

1.2. History of microglia

Microglia were also previously known as “Hortega cells”, after Pio del Rio-Hortega first described these cells in the CNS, around 1932 (Kettenmann et al., 2011). According to studies performed by del Rio-Hortega, using a modified silver staining technique, microglia are of mesodermal origin and are homogenously distributed cells of CNS (Fig. 1), known for their inflammatory response, towards any pathological event disturbing the homeostasis of CNS (Kettenmann et al., 2011). Subsequently, many studies were performed, that aimed to describe these microglial/“Hortega cells” and characterise their functions, but the advances made by Pio del Rio-Hortega still set the benchmark (Kettenmann et al., 2011).

1.3. Conventional activation paradigm

1.3.1. Diverse microglial phenotypes

Taking advantage of modern immunohistochemical methods, it is possible to identify all microglia in the brain using antibod-

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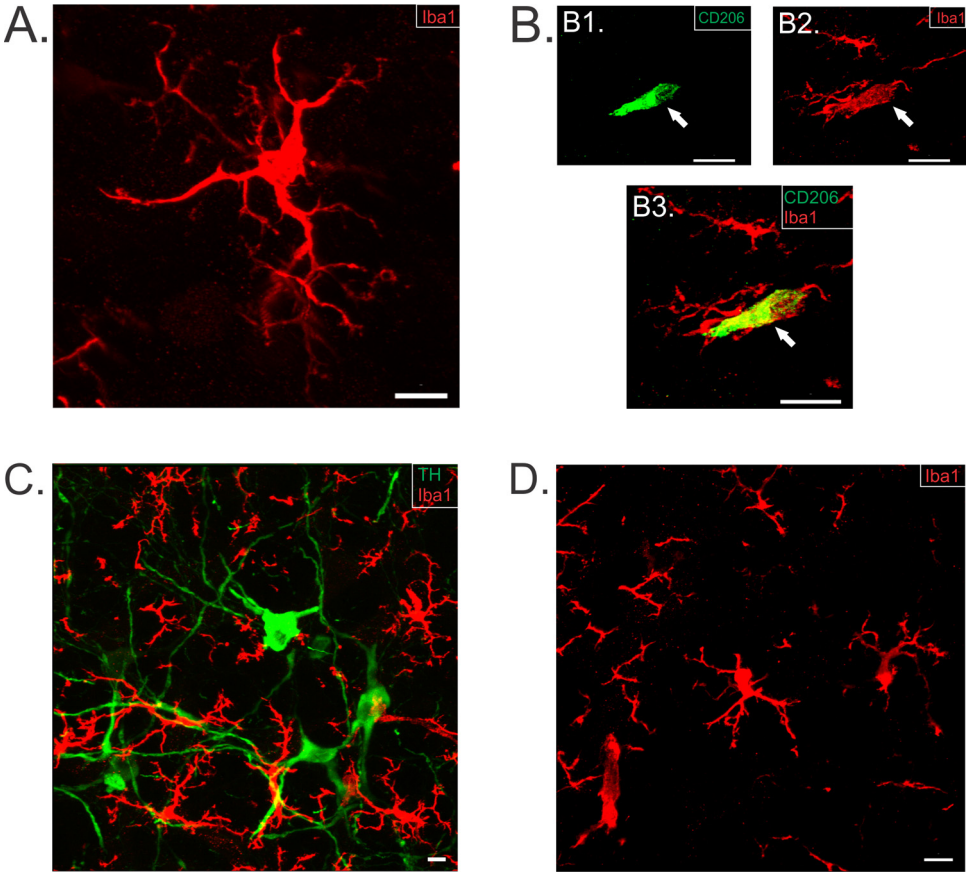

Fig. 1. Microglia in the SNS A. Microglia in the surveilling state in the brainstem of Sprague-Dawley (SD) rat. B. M2 microglia in the brainstem of a SD rat. B1. M2 (CD206 positive, green) microglia. B2. Iba1 (red) positive microglia acquiring an amoeboid morphology. B3. Merged image of B1 and B2 showing co-localization of CD206 expression with Iba1 in M2 microglia. C. Microglia (Iba1, red) are intermingled with the TH-ir (tyrosine hydroxylase-immunoreactive, green) neurons throughout the RVLM. D. Microglia (Iba1, red) in the CVLM region. Scale bar = 10 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ies to different markers in addition to the morphology of the cell. Microglial morphology is a useful indicator of its activation state and its potential role. Broadly speaking, microglial morphologies have been classed into 3 categories; M0, M1 or M2 (Butovsky et al., 2014; Hu et al., 2012). The M0 microglial phenotype (Figs. 1 and 2), represents microglia in their resting/surveilling stage, characterised by a small cell body (≈10 μm diameter) (Kozlowski and Weimer, 2012) and long thin processes/ramifications. On the other hand, M1/M2 phenotypes (Figs. 1 and 2) share similar morphological characteristics; these are amoeboid in shape with shorter or no processes, but differ in their activities. Iba1 (ionized calcium binding adaptor molecule 1) is a calcium binding protein that is also a pan microglial marker. M0, M1 and M2 microglia all express Iba1. Expression of CD16 and CD32 is specific to M1 microglia, whereas expression of CD206 is specific for M2 microglia (David and Kroner, 2011; Hu et al., 2012).

The M0, surveilling microglial phenotype, monitors the local environment for the presence of pathogens, or for changes in the extracellular concentration of constitutively expressed neurochemicals (including neurotransmitters such as neuropeptides, glutamate or GABA) (Nimmerjahn et al., 2005; Tremblay et al., 2011) (Fig. 2). Polarization of the M0 phenotype to an M1/M2 phenotype, signifies a marked activation of microglia. The M1 phenotype, also known as the neurotoxic microglial phenotype, is associated with release of molecules such as IL-6, TNF-α and IL-1β (Crain et al., 2013) (Fig. 2) that are all associated with apoptosis of cells that express receptors for these molecules. The M2 phenotype, which is a neuroprotective phenotype, releases molecules such as IL-10. The M2 phenotype is anti-apoptotic, and promotes tissue repair (Crain et al., 2013) (Fig. 2).

1.3.2. Activation pathway

Most of our understanding related to the activation of microglia, is based on findings from experiments performed *in vitro,* in either cultured microglia, or in freshly prepared brain tissue slices; this situation may differ *in vivo* .

The conventional microglial activation paradigm is divided into 3 distinct stages: a **withdrawal**, a **motility** and a **locomotory** stage (Stence et al., 2001). The ramified resting/surveilling microglia (M0), on sensing the presence of an activation signal, enters a **withdrawal** stage, where these cells start to retract their existing processes. Whilst still withdrawing their existing branches, microglia initiate the process of developing new, short ramifications that are highly dynamic in nature, enabling microglia to become highly motile. Replacement of long microglial fimbria, with short highly dynamic protrusions, permits microglia to enter a **motile** state. In their motile stage, microglia develop a large cell body and very short processes, enabling them to migrate towards the site of injury and participate in inflammatory (M1) or non-inflammatory (M2) related activities (Fig. 2). This last state is known as the **locomotory** stage (Stence et al., 2001).

In addition to the M0 morphological polarization to either M1 or M2, microglia also undergo intracellular re-arrangements, enabling them to translocate their cell body through the tissue. Recent studies indicate that the non-muscle myosin II B (NMIIB) is one of many intra-microglial molecules involved in microglial movement