Chapter 8

Central Nervous System Resident Cells in Neuroinflammation: A Brave New World

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1. Introduction

Neuroinflammatory and central nervous system (CNS) demyelinating diseases have most of its pathological features based on immune cells infiltrating brain parenchyma and leading to neuronal death and focal or diffuse destruction of the CNS architecture, as extensively reviewed (Rodriguez 2007; Weiner 2008; Shie FS 2011). In this context, the role for resident and infiltrating inflammatory cells is very relevant, as they may be both the trigger and the maintainers of the neuroinflammatory process (Almolda B 2011). The combination of interesting concepts of neurology to the immunology laboratory, has rendered glial cells with a more dynamic and immunological shape.

Stroke and Multiple Sclerosis (MS) are on the opposite sides of the coin of the neuroimmune diseases, depending on innate and adaptive immunity, respectively. That is the main reason why stroke is denominated as a neuroinflammatory rather than an autoimmune disease, and the opposite for MS. Despite this difference, which relies on the fact that cognate antigens for T cells were described for MS but not for stroke, central nervous system resident cells play a pivotal role on both cases. This greatly evidences the importance of brain parenchymal cells in the pathogenesis of neuroinflammatory and neurodegenerative diseases.

Innate immune system greatly differs from adaptive immune system in many ways. Innate immunity is much older concerning its phylogeny, whereas adaptive immunity is relatively recent. It is interesting to mention that the first lymphocytes appeared with the gnatus cartilaginous fish, such as sharks and rays (Matsunaga T 1998), whereas in 1882 Metchikoff E demonstrated that starfish had singular phagocytic cells which are now believed to be the macrophage ancestral, as reviewed (Janeway CA Jr 2001). Besides its phylogenetic discrepancies, innate and adaptive immune systems also greatly differ in function and

recognition. One important difference is that lymphocytes express Recombinase Activating Genes (RAG), conferring unique T and B cell receptors. Further differences between innate and adaptive immunity are described in Table 1 (adapted from (Abbul K Abbas 2006).

Specific Feature	Innate Immunity	Adaptive Immunity
Specificity	Macrophages, dendritic cells, neutrophils recognize Pathogen Associated Molecular Patterns (PAMPs) as Lipopolissacharide, peptidoglycan, ssRNA, dsRNA, unmethylated CpG nucleotides, mannose rich glycans among many others.	T lymphocytes recognize by its T cell receptor (TCRs), peptides presented by antigen presenting cells in the context of the MHC molecules. B cells recognize through the B cell receptor (BCRs) several different molecules, such as DNA, RNA, Lipids, Carbohydrates that may or may not be associated to carrier proteins.
Receptors	Pattern Recognition Receptors (PRRs) such as Toll-like receptors 1-12, NOD, Dectin-1, NLRPs.	T cell receptor (TCRs) and B cell receptor (BCRs)
Clonality	No	Yes. Each cell expresses only one type of TCR or BCR.
Self-tolerance	No mechanism to maintain tolerance to self.	T and B lymphocytes undergo selection mechanisms in the thymus and bone marrow, respectively.

Table 1. Table one pinpoints some important differences between innate and adaptive immune system.

In this sense, the discrepancy between stroke and MS pathology is mainly due to the fact that Multiple Sclerosis (MS) and its murine model Experimental Autoimmune Encephalomyelitis (EAE) are secondary to a peripheral still-to-be-defined activation of T CD4 cells specific for myelin derived epitopes. On the other hand, during stroke, no cognate antigens have been clearly established so far, and innate immune cells such as macrophages and neutrophils play the master role.

Acute stroke, defined as the occlusion or hemorrhage of brain blood vessels, culminates in neuronal and glial cell death and the local release of pro-inflammatory molecules (S Stoll G 2010). These molecules, named Danger Associated Molecular Patterns (DAMPs), such as Heat Shock Proteins (HSPs), degraded extracellular matrix, F-actin filaments (Ahrens S 2012), ATP and many others (Seong Seong 2004), may induce blood brain barrier (BBB) disruption and activation of resident cells (Ceulemans AG 2010; Stoll G 2010). This is

followed by polymorphonuclear and mononuclear cells infiltration establishing the inflammatory foci (Stoll G 2010; Thiel A 2011). It is noteworthy that ischemic stroke comprises more than 80% of the cases, caused by embolus or thrombus (Stoll G 2010). Stroke is also considered a heavy burden for Public Health Policies as almost 80% of patients suffer from further disability [18]. Stroke lesions are time-dependent, and longer periods of ischemia induce greater lesions than shorter periods. This is intimately linked to the reminiscent blood supply offered to the tissue. After stroke, two well defined regions are described: i) the region with completely abolished blood supply is the core, with intense necrosis, and ii) peripherally to the core is the penumbra, with the presence of inflammatory infiltrate.

Acute lesions are immune independent, and most cell death is due to altered ionic balance, enhanced water input to the cell body resulting in edema, high calcium influx and secondary high glutamate release (Dirnagl U 1999; Ceulemans A 2010). For instance, glutamate release spreads early throughout brain parenchyma inducing neuronal death by a phenomenon called excitotoxicity, mediated by ionotropic glutamate receptors, such as NMDAR (N-methyl-D-aspartate) (Ceulemans A 2010). Besides biochemical alterations, the release of inflammatory molecules, as Heat-shock proteins (HSPs), histamine, prostaglandins, TNF-α, is also observed in the injured area. Altogether, these inflammatory factors released after necrotic cell death induce the activation of resident cells, such as astrocytes, microglia, endothelial cells and even neurons, to secrete more pro-inflammatory cytokines and chemokines, and not only recruiting but also favoring the migration of inflammatory cells to the ischemic site. These mechanisms will be discussed in this chapter.

Secondary to the acute phase, a leukocyte-dependent period emerges. Due to the disruption of the BBB, and the local secretion of chemokines and other leukocyte-recruiting factors, inflammatory cells such as neutrophils, macrophages and even lymphocytes arrive from the periphery and then interact with brain parenchymal cells establishing the inflammatory process itself. Neutrophils and macrophages secrete high amounts of innate immunity derived cytokines such as IL-1 , IL-6, IL-8, IL-12, IL-15, IL-17, IL-18, IL-23, TNF-α, LIF, and chemokines as CCL2, CCL6, CCL21 and many others (Charles A Janeway 2002; Ghiringuelli F 2007; Suzuki T. 2007; Song C 2008; Weiner 2008). Just to mention, several other inflammatory factors were also found in ischemic area, such as matrix metalloproteinases (MMPs) (Del Zoppo GJ 2012), Adenosine Triphosphate (ATPs) (Arbeloa J 2011), High Mobility Group-box 1 (HMGB1)(Wang H 1999), HSPs (Romi F 2011), leucotrienes, prostaglandins, glutamate (Dhawan J 2011), leptin (Avraham Y 2011), complement factors and many others (Dirnagl U 1999; Ceulemans AG 2010). However, it is interesting to mention that these factors may also be secreted by many resident cells, such as microglia, astrocytes and also endothelial cells, overlapping the role of resident Vs. infiltrating cells. To make an example, IL-1β, whose source may be both macrophages and astrocytes, greatly contributes to the activation of the vascular beds facilitating adherence and transmigration of circulating leukocytes, as well as to the increased intracellular calcium in neurons and astrogliosis (Stoll G 2010). To corroborate

the importance of IL-1, recently the use of Anankira®, the recombinant IL-1Ra (IL-1 receptor antagonist) has been proposed to be used in therapy, as an attempt to abrogate the deleterious IL-1-derived features (Emsley HC 2005).

Thus, it is clear that during stroke, the importance of different cellular populations has been established; no matter these cells are infiltrating macrophages and neutrophils or resident astrocytes and microglia. Thus, therapeutic approaches whose mechanisms focus on the reduction of local inflammation and cellular recruitment may be consider as promising alternatives for reducing the overall disability so usually observed in these patients.

Very different from stroke, where the inflammatory process starts inside the CNS, during MS, self-reacting T CD4 cells are somehow activated in the periphery and further migrate to the target organ. In this context, there are two main differences between MS and stroke: i) the need for activated T cells to gain access to the CNS, and ii) the disease is autoimmune in nature, thus cognate antigens, such as myelin and proteolipoprotein epitopes are well described. Although it is a consensus that these self-reacting cells are specific for myelin-derived antigens (Weiner HL 1993; Korn T 2007; Rodriguez 2007), the trigger for these cells to be activated in the periphery is still matter of intense debate. Other possibilities related to MS incidence and prevalence range from association to certain HLA haplotypes, as for HLA-DRB1*1501 e HLA-DRB5*0101, HLA-DQB1 (Oksenberg Jr 2005), to genetic polymorphisms, and although not very accepted, even viral infections, as recently reviewed (Owens GP 2011).

MS is a neuroinflammatory and demyelinating disease of the CNS that leads to great disability in younger adults, and most of its incidence is observed in northern countries of Europe and also the United States (WHO 2006). Multiple Sclerosis stands for multiple scars, referring to the inflammatory plaques where myelin is lost mostly in perivascular regions of the white matter and whose observation by Magnetic Resonance Imaging (MRI) is considered the most reliable diagnostic approach. Myelin sheets are produced by neuron-surrounding oligodendrocytes, the main target cells during MS. Death of these cells results in axonal dissection, followed by neuronal lost and thus reduction in neuronal impulse transmission, resulting in weakness, fatigue, numbness and overall disability. Concerning its onset and evolution, MS may be separated in i) primary progressive ii) secondary progressive and also iii) the relapsing-remitting type. Usually the disease starts with a relapse-remitting pattern of the symptoms followed by a progressive stage of increasing disability, considered the more severe cases (Weiner 2008). Many patients evolve to a more chronic stage of the disease, with no relapses, but a more prominent progressive lost of motor coordination, sensory and autonomic function is observed. It is noteworthy that many of these symptoms are tightly regulated by the local inflammatory milieu in the CNS, as well as by the activation status of the resident cells.

Much of our understanding concerning the molecular and cellular mechanisms of MS was obtained after observations made on its murine model, Experimental Autoimmune Encephalomyelitis (EAE). With historical relevance, it is worthy to mention that the EAE model started after Prof. Thomas Rivers decided to understand why many years before,

Prof. Louis Pasteur experimental animals used to develop paralysis after vaccination against rabies. Rivers proceeded with the same protocol as Pasteur, immunizing rabbits with spinal cords extracts from rabbits that were inoculated with the rabies virus. At the same time he included a control group which consisted of extracts from non-contaminated animals. Interestingly, animals that received these extracts also developed paralysis, highlighting the fact that the secondary paralysis was not a rabies virus-dependent observation. Interestingly, the features develop by the animals was very similar to those observed by Pasteur many years earlier. It was also very exciting to recall that at that time Freund was describing its complete adjuvant (Complete Freund Adjuvant), what made Rivers research much easier, as the immunization for EAE induction changed from around 20 inoculations of brain extracts to only one injection. Then, one of the most used model to study not only neuroinflammation and neurodegeneration but also autoimmunity mechanisms had been brought to scientific community, as reviewed (Baxter 2007).

MS was previously considered to be the prototype of a Th1 type of autoimmune disease, where IFN-γ-secreting T CD4 (Kroenke MA 2007; Dardalhon V 2008) and CD8 (Michael P. Crawford 2004; Goverman J 2005) cells are greatly relevant and widely found in the periphery, liquor and brain parenchyma (Matusevicius D 1999). On the other hand, many contrasting data were obtained using the EAE model. In fact, it was known by a long date that mice deficient for IFN-γ were more susceptible to EAE induction when compared to control animals (Chu CQ 2000). This is in fact corroborated by other autoimmune disease model, the Experimental Autoimmune Uveitis (EAU) (Jones LS 1997). This was much unexpected, and for a great period of time a matter of intense speculation. Moreover, corroborating these findings, studies using animals deficient for p19, p35 and p40, the subunits for the heterodimers IL-12 (p35 + p40) and IL-23 (p19 + p40), had demonstrated that, unexpectedly, IL-23 was more relevant then IL-12 in these same models, raising still more questions in the field of EAE pathology (Cua DJ 2003). Thus, for many years there was a gap in the understanding on the actual role for IFN-γ-secreting Th1 cells in the EAE model. It is worthy to mention that much less was known for the human disease.

The final answer for that was obtained many years later when, using the EAE model, a new population of T cells was described as the main players in the pathogenesis of EAE The so-called Th17 cells secrete high amounts of the cytokines IL-17A, IL-17F (Ivanov I 2006), IL-21 (Zhou L 2007) and GM-CSF (Codarri L 2011), and its master regulator is the rorc gene which encodes the gene for the retinoic acid orphan receptor, RORγt (Ivanov I 2006). To further corroborate this hypothesis, it was demonstrated that IL-17 knockout mice are resistant to EAE induction, which was very contrasting with data obtained from IFN-γ knockout mice, as reviewed (Komiyama Y 2006). Besides, it became clear that these cells arise in the periphery in the dependence of two cytokines, IL-6 + TGF-β, whose activity was demonstrated to be inducers of rorc expression and thus Th17 commitment. Corroborating previous data, it was clarified that IL-23 is the maintenance factor for these cells to remain viable and active. In fact, IL-23r deficient cells are unable to be maintained viable and thus induce disease (Cua DJ 2003; Zhou L 2007). Thus, the findings in p19 KO mice highlighted

the fact that these mice were resistant to EAE induction due to the reduced survival of encephalitogenic Th17 cells in the periphery. However, it is well accepted that both Th1 and Th17 cells are found in focal lesions in MS and EAE, comprising around 20-25% and 10-15% of the CNS infiltrating T CD4 cells respectively (Murphy AC 2010; Peron JP 2010). Moreover, it is well accepted the fact both populations intimately interact with CNS resident cells, as astrocytes, microglia and neurons.

Aside from the discussion whether this or that population of CD4 cells is more important, it is a fact that a mixed population is found in the inflamed brain parenchyma, and in most cases Th1 cells outnumber Th17 cells. Interestingly, it has now been established that each population possibly infiltrate the CNS through different mechanisms. Actually, infiltrating cells may reach the CNS by three separate routes: 1) by direct transmigration through the capillary and post-capillary venules of the BBB and 2) from the blood to the cerebrospinal fluid at the choroid plexus and 3) migration through meningeal vessels to the subarachnoid space (B. 2006). In this sense, it has been recently demonstrated that Th1 cells use the α4β1 integrins (Very Late Antigen- 4 – VLA-4) to infiltrate the spinal cord during EAE. The blockade of VLA-4 did not block Th17 cells to infiltrate the brain, expressing both IL-23R and CCR6. Accordingly, T cell-conditional α4-deficient mice were not resistant to actively induced EAE but showed an ataxic syndrome with predominantly supraspinal infiltrates of T cells (Rothhammer V 2011). Corroborating this idea, it was previously showed that Th17 cells constitutively express the chemokine receptor CCR6, whose ligand, CCL20 is also constitutively expressed by choroid plexus cells. Thus, it is clear that Th1 and Th17 cells differ not only in phenotype and function, but also in its ways to gain access to the CNS (Jäger A 2009; Murphy AC 2010).

It is well established that after the entry of Th1 and Th17 cells to the brain parenchyma, these cells need to be reactivated upon encounter with MHC-Ag bearing cells on a second hit phenomenon. After re-activation in situ, Th1 and Th17 lymphocytes release its contents, most of which are pro-inflammatory cytokines, such as IFN-γ, TNF-α and IL-6 for Th1, and IL-17A, IL-17F, IL-21 and IL-22 for Th17 cells. In this context, CNS resident cells play an important role, not only because of its capacity to second hit T cells, but also because they express cytokine receptors and Toll-like receptors (TLRs), very important for the amplification of the inflammation. Thus, studies on the biology of astrocytes, microglia, endothelial cells and also neurons during inflammatory processes will greatly contribute to the understanding of the pathogenesis of neuroinflammatory and neurodegenerative disease.

1.1. Central nervous system resident cells and stroke

Due to the high necessity for blood supply and oxygen, brain resident cells are very sensitive to hypoxic injury. Neurons are the most affected, followed by astrocytes, microglia and finally endothelial cells (Dirnagl U 1999). During stroke, necrotic cell death is followed by the release of intracellular content, such as pro-inflammatory cytokines, that greatly accounts for the initiation of the process. In fact, activation of astrocytes and microglial cells

during stroke, either by the early necrotic cell death or by the later inflammatory cells-derived cytokines, is an important process directly involved with the disease pathology (Dirnagl U 1999; Ceulemans A 2010). However, we must not exclude the role for infiltrating macrophages, a rich source of cytokines and chemokines, found in the ischemic foci as soon as 10 hours.

Microglial cells are CNS resident phagocytic cells, with great resemblance to macrophages, however, it has been recently demonstrated that these macrophage-like cells in fact differ in ontogeny. Microglial cells were believed to derive from a myeloid precursor whose marker is the transcription factor PU.1. However, PU.1 knockout mice were absent not only of microglia but also macrophages, showing that microglial cells were in fact derived from a myeloid lineage, but PU.1 was a not a specific marker [1, 2]. Interesting results have demonstrated that bone marrow (BM) transplantation restored microglia presence in the central nervous system, what led to the interpretation that microglial cells do in fact derive from a common precursor in the BM [1] (Kettenmann H 2011). However, many of these experiments were performed after whole-body or central nervous system (CNS) irradiation. Using a parabiotic system, where the circulatory systems of two mice are connected, experiments demonstrated that no donor-derived cells are found in the CNS of parabiotic mice [3]. Recent reports using Green Fluorescent Protein (GFP) couple to the fractalkine receptor (CX3CR1- GFP) and Runt- related transcription factor 1 (Runx1) - Cre reporter mice described the presence of fetal yolk sac- derived microglial precursor cells at day E9.5. On day E10.5, microglial cells are already found in CNS, as circulatory system has been established. Thus, microglial are generated upon primitive haematopoiesis in the yolk sac, independent from bone marrow haematopoiesis [4]. Actually there is still a search for a reliable marker to indentify microglial cells by flow cytometry. One option is to use the markers CD45 and CD11b, where CD11b+CD45low cells are considered microglia and CD11b+CD45high are considered macrophages.

At resting state, microglial cells have a body shape very similar to dendritic cells, as they posses many dendrites or branches. Those are believed to be actively involved in the clearance of cellular debris as well as in the immunesurveillance, as some viruses and bacteria may reach the brain [13-14]. Moreover, it was already demonstrated that after ischemic brain injury, microglia migrate to the penumbra covering the remaining living neurons. As these neurons die by apoptosis, proper caping by microglia results in fast phagocytosis and thus avoiding more inflammation. This was demonstrated to be dependent on the expression of the LFA-1 integrin.

Non-activated microglia are described to be very quiescent with an anergic-like state. Resting microglia have very little capacity to prime naïve T cells as they express very low levels of MHC II molecules, and are negative for CD80, CD86 and CD40 (Kettenmann H 2011).This status seem to be maintained by several different mechanisms. For instance, it was recently demonstrated that, microglial cells express a high amount of the miRNA-124. This oligonucleotide directly inhibits the CCAAT/enhancer-binding protein-α (C/EBP-α) and also its target, the above mentioned PU.1. Further, this inhibition down-regulates CD45

and MHC II expression and also TNF-α secretion [15]. Interestingly, peritoneal macrophages do not express miRNA-124 and bone marrow derived macrophages are rendered less activated when transfected with miRNA-124 [15]. Thus, the specific expression of such miRNA greatly accounts for the anergic state of microglial cells.

The intimate contact with norepinephrine-releasing adrenergic neurons, as in the locus coeruleus may be also responsible for maintaining microglia hyporesponsive. Microglial cells express β2-adrenergic receptors associated to G stimulatory proteins. These proteins trigger the activity of adenilate cyclase increasing cAMP synthesis. Elevated intracellular cAMP concentrations activate Protein Kinase A (PKA) which may have many suppressive activities. PKA phosphorylates Csk and Ezhrin, whose kinase activity phosphorylates inhibitory proteins of Lck (Ruppelt A 2007). High cAMP levels also phosphorylates CREB (Cyclic AMP Responsive Elements Binding Protein) that translocates to the nucleus [16, 17]. CREB interacts with CREB Binding Protein (CBP) activating several promoters, such as IL-10. PKA is also able to phosphorylate the Nuclear Factor of Activated T Cells (NF-AT). It is worthy to remember that NF-AT is inactive at its phosphorylated state. Altogether these are some of the mechanisms that greatly contribute to the hyporesponsiveness of microglial cells.

Although at resting state microglial cells are very "polite", after activation they start to express a whole different set of genes, acquiring the capacity not only to secrete significant amounts of cytokines, chemokines and other inflammatory molecules, but also to present antigens and activate CNS infiltrating T cells. In fact, there are at least three acceptable ways to activate microglial cells: i) with pro-inflammatory cytokines, such as IFN-γ, TNF-α and IL-17; ii) engagement of TLRs ligands, such as LPS, PGN, HSPs, and iii) the phagocytosis of myelin debris from demyelinating regions. Altogether, these are the main mechanisms by which microglial cells subverts the anergic-induced state, and thus acquiring an active role in neuroinflammation, as observed during acute brain ischemia.

It was recently proposed that microglia posses all inflammasome derived machinery, thus conferring these cells positive for IL-1 / IL-18. IL-1β interacts with its receptor activating a signaling pathway that is shared with Toll-like receptors (TLRs) through MyD88 and culminating in NF-κB phosphorylation and translocation to the nucleus. IL-1R was shown to be widely found in rat hippocampus, but preferentially at synaptic sites. More interesting, it was also demonstrated that IL-1R co-localizes with the ionotropic glutamate receptor NMDA through its GluN2B subunit (Gardoni F 2011). Just to mention, a few overall effects of IL-1β are: induces rapid local inflammation and up-regulates the synthesis of other cytokines and costimulatory molecules, as CD80, CD86 and CD40. IL-1β also disturbs ionic balance, increases Ca2+ influx in neurons leading to degeneration, facilitates BBB disruption and most important, it is a potent stimuli for endothelial cells to express adhesion molecules which amplifies local inflammatory cells recruitment (Ceulemans AG 2010).

IL-6 is not found in the CNS at resting state. On the other hand, it is widely found after stroke, and may be secreted by many resident cells, as microglia, astrocytes, endothelial cells and also infiltrating monocytes and T cells. Interestingly, microglia are not only able to

secrete IL-6 but also they express IL-6R, evidencing a positive feedback loop. IL-6 signaling in microglia phosphorylates STAT-1, STAT-3 and ERK, culminating in MHC II, CD40 and IL-12p70 up-regulation and increasing inflammation (Lin HW 2009). However it is also accepted a dichotomy for IL-6 function, as many reports have also demonstrated its protective role after stroke. In this concern, it is interesting to mention that IL-6 up-regulates the transcription of the suppressive molecule IL-1ra (IL-1 Receptor Antagonist), thus blocking some of the deleterious functions of IL-1. In fact, higher IL-6 levels may positively correlate with a better neuropathological outcome (Emsley HC 2005).

It is noteworthy that microglial cells secrete so many inflammatory factors, such as IL-1, IL-4, IL-6, IL-17, TNF-α, synthesize reactive oxygen and nitrogen species, secrete matrix metaloproteinase as MMP-2, MMP-9, and other factors. Microglia also express several membrane receptors, as TLR-2, TLR-4, NLRP3 (Nalp3), gp91 phox (Dohi K 2010), ciclooxinagenase-2 (Cox-2), oncostatin M, heme-oxigenase -1 (HO-1) [10, 15-17]. Onconstatin M shares the IL-6R beta chain and thus phosphorylates STAT-3 and also NF-κB transcription factors, up-regulating TNF-α secretion and iNOS expression (Baker BJ 2010). It is also known that activated microglia secrete high amounts of glutamate and TNF-α. These molecules are able to induce apoptosis of neurons but especially of oligodendrocytes, leading to demyelination, a hallmark of MS and EAE. For this reason, it has been more and more evidenced that microglial cells have a very important role in the pathogenesis of several different inflammatory diseases of the CNS, as stroke [13, 18], multiple sclerosis [17], Alzheimer disease, amyotrophic lateral sclerosis and also different types of brain and liquor infections, as reviewed [19].

The products of NADPH oxidase activation, the reactive oxygen species (ROIs), are also a very relevant source for cellular injuries. In this concern, ischemia can also induce this pathway whose importance in the pathogenesis of stroke is well established. The voltage-gated proton channel Hv1 counterbalances NADPH oxidase and subsequent cellular loss of electrons with protons. It has recently been shown that mouse and human brain microglia, but not neurons or astrocytes, expressed large Hv1-mediated currents and that Hv1 was required for NAPDH oxidase activity and ROI generation in brain microglia in situ and in vivo. Thus, Hv1 knockout mice are less prone to develop neuronal death and brain damage after stroke. These indicate that, aside the cytokine and glutamate burst, ROIs are responsible for a substantial fraction of brain damage at early time points after ischemia, and rendering Hv1 with a promising therapeutic approach (Wu LJ 2012).

In summary, there is a never ending list of features, such as released factors, receptors expressed, ROIs, membrane molecules, MMPs, that points out microglia as a very important resident cell population, with an unquestionable relevance in the mechanisms of triggering and perpetuating CNS inflammation. On the other hand, although mostly shown as an important pro-neuroinflammatory agent, it is conceivable that microglial cells may have a modulatory function, in an attempt to reduce neuroinflammation. In fact, it has been demonstrated that microglia may also secrete anti-inflammatory molecules such as IL-10 [20], TGF-β, IL-1Ra and express suppressive molecules as Suppressor of Cytokine Signaling

(SOCS-3) [21], the tryptophan depleting enzyme indoleamine-2,3-dioxigenase (IDO) [22] and also IL-4 (Ponomarev ED 2007). It has recently been accepted that, as observed in macrophages, microglial cells may also be divided in those with pro-inflammatory activity, called Microglia 1 (M1) and those with regulatory activity, called Microglia 2 (M2), as extensively reviewed (Martinez FO 2009). Such findings, much contributes towards the great relevance of microglial cells during neuroinflammatory diseases. However, further studies must be performed to a better understanding of the suppressive activities of microglial cells.

Activated astrocytes also play a significant role in the pathogenesis of stroke. Astrocytes may not only secret many cytokines, chemokines and MMPs but also undergo astrogliosis, which is an exacerbated proliferation of astrocytes as a wound-healing like process. Astrocytes may secrete significant amounts of TNF-α, interestingly with both neuroprotective and neurotoxic properties. TNF-α belongs to a great family of cytokines and also membrane bound molecules, such as Fas, FasL, Glucocorticoid Induced TNF Receptor (GITR) and several others (Abbul K Abbas 2006). It has been proposed that TNF-α follows a biphasic pattern of secretion after brain ischemia, and the first wave is detected as soon as 1-3 hours probably, and the second after 24-36 hours. This fluctuation may be a consequence of two separate sources for this cytokine, as it may be first derived by the tissular injury itself, and the second one derived from resident cells activation and also from infiltrating inflammatory cells (Ceulemans AG 2010). Moreover, TNF-α using its death domains, induces apoptosis of endothelial cells, and thus promoting CNS infiltration, as it disrupts BBB (Ceulemans AG 2010). However, contrasting data showing that previous treatment with TNF-α protect from neuronal death, although the mechanisms are still to be elucidated. AVC

Transglutaminase 2 (TG2) is a Ca(2+)-dependent transamidating enzyme ubiquitously expressed in the body. TG2 is the predominant form of transglutaminase expressed in the mammalian nervous system. Previously, it was shown that TG2 can affect both cell death and cell survival mechanisms depending on the cell type and the stressor. Intriguingly, infarct volumes in TG2 knockout mice were significantly reduced when compared to controls. Neurons from TG2 knockout mice showed decreased viability in response to oxygen-glucose deprivation, which was not observed in astrocytes, as they were resistant to oxygen-glucose deprivation in situ. Interestingly, wild type and knock out neurons were protected against oxygen glucose deprivation when they were co-cultured with astrocytes from TG2 knockout mice (Colak G 2012). Therefore, the decreased stroke volumes observed in TG2 knock out evidences that its expression is more important in astrocytes. Altogether, this serves to corroborate the idea that neuron-astrocyte crosstalk plays a significant role in mediating stroke pathogenesis.

Matrix metalloproteinases (MMPs) are extracellular matrix remodeling enzyme, very important for tecidual infiltration. Recent data on non-nervous system tissue showed intracellular and even intranuclear localizations for different MMPs. In this concern it was recently demonstrated that MMPs localization to the nucleus of neurons may correlate to

apoptotic cell death. More interesting, cells expressing MMP-9 in the nuclear compartment also co-expressed activated-caspase 3, linking MMP-9 expression to neuronal and glial death (Del Zoppo GJ 2012). The research has shown for the first time the localization of MMP-9 to the nucleus of human neurons. Altogether this gives more relevance to MMPs secretion in the CNS as it correlates not only with facilitation of the cellular infiltration.

Moreover, the mechanisms through which astrogliosis is orchestrated is still considered to be unknown. However, it has been recently demonstrated that CD36 plays a pivotal role in astrocytic survival, and proliferation In fact, CD36 deficient and CD36 -siRNA treated animals had reduced astrogliosis and wound healing. Thus, it is now accepted that CD36 signaling intimately correlates with astroglial proliferation (Bao Y 2012).

The presence and the role of certain types of dendritic cells (DCs) in the CNS is constant matter of intense debate. Things get even darker when it concerns stroke pathogenesis. Some reports have shown that both resident and peripheral DCs may route to CNS after ischemic injury. Using CD11c-GFP animals, it was clearly shown that as soon as 24 hours after medial cerebral artery occlusion (MCAO), brain dendritic cells (bDCs) are found peripherally to the penumbra area. Using bone marrow chimeras, where both wild type and CD11c - GFP animals were irradiated followed by bone marrow reconstitution interestingly demonstrated that bDCs migrate to the penumbra, whereas peripherally recruited DCs are mostly found in the core. These cells were highly positive for the expression of the dendritic cells marker CD11c and several T cell activation-related molecules, as MHC II, CD80 and CD86. However, this was not observed early after MCAO occlusion, and thus, the initial activation observed in the CNS is most probably dependent on microglial activation rather than on bDCs (Felger JC 2010).

As professional antigen presenting cells as DCs are important in the pathogenesis of stroke, it is conceivable that T cells may also have a relevant role due to antigen recognition. However, one may not forget that, differently from MS, stroke is not an autoimmune disease, and the cognate epitopes recognized by T and B cells after ischemia are still matter of debate. However, elegant researches have demonstrated a pivotal role for T cells in stroke lesions, mostly on a later period after ischemia. In fact, IFN-γ deficient mice develop much smaller lesions than control animals. Although this IFN-γ may derive from different sources, it is accepted that Th1 cells play a relevant role in this phenomenon. Moreover, T CD4 deficient animals are much more resistant to ischemic injury and this may corroborate the role for these lymphocytes, as recently reviewed (Brait VH 2012). This only contributes to the hypothesis that T cells may have an important role in the ischemic brain lesions.

IL-17 is the hallmark of the T CD4 lymphocytes with Th17 phenotype (Ivanov I 2006; Iwakura Y 2006). It is now accepted that many other cell types may also secrete this cytokine, as microglia and astrocytes (Kawanokuchi J 2008), alveolar macrophages (Song C 2008), and many others. Several important roles for the biological function of IL-17 have been evidenced during neuroinflammation. For instance, it was demonstrated that human BBB endothelial cells express IL-17R constitutively. Thus, bioactive IL-17 facilitates the disruption of the BBB reducing the tight cellular interaction of endothelial cells through

GAP junctions (Kebir H 2007). Moreover, IL-17 is also considered a potent neutrophil recruiting factor, increasing cellular infiltrate. To further corroborate the importance of IL-17 on the pathogenesis of stroke, it was interestingly demonstrated that IL-17 deficient animals have smaller brain focal lesions when compared to wild-type controls. More interesting, using conditional deletion approaches, it was well established that this IL-17 was secreted by T γδ lymphocytes. The abrogation of IL-17 secretion only in this population resulted in an increased resistance in developing lesions. It is interesting to mention that these observations were performed on day seven after MCAO (Shichita T 2010). Thus, these observations pointed not only to the importance of the IL-17 cytokine but also to the pivotal role played by T γδ lymphocytes. It is worthy to mention that myelin sheets are lipid-rich structures and T γδ cells recognize lipidic antigens.

In summary, the importance of CNS resident cells during neuroinflammatory processess is out of question. The primary secretion of inflammatory factors that follows necrotic cell death is the first trigger for local inflammation. This greatly increases endothelial permeability resulting in the recruitment of inflammatory cells from the periphery. These cells will physically interact with CNS resident cells, and thus, perpetuating the process. It is interesting to mention that some anti-inflammatory factors may also be detected. However, the knowledge about its regulatory functions is just starting, at least after ischemic brain injury.

1.2. Central nervous system resident cells and MS/EAE

Most autoimmune diseases result from the peripheral activation of T CD4 cells against endogenous peptides, as is the case for arthritis, Systemic Lupus Erithematosus (SLE), myastenia gravis (MG), ankilosing spondilytis (AS), Vogt-Kainag-Harada Syndrome (VKH) and multiple sclerosis (MS). It is well accepted that in MS such antigens are myelin-derived epitopes. After activation in the periphery, these T cells migrate to the target organ where they need to be reactivated on a second hit phenomenon. Through this phenomenon a new set of genes are transcribed and translated. For instance, pro-inflammatory cytokines, chemokines, and T cell-activating membrane receptors are promptly up-regulated. MHC I and II, CD80, CD86, CD40, TLR-2, and many cytokines receptors, as IFN-γR and IL-17R are up-regulated in microglia after CNS infiltration of encephalitogenic T CD4 cells. In this concern, which population of CNS resident cells is indispensable for this in situ interaction with infiltrating T cells is still a matter of intense debate, and microglia and resident dendritic cells (DCs) are the main candidates.

Multiple sclerosis (MS) and its murine model, EAE, are characterized by an autoimmune response against myelin-derived epitopes, which culminates in inflammatory infiltrate, gliosis, damage of the myelin sheath and also neuronal death (Neumann 2003; Rebenko-Moll NM 2006; Rodriguez 2007). Many studies have focused on the correlation between different cell types infiltrating the CNS during EAE and the clinical features of the disease. For instance, it has been shown that T CD4+ (Kroenke MA 2007) and CD8+ (Goverman J 2005) cells, as well as macrophages and microglial cells are involved in EAE pathogenesis (Weiner 2008). It is worthy to remember that, as discussed in the introduction, MS pathogenesis is orchestrated by both Th1 and Th17 encephalitogenic cells.

The discovery of DCs by Ralph Steinman was considered a huge breakthrough in the immunology field, rendering Steinman with a Nobel Prize in 2011. Since then, DCs are widely studied and used in many different fields, and it is now accepted as CNS resident cells, as bDCs. In the search for resident DCs in the brain, the use of CD11c –GFP animals was very useful. It was clearly demonstrated that GFP+ cells were find in brain parenchyma of naïve animals, and thus called brain dendric cells or bDCs. More interesting, it was shown that bDCs were found in the circunventricular organs, whose characteristics are not to be isolated by the BBB. Thus, it seems clear that bDCs may have an important role in brain surveillance. Moreover, and corroborating this idea, bDCs have migrating abilities, as they route to lesions of kainic acid-induced neuronal death. Concerning bDCs, is established that they are important players in the second-hit of brain and spinal cord infiltrating T CD4 cells. Using the murine model for EAE, it was demonstrated that bDCs not only present CNS-derived antigens to T cells, but they also input both Th1 and Th17 phenotype to naïve T CD4 cells (Felger JC 2010). It is interesting to remember that, as discussed in the introduction, both populations play a very relevant role in MS pathogenesis. Corroborating this idea, another research has shown that CD14 infiltrating monocytes acquire the CD83+CD209+ dendritic cell phenotype with a significant capacity to prime T cells after passing the BBB. These cells were able to secrete IL-12p70, IL-6 and TGF-β and thus generating Th1 and Th17 cells .

Although questioned by some researchers, it is believed that under resting conditions microglial cells do not express significant levels MHC I and II molecules, impairing its ability to activate T cells. This, greatly differ from resident dendritic cells, what points out for a most likely role for bDCs in the activation of infiltrating T cells than microglial cells. However, further presentation of myelin-derived epitopes by microglia to T cells triggers T cells to release a great deal of pro-inflammatory cytokines, as IFN-γ and TNF-α for Th1, and IL-17, IL-22 and GM-CSF for Th17 cells, establishing focal inflammation (Murphy AC 2010; Almolda B 2011). Microglia also secrete significant amounts of IL-23, as observed from human samples. Moreover, besides Th17 cells, both microglia and astrocytes are able to secrete IL-17 (Kawanokuchi J 2008). It has also been established that these populations also constitutively express IL-17R. It is interesting to mention that in vitro activation of astrocytes and microglial with rIL-17 resulted in interesting outcomes, such as the up-regulation of MCP-1, MCP-5, MIP-2 and KC. Thus, it is unquestionable that IL-17 greatly contributes fort the amplification of the inflammatory infiltrate, and its source may be resident cells as well as Th17 infiltrating lymphocytes.

Interestingly, using a microglial paralysis approach, it was clearly shown that functional microglia are fully needed for the establishment of the CNS inflammation. CD11b-HSVTK transgenic mice, which express herpes simplex thymidine kinase in macrophages and microglia after ganciclovir treatment impairs microglial activation, as observed by the interruption on the release of nitrite, proinflammatory cytokines and chemokines. As a consequence, mice are more resistant to EAE induction, with reduced CNS lesions, myelin degradation, cellular infiltration and cytokine release (Heppner HL 2005).

Due to its resemblance with macrophages, it is conceivable that microglia expression all inflammasome associated molecules, which in fact happens to be true. Microglia secrete not

only IL-1β, but also IL-18 and IL-33, all of them caspase-1 derived cytokines. As discussed in the stroke session, IL-1 β has a very important role in the initiation of the inflammatory infiltrate. NOD (Nucleotide-binding domain, Leucine-rich Repeat containing family) like receptors (NLRs) have also been reported as important DAMP receptors in the CNS, being easily found in microglia (Chakraborty S 2010). Altogether, inflammasome-derived cytokines as well as NLRs and TLRs activation greatly contributes for local inflammation and thus influencing overall outcome.

Although it was known that IL-17 had a very important role in the pathogenesis of MS and EAE, either from resident cells or from Th17 cells, it was not well established the target of IL-17. Thus, it was elegantly demonstrated that IL-17-derived action is mostly played by astrocytes. Using conditional deletion of the Act-1 gene, that encodes an adaptor molecule of the IL-17R, it was clearly demonstrated that astrocytes change into a much activated state after engagement with IL-17. It is interesting to mention that IL-17 induced the expression of CXCL1, CXCL2, CXCL9, CXCL-12, IL-6, MMP-3 and MMP-9 in vitro and in vivo. Thus, NesCre / Act-1flox/flox mice are very resistant to EAE induction, with reduced CD4, CD19 and monocytes infiltration of the CNS. More interesting, Act-1 abrogation from microglia/macrophage or endothelial cells did not show any difference (Kang Z 2010) .This points out not only the main target of IL-17 activity, but also the importance of the CNS resident cells as astrocytes in the pathogenesis of EAE. Moreover, using astrocytes cultures, researchers demonstrated that these cells are able to induce Th1 and Th17 cells (Li Y 2007).

In fact, neuroglia cells, as astrocytes, microglia constitutively express many cytokines and chemokines receptors, such as IL-1R, IL-4R, IL-10R, IL-12R, TNFR1, TNFR2, GM-CSFR, IFN-γR, IL-17RA, CCR2, CCR5, TLR-2, TLR-4 among many others (Sedjwick 1991; Lin HW 2009). The engagement of these cytokines with its receptors triggers a cascade of events resulting in the expression of molecules such as, MHC I and II, iNOS, CD45, CD80, CD86, IL-1, IL-6, MMP-2, MMP-9, CCL2, among many others. Thus, the direct interaction of infiltrating encephalitogenic Th1 and Th17 cells with CNS resident cells, as microglia and astrocytes, is of great importance for the understanding of the pathogenesis of MS and also other neuroinflammatory diseases.

At last but not least, it is interesting to mention the role of oligodendrocytes during MS. In fact, oligodendrocytes are the main targets of peripherally activated T CD8 cells, whose antigen recognition induces oligodendendrocyte death by apoptosis, and thus demyelination, the hallmark of MS and EAE (Neumann 2003). Oligodendrocytes may be killed by several distinct mechanisms, such as FasL – Fas interaction, granzymes from cytotoxic T CD8 cells and also high amounts of TNF-α (Neumann 2003). More recently it has also been demonstrated that during EAE, the neuroinflammatory process significantly reduces the amount of Connexins (Cx), such as Cx32, Cx43 and Cx47. This reduction is associated with a disturbance in the gap junctions of oligodendrocytes and altered ionic balance, inducing cell death (Markoullis K 2012). In this context, besides the aforementioned ways to induce demyelination, intercellular interaction among glial cells also seem to have a pivotal relevance. The integrity of the myelin sheet is mandatory for a well functioning CNS. However, this integrity is breached during MS, damaging and destroying neuronal ability to transmit information, leading to a severe overall impairment.

2. Conclusions

The term glia, stands for glue, referring to the architectural role of these populations, such as astrocytes and microglial cells. Nowadays however, much new information was obtained concerning their biology, not only at resting state but also during neuroinflammatory processes. In this concern, microglia and astrocytes had gained more and more attention in the neuroimmunology field, specially due to its great capacity to generate important inflammatory factors, and also to directly interact with infiltrating T cells or monocytes. This results in the amplification of neuroinflammatory process and establishing the lesions themselves. In fact, it has been recently proposed a shift from T cell to innate immune dependence during the chronic phase of multiple sclerosis. Overall, data pointed out to microglial cells and astrocytes as master players during neuroinflammation, and therapeutic approaches focusing on reducing it activation status may greatly contribute to the treatment of neurological diseases, such as MS and stroke.

	Relevance in Stroke	**Relevance in EAE / MS**
Microglia	Express several receptors as IL-1R, IL-4R, IL-10R, IL-12R, TNFR1, TNFR2, GM-CSFR, IFN-γR, IL-17RA, CCR2 and CCR5.	Express several receptors as IL-1R, IL-4R, IL-10R, IL-12R, TNFR1, TNFR2, GM-CSFR, IFN-γR, IL-17RA, CCR2 and CCR5.
	Migrate to the penumbra caping dying neurons to reduce inflammation.	After activation up-regulates MHC I and II, CD40, CD45, CD80 and CD86 to activate infiltrating T cells.
	Hv1 expression correlates with NADPH oxidase activity and neuronal injury.	Secrete MCP-1, MCP-5, MIP-2 and KC after IL-17 activation.
	Secrete IL-1, IL-6, IL-12, IL-17, IL-23, TNF-α.	Secrete IL-1, IL-6, IL-12, IL-17, IL-23, TNF-α.
Astrocytes	Transglutaminase expression up-regulates cell death.	Undergo intense proliferation orchestrated by CD36
	Secrete IL-6, IL-12, TNF-α, MMP-3 and MMP-9.	After activation by IL-17 secrete high amounts of CXCL1, CXCL2, CXCL9, CXCL-12, IL-6, MMP-3, MMP-9, MCP-1, MCP-5, MIP-2
bDCs	bDCs migrate to the penumbra whereas peripherally recruited DCs migrate to the core.	Activate infiltrating T cells.
	May be involved in the activation of Tγδ lymphocytes.	Induce both Th1 and Th17 cells.

Table 2. Features highlights the importance of separate features in stroke and EAE/MS.

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