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Parenchymal accumulation of CD163$^+$ macrophages/microglia in multiple sclerosis brains

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ABSTRACT

Reactive macrophages/microglia exert both protective or damaging effects in multiple sclerosis (MS), which contribute to the relapsing–remitting nature of MS. CD163 is considered a marker of M2 (alternatively activated) macrophages. In the MS brain, CD163$^+$ perivascular macrophages express molecules for antigen recognition and presentation. Here we further investigated the accumulation of CD163$^+$ macrophages/microglia in the parenchyma of MS brains. CD163 expression pattern was investigated in different lesions of brain tissue specimens from five MS brains and five neuropathologically unaffected controls by immunohistochemistry. In the parenchyma of normal brain samples, immunoreactivity (IR) of CD163 was absent. In acute active lesions and at the rim of chronic active lesions of MS, strong accumulation of CD163$^+$ macrophages/microglia was seen. In chronic inactive lesions and in the center of chronic active lesion, CD163$^+$ macrophages/microglia were rare. Further, double-labeling showed that parenchymal and perivascular CD163$^+$ macrophages/microglia were myelin basic protein positive and HLA-DR$^+$, suggesting that CD163$^+$ macrophages/microglia could ingest and present antigen. In addition, in vitro incubating macrophage RAW264.7 cells with myelin turned LPS-induced inflammatory macrophages into an anti-inflammatory phenotype, indicating that myelin basic protein positive, CD163$^+$ macrophages/microglia in MS might have anti-inflammatory effects. The parenchymal CD163$^+$ macrophages/microglia, which had the capacity for antigen ingestion and presentation, might contribute to the resolution of inflammation in MS.

1. Introduction

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS), which primarily affects young adults and in most cases leads to chronic disability through deficits of sensation, motor, autonomic and neurocognitive functions (Ramagopalan et al., 2010). Clinically, MS is characterized in its initial phases by recurrent episodes of neurologic dysfunction (exacerbations/relapses), each followed by a variable extent of functional recovery (remission). In most MS patients, this initial relapsing–remitting disease phase evolves clinically over years into a secondary progressive course (Steinman, 2009; Ramagopalan et al., 2010; Tremlett et al., 2010). The relapsing–remitting nature of multiple sclerosis strongly suggests the presence of potent counter-regulatory mechanisms that prevent infinite expansion of the inflammation and demyelination (Steinman, 2009). Understanding the involved counter-regulatory mechanisms might provide novel therapeutic options.

The pathologic hallmarks of MS are inflammation and demyelination/axon loss in the CNS (Hu and Lucchinetti, 2009). The inflammatory lesion of MS is orchestrated by massive infiltration by a heterogeneous population of cellular and soluble mediators of the immune system (Nataf, 2009). Reactive microglia/macrophages are active participants in the development, and expansion of MS lesions (Breij et al., 2008; Koning et al., 2009). It has been observed that clusters of activated microglia are present even before demyelination in MS (Marik et al., 2007). Furthermore, infiltrating macrophages and activated microglia are the predominant cell types present in expanding MS lesions (Prineas and Wright, 1978) and they are actively involved in myelin phagocytosis (Breij et al., 2008). In addition, activated microglia and macrophages synthesize a cornucopia of different cytokines, trophic factors, extracellular matrix components and neurotransmitter-like molecules that could exert a protective or a damaging effect on the adjacent cells (Gandhi et al., 2010).

Abbreviations: AAL, acute active lesion; CAL, chronic active lesion; CIAL, chronic inactive lesion; CNS, central nervous system; EAE, experimental allergic encephalomyelitis; Hb–Hp, hemoglobin–haptoglobin; HPF, high-power field; LPS, lipopolysaccharide; IR, imunoreactivity; iNOS, inducible nitric oxide synthase; IFN-γ, interferon-γ; IL-12, interleukin-12; MS, multiple sclerosis; SEM, standard errors of means; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α.

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The functional heterogeneity of reactive macrophages/microglia in MS could be due to the existence of different forms of macrophages. It is well established that recruited macrophages can differentiate into two extremes distinct subsets that are categorized as either classically activated (M1) or alternatively activated (M2). M1 macrophages induced by lipopolysaccharide (LPS) or inflammatory cytokines, like interferon-γ (IFN-γ), are characterized by high expression of interleukin-12 (IL-12), IL-23, tumor necrosis factor-α (TNF-α), inducible nitric oxide synthase (iNOS), etc., and are mainly considered to cause autoimmune tissue damage or host defense to infection. M2 macrophages induced by IL4, IL13, IL10 or transforming growth factor-β (TGF-β), highly express anti-inflammatory molecules, such as IL-10 and TGF-β, extracellular matrix molecules, like fibronectin, and scavenger receptors, which underlie their roles in anti-inflammation and tissue repair (Benoit et al., 2008; Olefsky and Glass, 2010).

The normal CNS contains three populations of resident macrophages: microglial cells, meningeal macrophages, and perivascular macrophages. Perivascular macrophages are distinct from parenchymal microglia in their location, morphology, expression of myeloid markers, and turnover in the CNS (Cosenza et al., 2002; Guillemin and Brew, 2004). The rapid recruitment of blood-borne monocytes, the activation of resident microglia and perivascular macrophages, together with the recruitment of T cells, are among the most consistent changes observed in MS and its autoimmune animal models of experimental allergic encephalomyelitis (EAE). Both brain-derived microglia and blood-borne macrophages are crucially involved in many consecutive stages of autoimmune demyelination in EAE and MS (Raivich and Banati, 2004).

CD163 is a member of the scavenger receptor cysteine-rich family class B. While the expression of CD163 was recently found on dendritic cells (Marquet et al., 2011) its expression is mainly on monocytes and macrophages (Van den Heuvel et al., 1999; Van Gorp et al., 2010). CD163 functions as an endocytic receptor for hemoglobin–haptoglobin (Hb–Hp) complexes and as such is proposed to mediate the clearance of free Hb from the circulation (Kristiansen et al., 2001). Recently CD163 has also been found as receptor for TNF-like weak inducer of apoptosis (TWEAK). Upon binding to CD163-expressing macrophages, TWEAK is internalized and degraded (Bover et al., 2007). CD163 expression is induced by IL-10, IL-6, and glucocorticoid but is downregulated by LPS and IFNγ (Buechler et al., 2000; Philippidis et al., 2004; Van Gorp et al., 2010). Interestingly, CD163 is considered as a marker of alternatively activated or anti-inflammatory macrophages (Abraham and Drummond, 2006; Komohara et al., 2006). $CD163^+$ macrophages are found during the late downregulatory phase of acute inflammation (Zwadlo et al., 1987) and in chronic inflammation (Topoll et al., 1989). It is known that in the normal and MS brain, $CD163^+$ perivascular macrophages express molecules for antigen recognition and presentation (Fabriek et al., 2005), but the parenchymal distribution of $CD163^+$ macrophages/microglia is not clear yet. Therefore, we have studied the accumulation of $CD163^+$ macrophages/microglia in human MS brains.

2. Materials and methods

2.1. Patients

Tissue sections of adult brains were studied, including 5 neuropathologically normal brains and 5 MS brains (sections from lesions in acute and chronic MS). Except for one case, a stereotactic biopsy, 6 to 10 blocks from different affected regions of brain parenchyma were used for histology and immunohistochemistry for routine MS classification and analysis of CD163 expression. Lesions were classified according to standard histopathological criteria as previously published (van der Valk and De Groot, 2000) using routine staining for inflammatory cells (expression of CD68, CD3, CD4, CD8 and CD20) and the presence of myelin breakdown products (myelin basic protein, myelin oligodendrocyte glycoprotein). In our study, three types of lesions were identified, (I) acute active lesion (AAL): clusters of $CD68^+$ reactive microglia/macrophages but without apparent myelin loss, (II) chronic active lesion (CAL): a hypocellular demyelinated gliotic center and a hypercellular rim containing numerous macrophages and (III) chronic inactive lesion (CIAL): a few leukocytes and extensive GFAP immunoreactivity. In total 28 AALs, 51 CALs and 38 CIALs were available for analysis. Two experienced neuropathologists performed histological diagnosis and classification. Relevant clinical information and neuropathological observations of investigated cases were reported in our previous study (Zhang et al., 2008). Tissue specimen procurement was performed according to the ethical guidelines of the University of Tuebingen.

2.2. Immunohistochemistry

After dewaxing, sections were boiled (in a 600 W microwave oven) for 15 min in citrate buffer (2.1 g citric acid monohydrate/L, pH 6). Endogenous peroxidase was inhibited with 1% H_2O_2 in pure methanol for 15 min. Sections were incubated with 10% normal pig serum (Biochrom, Berlin, Germany) to block non-specific binding of immunoglobulins and then with mouse monoclonal antibodies against CD163 (Serotec, Oxford, Great Britain; dilution 1:100). Antibody binding to tissue sections was visualized with a biotinylated rabbit anti-mouse IgG $F(ab)_2$ antibody fragment (DAKO, Hamburg, Germany). Subsequently, sections were incubated with a Streptavidin–Avidin–Biotin complex (DAKO, Hamburg, Germany; dilution 1:100), followed by development with diaminobenzidine (DAB) substrate (Fluka, Neu-Ulm, Germany). Finally, sections were counterstained with Maiers hemalaun. In negative controls, the primary antibodies were omitted.

2.3. Immunostaining evaluation

After immunostaining, CD163 localization was examined by light microscopy. In order to semi-quantify CD163 expression, the numbers of parenchymal $CD163^+$ cells were counted at different lesions as following: sections were randomly numbered and observers were not aware of the lesion type. For counting, each lesion was scanned at 40 times magnification for the regions of highest $CD163^+$ cell count (hot spot), followed by counting the number of $CD163^+$ cells within a single high-power field (HPF, ×400 magnification) by two investigators independently. Three HPFs were counted for each lesion and the mean value was taken for this lesion. In each field studied, only positive cells with the nucleus at the focal plane were counted. During the counting, parenchymal $CD163^+$ cells were easily distinguished from parenchymal macrophages, which were located in between the blood vessel endothelia, occasional perithelial cells and the basal membrane that separates the blood vessel from the surrounding neural parenchyma. Results of different lesion type were given as arithmetic means of positive cells per HPF and standard errors of means (SEM).

2.4. Double staining

In double labeling experiments, sections were pre-treated as described above and then incubated with the appropriate antibodies. Visualization was achieved by adding secondary antibody (biotinylated rabbit anti-mouse IgG) at a dilution of 1:400 in TBS-BSA for 30 min and alkaline phosphatase conjugated ABC complex diluted 1:400 in TBS-BSA for 30 min. Consecutively, immunostaining was developed with Fast Blue BB salt chromogen-substrate solution. Then sections were once more irradiated in a microwave for 15 min in citrate buffer and were immunolabeled as described above, but by omission of counterstaining with hematoxylin. The following antibodies were used: HLA-DR (1:500; Chemicon International, California, USA) or myelin basic protein (1:100; Dako Hamburg, Germany).