Dendritic cells in brain diseases

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ABSTRACT

Dendritic cells (DCs) are professional antigen presenting cells that constantly survey the environment acting as sentinels of the immune system, including in the CNS. DCs are strategically located near the cerebrospinal fluid, but they can potentially migrate to draining cervical lymph nodes either triggering immunogenic T cell responses or displaying tolerogenic functions. Under physiological conditions, the presence of DCs in the brain parenchyma is minimal but their numbers increase in neuroinflammation. Although DCs belong to a distinct immune cell lineage, they show various phenotypes and share certain common markers with monocytes, macrophages, and microglia. All these cells can express major histocompatibility complex class II, and acquire similar morphologies hampering their precise identification. Neuroinflammation is increasingly recognized in many brain disorders; here we review the literature reporting DCs in the inflamed brain in disease conditions and corresponding animal models of multiple sclerosis, stroke, brain tumors, Alzheimer's disease, Parkinson's disease, and epilepsy.

Abbreviation list:

Aβ	amyloid-β
ABRA	amyloid-β-related angiitis
AD	Alzheimer's disease
APC	antigen presenting cell
APP	amyloid precursor protein
Arg-1	arginase-1
B7-H1	human B7 homolog 1 (PD-L1)
Batf3	basic leucine zipper transcriptional factor ATF-like 3
BBB	blood-brain barrier
BDCA	blood dendritic cell antigen
BDCA-1	CD1c
BDCA-2	CD303
BDCA-3	CD141, thrombomodulin
BDCA-4	CD304, Neuropilin-1
BMDC	bone marrow-derived DC
C1q	1st C1 complex subcomponent of classical complement activation pathway
CAA	cerebral amyloid angiopathy
CCL	chemokine (C-C Motif) ligand
CCR	chemokine (C-C Motif) receptor
CD	cluster of differentiation
cDC	conventional DC
CNS	central nervous system
COX2	cyclooxygenase-2
CSF	cerebrospinal fluid
CX_3CR1	chemokine (C-X3-C motif) receptor 1
DAP12	DNAX activation protein of 12 kDa
DC	dendritic cell
DEC-205	CD205, Ly75
DNGR1	C-type lectin receptor marker of DC lineage (also known as CLEC9A)
EAE	experimental autoimmune encephalomyelitis
ERK	extracellular signal-regulated kinases
EYFP	enhanced yellow fluorescent protein
F4/80	EGF-like module-containing mucin-like hormone receptor-like 1
Fc	immunoglobulin receptors
FCD	focal cortical dysplasia
Flt3	FMS-like tyrosine kinase 3
FPR1	formyl peptide receptor 1
G-CSF	granulocyte-colony stimulating factor
GFP	green fluorescent protein
GM-CSF	granulocyte macrophage colony-stimulating factor
GM1	monosialotetrahexosylganglioside
Gr1	antibody clon RB6-8C5 recognising Ly6G and Ly6C
HLA	human leukocyte antigen
Iba-1	ionized calcium binding adaptor molecule 1
ICOS-L	inducible costimulator ligand
IDO	indoleamine 2,3-dioxygenase
IFN	interferon
IFNAR	type I interferon receptor
IL	interleukin

ILT7	CD85g
infDC	inflammatory DC
iNOS	inducible nitric oxide synthase
Lin^-	lineage-negative
Ly	lymphocyte antigen
MBP	myelin basic protein
MDDC	myeloid-derived dendritic cell
MDSC	myeloid-derived suppressor cell
MHC	major histocompatibility complex
MMP	matrix metalloproteinase
MOG	myelin oligodendrocyte glycoprotein
MS	multiple sclerosis
mTOR	mammalian target of rapamycin
NLRP3	NACHT, LRR and PYD domains-containing protein 3
PD	Parkinson's disease
PD-L1	programmed cell death-ligand 1
pDC	plasmacytoid DC
PGE2	prostaglandin E2
PI3K	phosphatidylinositol 3 kinase
RAGE	receptor for advanced glycation endproducts
RIG-I	retinoic acid-inducible gene 1
rtPA	recombinant tissue plasminogen activator
SCA1	stem cells antigen-1
SIRP1α	signal regulatory protein α, CD172A
TGF	transforming growth factor
Th	T helper
Tim-1	T cell immunoglobulin and mucin domain
TLR	toll-like receptor
TNF	tumor necrosis factor
Treg	regulatory T cell
TREM2	triggering receptor expressed on myeloid cells 2
VEGF	vascular endothelial growth factor
XCR1	chemokine (C Motif) Receptor 1

1. Introduction

Neuroinflammation is a common denominator in many neurological and even psychiatric diseases. Neuroinflammation is a reaction to a disturbed environment that under physiological conditions can take place at very low levels, designed to restore balance in the central nervous system (CNS). Neuroinflammation in disease conditions can be a strong reaction in response to neuronal cell damage or neuronal death, infection, acute brain damage – as in trauma or stroke – accumulation of toxic products, autoimmune responses, tumors, genetic conditions, vascular dysfunction, altered function of neuronal networks and neurotransmitter systems, stress or imbalance in the autonomic nervous system, and possibly alterations in the communication between the brain and the immune system. But to what extent neuroinflammation drives disease onset and progression, or contributes to repair and regeneration is not well understood. Neuroinflammation is a global process that often encompasses the brain and involves peripheral responses with cellular players either resident in the brain or traveling from the periphery, or even acting from the periphery. Many of these cell players interact either locally or from a distance through signaling molecules and nerve wire connections. Therefore, it is likely that studying one cell type will only provide a partial view of the whole process. Keeping this in mind, we set this work to revise some of the current knowledge on the participation of dendritic cells (DCs) in various neuroinflammatory conditions. DCs are the archetypal antigen presenting cells that sense foreign molecules, or access self-proteins abnormally present in the milieu, and present them to T cells to either mount an immune response or induce tolerance. These cells are reported in various brain diseases but their actual role is largely unknown. We aimed to take into account the literature from the immunology and neuroscience fields that provide diverse approaches and sometimes lead to confusing designations of cell types and functions, adding complexity to the difficult task of cell type identification and functional characterization. In the context of neuroinflammatory conditions, we will address the literature about similarities, differences and overlaps between DCs (understood as a unique bone marrow cell lineage), monocyte-derived DCs (MDDCs), inflammatory dendritic cells (infDCs), and reactive microglia. None (or very few) of these cellular phenotypes occur in the brain parenchyma under physiological conditions, where resident microglia display different phenotypes and peripheral immune cells tend to keep away from the brain parenchyma exerting immunosurveillance functions from the choroid plexus, meninges, and perivascular spaces.

1.1 Antigen-Presenting cells

The adaptive immune response is based on T lymphocyte recognition of antigens that are presented by specialized cells called antigen-presenting cells (APCs). These cells display small peptides derived from processed antigens bound to major histocompatibility complex (MHC) class I and II molecules. Presentation though MHC I and MHC II depends on the intracellular antigen degradation pathway [1]. Antigens derived from exogenous proteins are processed by lysosomal enzymes of the endocytic pathway and are presented by MHC II. In contrast, presentation through MHC I molecules relies on cytosolic antigen processing in the endoplasmic reticulum which normally involves endogenous molecules, with the exception of a specialized phenomenon called 'cross-presentation' that requires the translocation of exogenous proteins from the lysosomal compartment to the cytosol [2]. Naïve T cells that recognize peptides bound to MHC molecules can become effector T cells. Mechanisms of central [3] and peripheral tolerance [4] ensure the elimination of anti-self-reactive T cells. Immature APCs presenting self-antigen to T cells in the lymph nodes induce T cell anergy, death, or regulatory T cells (Treg) ensuring tolerance to self [5]. However, the repertoire of peptides presented by MHC molecules in non-lymphoid peripheral organs may exceed that in the lymphoid organs [6] and might contribute to the initiation and maintenance of autoimmune conditions [7]. The context of the interaction between T cells and APCs determines priming or tolerization of naïve T cells [8]. Therefore APCs play a crucial role in the mechanisms of tolerance, and the properties of these cells and their local environment are determinant to induce tolerance or immunity.

1.2 Types of APCs

Cells capable of upregulating MHC II expression and antigen presentation include DCs [9], macrophages, monocytes, and in the brain, also microglia [10]. Furthermore, it is now becoming apparent that under certain circumstances some non-APC cells can acquire antigen-MHC I or MHC II complexes from neighbouring cells through either a process of cell–cell contact-dependent membrane transfer called trogocytosis, or by transfer of these complexes after secretion of membrane vesicles such as exosomes [11]. Here we will address the main types of APCs focusing on the DCs due to their superior ability, compared to other APCs, to sense, process and present antigen, migrate to lymph nodes, and prime

naïve T cells [12]. A summary of the main DC types and DC-related cells is shown in Table 1 and schematically represented in Fig. 1.

1.2.1 Classical Dendritic cells

DCs are bone marrow derived cells playing a major role in immunosurveillance for their ability to sample the environment, detect the presence of antigens and induce T cell responses. Most DCs belong to the 'conventional or classical' type and are called cDCs. To accomplish the role of sampling the environment, cDCs are strategically located in the different peripheral organs where they reside and acquire tissue-specific characteristics. Key features of tissue cDCs are migration from peripheral tissues to regional lymph nodes [13], maturation and T cell stimulation [14]. The typical example is the Langerhans cells located in the interstitial spaces of the epidermis, bronchi and mucosae that traffic from the tissue to the draining lymph nodes to present antigen to T cells [15]. Peripheral cDCs enter the lymphatic endothelium and migrate to the draining lymph nodes via afferent lymphatics [16], with the aid of chemokine/chemokine receptor signalling, involving, amongst others, molecules such as CCR7, CCL19 and CCL21 [17]. Consequently, there are two main types of cDCs in the lymph nodes with distinct functions, i.e. the resident cDCs and the migratory tissue-derived cDCs [18]. cDC maturation is required to upregulate MHC II and co-stimulatory molecules. These populations are composed of phenotypically heterogeneous cells and different subsets of resident and migratory cDCs with specific features are defined by the expression of certain markers, e.g. CD8 for lymphoid resident cDCs, CD103 and CD11b for migratory cDCs, which are hallmarks of their functional specialization. A common marker of cDCs is CD11c, but as we will see later, the expression of this molecule is not exclusive of this cell type [19]. The rich assortment of DC phenotypes and functions complicates the study of these cells. For extensive information the reader is referred to specialized reviews addressing this topic in detail [14]. Notably, many cDC markers differ between humans and rodents, confounding the translation of experimental animal studies to the human biology [20, 21]. This is particularly relevant for lineage tracing since the current knowledge of DC ontogeny and differentiation from bone marrow precursor cells mostly derives from studies in mice. Although the ontogeny of cDCs is, to some extent, still a matter of debate, developmental precursors that have recently been identified strongly support that DCs are a distinct immune cell lineage [19, 22]

1.2.2 Plasmacytoid dendritic cells

A rare subset of DCs called plasmacytoid dendritic cells (pDCs) is found in the blood and the lymph nodes. These cells have crucial functions in the activation of B cells and generation of plasma cells in response to viral infections [23]. pDCs do not express CD11c but express other characteristic markers [24]. Upon stimulation, pDCs can migrate to the lymph nodes [25] and are known for the ability to produce large amounts of IFNs in response to viral infections [26]. However, it has also been shown that maturing pDCs selectively upregulate the expression of inducible costimulator ligand (ICOS-L) and can induce the differentiation of naive CD4 T cells to Treg cells [27]. Therefore, pDCs can either induce immunogenic T cell responses or show tolerogenic functions by inducing $CD8^+$ T cell deletion, $CD4^+$ T cell anergy, and Treg differentiation. For detailed information on pDCs and pDC functions, the reader is referred to specialized reviews on this topic [28, 29].

1.2.3 Other non-classical DC-like cells

Blood monocytes can be differentiated *in vitro* to DC-like cells in the presence of cytokines such as granulocyte–macrophage colony-stimulating factor and interleukin-4 [30, 31]. *In vivo*, monocytes can also develop into a DC-like population [32] and these cells are called MDDCs. These phenotypic changes in the monocyte population *in vivo* have been mainly identified under inflammatory conditions [33]. The distinct MDDC subsets *de novo* generated under inflammatory conditions (see below) are termed infDCs, with a potential role in inflammatory diseases [18]. Populations of monocytes producing high levels of TNF-α and inducible nitric oxide synthase (iNOS) found under pathological inflammatory conditions have been called Tip-DCs. However, these cells do not seem to play an intrinsic DC function since they are dispensable for T cell priming and their main role is innate immune defense [34]. Microglial cells react to brain damage and inflammation [35] and acquire MHC II expression but have a poor capacity to activate naïve or effector T cells [36] and their putative function will be discussed in the next sections in relation to various brain diseases.

1.3 Dendritic cells in the healthy brain

Dendritic cells were identified long ago in the meninges and the choroid plexus of rodents [37] and humans [38] as cells expressing MHC II and HLA-DR, respectively. More recently, the availability of transgenic mice expressing a fluorescent protein under the control of

CD11c [39] has allowed great progress in the field providing very valuable information about the presence of $CD11c^+$ cells in the brain under physiological and pathological conditions. Nevertheless, it is important to note that the expression of CD11c is not limited to cDCs, since other cells like monocytes [40], and microglia can express or upregulate CD11c expression under certain circumstances, particularly in inflammatory conditions [41]. Using these fluorescent reporter CD11c mice, Bulloch et al. [42] first described in an elegant study the presence of $CD11c^+$ cells in specific regions of the developing and adult mouse brain. Notably, the localization of $CD11c^+$ cells in these transgenic mice is in agreement with an immunosurveillance function in the brain [42, 43]. $CD11c^+$ cells are found in the control brain parenchyma in regions in contact with the CSF, such as along the ventricles and choroid plexus, and within the circumventricular organs, in neurogenic zones, such as the granule cell layer of the hippocampus and the rostromigratory path, as well as along nerve fibre tracts and in layer II of the piriform cortex [42]. $CD11c^+$ cells found in the meninges and choroid plexus that express the lineage marker DNGR1 [44] respond to the FMS-like receptor tyrosine kinase 3 (Flt3) ligand and are distinct from microglia [44]. Furthermore, $CD11c^+$ cells were identified in the brain parenchyma surrounding the basal laminae of blood vessels in a juxtavascular location rather than in the perivascular space, and $CD209^+$ cells were also identified in this location in the postmortem human brain [43]. Brain $CD11c^+$ cells display various morphologies, ranging from very stellate cells (the most frequent type) to bipolar and ovoid cells, suggesting various subtypes of DCs, and they express the typical microglia marker Iba-1 [42]. Interestingly, it has been reported that only $CD11c^+$ cells present in the brain parenchyma are $Iba1^+$, while those in the choroid plexus are not [43]. Therefore, it cannot be excluded that the $CD11c^+$ cells found in the brain under physiological conditions were derived from microglia [45]. In support of this possibility, Prodinger et al [43] showed increased numbers of $CD11c^+$ cells in organotypic hippocampal cultures.

1.4 DCs versus microglia

Microglial cells are the brain resident macrophages [46] and derive from primitive erythro-myeloid precursors in the yolk sac different from bone marrow precursor myeloid cells [47, 48]. Microglia are recognized as a heterogeneous population of cells that can undergo different states of activation and acquire various functions in response to the various pathological conditions [49, 50]. Microglial cells can upregulate CD11c expression [41] and, upon *in vitro* exposure to granulocyte macrophage colony-stimulating factor (GM-CSF),

resting microglia obtained from normal adult brain generated immature DC and potent allostimulatory $CD11c^+$ cells [51]. Therefore microglial cells are able to acquire some DC functions under certain conditions. However, the question of whether cells of the DC lineage access the brain parenchyma under disease conditions and play a differential role than activated microglia in disease pathogenesis and progression in the various CNS inflammatory diseases is not fully resolved. Nevertheless, we will see below that some distinct functions are recognized for these cells in certain neuroinflammatory conditions. Because of brain residence, microglia are the first to sense local alterations in the brain environment and their reaction can sometimes require further cooperation of DCs that need to traffic to the brain from the periphery. The possibility that brain DCs might differentiate from myeloid progenitors of the choroid plexuses has also been suggested [52]. Possibly the best distinction between DCs and activated resident tissue microglia would be the ability to migrate from the brain tissue to the draining lymph nodes since this migration is considered a property of DCs. After injection of MDDCs into the ventricles of normal rats, Hatterer et al. [53] detected the presence of the injected cells in the cervical lymph nodes, in contrast to injection of cells in the parenchyma that resulted in no significant cell migration. However, a previous study showed that, after intracerebral injection, bone marrow-derived DCs were able to reach the cervical lymph nodes [54]. Different routes for possible migration of immune cells from the brain to the cervical lymph nodes have been proposed (for review, see [55]). Very recent works have challenged the classic concept that the brain lacks lymphatics by the identification of lymphatic-like vessels in the meninges of mice [56]. CD11c+ cells were detected inside these lymphatic brain vessels suggesting that these cells, probably DCs, could exit the brain and reach deep cervical lymph nodes [56]. Further investigation is needed to find out whether brain $CD11c^+$ cells migrate to the draining lymph nodes and the relevant pathways in the different pathological situations.

1.5 DCs and inflammation

Inflammatory stimuli promote the migration and maturation of tissue-derived cDCs to the draining lymph nodes [13]. Also, infDCs accumulate in the inflamed tissues where they can locally present antigens to effector T cells [57]. infDCs seem to play a prominent role in T cell immunity under inflammatory conditions, and are recognized as essential to mount a response against pathogens [33, 58]. infDCs have been described in conditions like experimental autoimmune encephalitis (EAE) [59]. However, the studies of DCs under

pathological conditions do not often distinguish the origin of the DCs and it is frequently unknown whether they correspond to cDCs or to infDCs. Moreover, the distinction between infDCs, cDCs, and macrophages in the inflamed tissues is not trivial since these cells can share common markers [18]. The situation is particularly complex in the brain where tissue resident microglia is an heterogeneous population of cells [49, 50] that can acquire some features of DCs under inflammatory conditions [41, 43] typically expression of CD11c and MHC II. However, $CD11c^+$ cells found in the brain from EAE and toxoplasmic encephalitis mice have been characterized as distinct from microglia while rather resembling bone marrow-derived DC, but potentially some subsets of microglial cells also differentiate to $CD11c^+$ cells differentiate from the resident microglia cannot be excluded [51]. Although microglial cells are normally distinguished from blood-borne cells by their low or intermediate expression levels of CD45, the possibility that the different cells change their level of CD45 expression under certain inflammatory conditions cannot be fully excluded [51]. Furthermore, CSF-circulating DCs are not only able to survey the inflamed brain, but can also reach the cervical lymph nodes, as reported in a model of EAE [60]. Neuroinflammation certainly increases the presence of DC-like cells in the brain and provides a rich assortment of cellular phenotypes that, regardless of the origin, can present antigen and play crucial functions in T cell activation. Previous reviews have extensively addressed the role of DCs in the inflamed brain [61] and have compared DCs with microglia and macrophages in brain inflammation [62]. In the following sections we will summarize some of the current knowledge on the role of DCs in neuropathological inflammatory conditions.

2. DCs in Multiple Sclerosis and Experimental Autoimmune Encephalitis (EAE)

Multiple sclerosis (MS) is an inflammatory CNS disease characterized by primary demyelination of axonal tracks causing progressive paralysis and neurodegeneration. There are several forms of MS, the most prevalent being relapsing-remitting MS showing episodic worsening of neurological symptoms [63]. Neuropathological features include perivascular T cell and mononuclear cell infiltration in the CNS [64]. MS is thought to be an autoimmune disease after the observations in MS patients of T cells autoreactive to myelin-related proteins, such as myelin basic protein (MBP) (e.g.[65]) and myelin-oligodendrocyte glycoprotein (MOG) (e.g. [66]). Pro-inflammatory $CD4^+$ Th1 cells are viewed as important players in MS pathogenesis ([67]), and Th17 cells are expanded in MS patients [68].

However, besides $CD4^+$ T cell subsets, B cells, $CD8^+$ T cells, microglia and macrophages are increasingly regarded as relevant in the immunopathogenesis of MS [69]. DCs have important roles in T cell priming and polarization that sustain brain inflammation in MS [70].

2.1 Involvement of DCs in EAE

EAE is an animal model of multiple sclerosis where auto-reactive encephalitogenic T helper (Th) cells are causative of the onset of the disease [71]. EAE development requires antigen presentation by MHC II [72]. Presentation of myelin antigen by DCs to autoreactive T cells can lead to T cell expansion and polarization to encephalitogenic Th1 or Th17 effector cells [36]. Subsequent encounter with the antigen in the context of MHC II provides a restimulation signal to the T cells that can trigger the development of EAE [70]. DCs in the brain are very efficient in priming and polarizing T cells in EAE caused by a myelin-associated peptide antigen [57], and maintaining Th17 cell differentiation [73]. Despite all the studies showing the encephalitogenic activities of DCs, DCs do not seem to be sufficient or indispensable to induce EAE. After DC ablation, T cell priming could occur despite the absence of DCs [74]. In fact, mice lacking DCs developed aggravated disease compared to control mice, showing that a reduction of DCs interferes with tolerance, resulting in a stronger inflammatory response and suggesting that other types of APCs compensate for the loss of the immunogenic function of DCs in experimental MS models [75].

pDCs are a major infiltrating DC population in EAE that seem to play an important pathogenic role [76]. pDC ablation inhibited autoimmunity mediated by the expansion of myeloid-derived suppressor cells (MDSC) [77]. In contrast to their role in inducing Th cell priming, there is strong evidence suggesting that DCs can also reduce T-cell mediated inflammation and promote Treg differentiation. In this direction, pDC depletion increased the production of inflammatory cytokines by T cells and exacerbated EAE [78]. Furthermore, DC-specific expression of a myelin self-antigen protected against the development of EAE and this was associated with Treg cell induction via the expression of inhibitory PD-L1 on DCs [79]. After EAE induction, pDCs are recruited to lymph nodes and establish MHC II-dependent myelin-specific contacts with Th cells. Mice exhibiting a selective abrogation of MHC II expression by pDCs developed exacerbated EAE, showing that the interactions between pDCs and Th cells in secondary lymphoid organs promote the selective expansion of myelin-antigen-specific natural Tregs that dampen the autoimmune T cell response [79]. Therefore, it seems that pDCs might facilitate autoimmunity in the priming phase of EAE

[80], whereas pDCs recruited to the CNS seem to limit pathology by regulating T cell activation and cytokine production [78].

2.2 DCs versus microglia and macrophages in EAE

In EAE microglial cells acquire CD11c expression and APC functions [51, 81]. Furthermore, $CD11c^+$ cells can expand locally through proliferation in the brain at early stages of EAE [51, 82]. Both microglial cells acquiring APC functions and DCs recruited from the periphery play a role in EAE (reviewed in [83]). The important role of microglia in EAE was identified in mice deficient in microglia showing delayed EAE onset and reduced clinical severity [57]. Microglial phenotypic changes and myelin-antigen presentation occur early after EAE induction, and are followed by delayed infiltration of DCs [84, 85]. The rapid microglial activation is associated to alterations of blood-brain barrier (BBB) permeability during the asymptomatic phase of EAE induction [85]. Interestingly, the constitutive presence of myelin antigen was identified in a small number of microglial cells in the control mouse brain and it increased after EAE, suggesting that these cells might be the first to be encountered by encephalitogenic T cells [84]. In contrast, the infiltrated DCs did not carry myelin antigen in the initial stages but they progressively acquired it, coinciding with the onset of EAE symptoms [84]. Using the CD11c-GFP transgenic mice, the $CD11c^+$ cells were first observed in the meninges and later in the parenchyma suggesting infiltration of $CD11c^+$ cells from the periphery [85]. Although in this study the distribution of $CD11c^+$ cells was more limited and different than that of microglial cells, which were identified by the expression of CX_3CR1 using the $CX_3CR1^{-/GFP}$ reporter mice, upregulation of CD11c in a subset of microglia cannot be excluded. Therefore, both resident activated microglia and peripheral DCs might present myelin antigen in EAE, but these cells likely play differential roles whereby the reaction of microglial cells seems to be crucial for the initial local events during the subclinical phase of EAE before peripheral DCs have reached the brain parenchyma (Fig. 2). At later stages of EAE development microglia might exert a down-regulatory role limiting the expansion of autoreactive T cells through NO production, which inhibits T cell proliferation [81]. However, DC recruitment from the periphery is required for the accumulation of autoreactive T cells that will trigger the manifestation of the clinical EAE symptoms [71].

The inflammatory lesions in EAE are rich in macrophages and depletion of macrophages markedly suppresses the clinical signs of EAE [86]. However, the distinction of microglia from macrophages is also very difficult. It cannot be made on a morphological basis, and both

populations express many common cell markers. Strategies of parabiosis [82] and the use of double-heterozygous knock-in mice with distinct fluorescent labels in microglia (CX_3CR1+) and infiltrating monocytes (CCR2+)[87] have been very useful to differentiate these two cellular populations in the inflamed CNS. Using parabiotic mice, Ajami et al. [82] showed that monocyte infiltration was crucial for EAE development and that monocytes did not contribute to the pool of brain microglia. Likewise, using the $CCR2^{rfp/+}$ $CX_3CR1^{gfp/+}$ mice, Yamasaki et al. [88] showed that monocyte-derived macrophages initiate demyelination in EAE whereas phagocytic microglia seemed to play a less prominent function.

Therefore DCs, microglia and macrophages are important local immune cellular players in EAE with distinctive roles, and the relative relevance of these cell types seems to change during the various stages of the disease. Potential differential functions might include a prominent role of DCs in migration to the draining lymph nodes and T cell priming, microglia seem to be required to provide a suitable inflammatory local milieu, while macrophages are involved in demyelination, amongst other functions. For translational purposes, results obtained in EAE will require validation in the human disease.

2.3 DC activation and signaling

The identification of signaling pathways in DCs involved in priming and polarization of Th cells has potential interest for therapeutic purposes. Interferon-β (IFN-β) is widely used to treat MS [89]. Mice with EAE showed elevated levels of IFN-β in the CNS but not the blood [90] and IFN-β deficiency exaggerated the severity of EAE [91]. Also, the genetic blockade of type I IFN receptor (IFNAR) exacerbated EAE, possibly by facilitating Th17 cell effector differentiation [92]. However, the worse clinical signs, higher inflammation, demyelination, and lethality in IFNAR-deficient mice were attributed to lack of this receptor in monocytes, macrophages and microglia, rather than in DCs, and the deficiency did not modify Th polarization [90]. Absence of IFN-β or IFNAR induced a stronger production of proinflammatory molecules in EAE [90, 91] and the beneficial effects of this signaling pathway are related to inhibition of NLRP3 inflammasome activation [93]. However, the engagement of IFNAR on DCs, but not on macrophages or microglia, was required for the suppressive effect of RIG-I-like helicase stimulation on the maintenance and expansion of committed Th1 and Th17 cells in EAE [94].

The T-cell immunoglobulin mucin, Tim-1, which is a receptor for phosphatidylserine, is constitutively expressed on DCs. Its expression further increases after DC maturation, upregulates co-stimulatory molecule expression and proinflammatory cytokine production, and shifts the balance between effector and Treg cells towards an enhanced immune response [95]. Accordingly, an agonistic anti-Tim-1 antibody worsened EAE in susceptible mice and also impaired tolerance and induced EAE in a genetically resistant strain of mice [95]. Also, integrin αvβ8 expression on DCs plays a critical role in the differentiation of Th17 cells by activating TGF-β, which is required for conversion of naive T cells to Th17 cells. Th17 cells were nearly absent in mice lacking αvβ8 expression on DCs, and these mice showed near-complete protection from EAE [96]. However, the role of TGF-β and DCs in EAE is complex because DCs also have a function in establishing the cytokine milieu that is required for T cell polarization, and TGF-β helps to control autoimmunity by reducing the capacity of DCs to prime T cells. Functional inactivation of TGF-β signalling in DCs caused strong CNS inflammation, high frequency of T cells invading the CNS, increased levels of Th1 and Th17 cytokines in the periphery, and lack of EAE remission [97].

DCs need to express the chemokine receptor CCR4 for EAE induction since mice deficient in CCR4 were resistant to developing clinical signs of EAE and showed reduced IL-23 and GM-CSF expression in the CNS, suggesting that CCR4 in DCs could be a target for therapeutic intervention [98].

2.4 DCs in Multiple Sclerosis

Inflammation is strongly prominent in MS [99], and evidence for pro-inflammatory DC activity has been reported, particularly in the secondary progressive phase of the disease [100]. Genetic variations in the MHC II gene are strongly associated to MS susceptibility [101]. MHC and co-stimulatory molecules are expressed on ramified myeloid cells surrounding plaques, and on non-myelin- and myelin-containing myeloid cells within plaques [102]. DCs have been found preferentially in areas of MS lesions such as the periventricular areas, adjacent tracts, and the optic nerve [43]. Clusters of activated HLA-DR$^+$ microglia were frequently found in white matter regions devoid of leukocyte infiltration or apparent neuropathological signs (also called preactive lesions) and not related to alterations of the BBB, suggesting brain intrinsic innate immune alterations [103]. These regions showing microglial activation have been regarded as an early stage of tissue injury

[104]. However, isolation of these microglial cells showed alterations in levels of Fc-γ receptors in MS patients versus controls but they were unresponsive to proinflammatory challenges [105]. It has been suggested that rather than preceding demyelinating lesions, this microglial activation could be secondary to axonal degeneration in regions of active demyelination [106].

Impaired tolerogenic activity could be relevant in the pathogenesis of MS. A decrease of the toll-like receptor (TLR)-7-induced IFN-α secretion by pDCs from MS patients compared to controls could reflect altered immunoregulatory mechanisms in MS [107]. A better knowledge of the role of DCs in patients with MS is crucial, especially for therapeutic purposes, since many drugs that are being tested or already approved for MS treatment may act by modulating DCs. IFN-β is widely used for the treatment of MS [89], and DCs might represent important cellular targets of anti-inflammatory type I IFN signaling both during the natural course of MS and of IFN-β therapy. A novel oral treatment for MS, laquinimod, that is being tested in phase III clinical trials also exerts diverse immunomodulating actions in DCs. Laquinimod down-regulates secretion of pro-inflammatory cytokines and enhances production of anti-inflammatory cytokines from peripheral blood mononuclear cells [108]. In EAE, laquinimod prevented further relapses and strongly reduced infiltration of Th and cytotoxic T cells in the CNS. In MS patients, laquinimod exhibits its disease-modulating activity by downregulating immunogenicity of DC responses. Chemokine and cytokine secretion by DCs was consistently reduced in laquinimod-treated patients with MS. Similarly to the animal model, both cDCs and pDCs were decreased in the blood, and laquinimod treatment modified the maturation of DCs demonstrated by an upregulation of CD86 expression *in vivo* [109]. Following encouraging phase II results that showed reduced inflammatory lesions with laquinimod as compared to placebo [110], the ALLEGRO and BRAVO trials showed reductions in relapse rates with laquinimod [111], and a third phase III trial is currently ongoing trying to confirm the effectiveness of laquinimod in MS. If positive, it will prove the value of immunomodulating agents acting on DCs to treat the human disease.

3. DCs in Stroke

Stroke is a very prevalent disease that causes acute brain damage and it is an important cause of death or permanent disability in the world [112]. The only effective therapy for

ischemic stroke until now has been the recanalization of the occluded artery with recombinant tissue plasminogen activator (rtPA), and more recently mechanical thrombectomy has shown significant benefits for acute ischemic stroke in several clinical trials [113]. However, brain lesions and clinical deficits occur almost always even after successful arterial recanalization. Thus, improving the current treatment of acute stroke will require impeding the tissue and cellular consequences and this could result from a better understanding of the complex interplay between the central nervous system and the immune system. Stroke causes necrotic brain cell death promoting a strong inflammatory response involving the release of danger signals from the injured tissue alerting the immune system [114]. Microglial cells are equipped with danger signal sensors, such as TLRs, and become strongly reactive after stroke releasing inflammatory mediators and chemokines attracting circulating leukocytes [115]. As we will discuss below, the presence of APCs has also been reported in the ischemic brain. The notion that antigen-mediated effects might have some relevance in the functional outcome of stroke [116] comes from experimental studies suggesting that the modulation of antigen-specific responses could protect the brain in stroke [117, 118]. Further knowledge about the role of DCs in stroke is needed to unravel the potential contribution of antigen presentation to the functional outcome of stroke.

3.1 Experimental stroke

The presence of cells with DC features in the brain after induction of experimental stroke has been reported in rats and mice. Kostulas et al. [119] reported the presence of cells expressing MHC II ($OX6^+$) in the ischemic rat brain following permanent middle cerebral artery occlusion (MCAo). MHC II was expressed by DCs ($OX62^+$) normally absent in the brain parenchyma but present in the meninges and choroid plexus. DCs invaded the ischemic core in the first hours after ischemia and progressively increased in number up to 6 days after ischemia, as well as progressively acquiring the expression of IFN-γ. They observed that parenchymal DCs ($OX62^+$) also expressed CD11b ($OX42^+$) suggesting that some microglia cells developed into DCs after stroke [119]. In the study of Reichmann et al. [120] using photochemically induced cortical ischemia in the mouse brain, DCs were detected in the periphery of infarction and also in degenerating corticothalamic fibre tracts and subcortical nuclei where they were seen for several weeks after the induction of stroke. In this study, the $CD11c^+$ cells ($CD11b^+$ $CD8^-$ $CD205^-$) were compatible with infDC; they showed an immature DC phenotype according to the pattern of MHC II and co-stimulatory molecule

expression, and on the basis of high levels of CD45 expression they seemed to be mostly blood-derived cells, at least in the ischemic zone. In contrast, the more ramified DC-like cells in remote degenerating regions were taken as derived from resident microglia.

In the more recent years, Felger et al. [121] showed the progressive accumulation of $CD11c^+$ cells in the ischemic tissue from 24h to 72h after transient MCAo, using the transgenic CD11c-fluorescent reporter mice. By generating radiation chimeras, they could show that peripheral $CD11c^+$ cells acquired an ovoid shape, and preferentially accumulated in the core of infarction. In contrast, DCs derived from brain resident cells were more ramified and were located at the periphery of the lesion, thus suggesting that in spite of a similar morphology, common markers, and expression of MHC II and co-stimulatory molecules, DC-like subsets can have differential functions depending on the cellular origin and regional location [121]. Furthermore, a large increase in $MHCII^+CD11c^+$ ($Lin^-F4/80^-$) cells expressing CD11b, but not CD103, and compatible with infDC, were found in the ischemic brain 6 days after photothrombotic stroke in mice [122]. This latter study also reported the presence of $CD11b^-$ $CD103^+$ cDC in the brain, but their numbers did not change after ischemia [122]. The presence of DCs in the brain after stroke has been documented in several studies reporting increased numbers of DCs 24h after ischemia in mice [123] and rats [124], and three days after ischemia in mice [125], together with increased expression of MHC II [126, 127]. Besides brain ischemia, experimental intracerebral hemorrhage also induces increased numbers of cells with DC features in the brain parenchyma, as detected after 12h [128] and 3 days [129].

3.2 Role of DCs and APCs in the ischemic brain

Certain treatments that were able to attenuate brain damage in experimental stroke reduced the number of DCs or MHC II expression in the ischemic brain. For instance, an anti-inflammatory treatment with a flavonoid called fisetin reduced brain $CD11c^+$ cells after ischemia, and did not modify the number of microglial cells but attenuated their activation status [125]. Interestingly, MHC II^+ microglia/macrophages in the ischemic rat brain were found to express dopamine D1 receptors, and treatment with levodopa/benserazide that promotes dopamine signalling and improves the functional outcome in experimental stroke, was found to reduce the expression of MHC II in the ischemic core and this effect was related to treatment-induced attenuation of delayed white matter fibre tract degeneration [127]. Also, treatment with recombinant T cell receptor ligands targeting myelin-specific T

cells after induction of ischemia reduced the numbers of DCs and was protective, suggesting that myelin antigen presentation and T cell autoreactivity might contribute to brain damage after stroke [130]. Many of these experimental findings thus support that DCs and microglia upregulate MHC II expression and suggest that myelin antigens might be presented to T cells after stroke.

However, one study reported that $CD11c^+$ DCs in the ischemic mouse brain upregulated the production of indoleamine 2,3-dioxygenase (IDO) increasing from 6h to 3 days and this effect was associated with increased Treg mobilization from the bone marrow to the circulation, thus suggesting an immunomodulatory beneficial effect of DCs [131] (Fig. 2). Cortical brain infarction causes secondary thalamic degeneration [132], which is exacerbated by chronic stress [133]. In this latter study, chronic stress was found to attenuate the microglia/macrophage reaction and downregulate the expression of MHC II in the thalamus. Although the nature of the MHC II expressing cells was not identified, it becomes clear that the expression of MHC II is not indicative of the actual role of the cells.

Taken together, these studies showed the presence of DCs in the brain after stroke and identified peripheral sources of brain DCs as well as microglia-derived DC-like cells. However, the functional relevance of these cells remains currently unknown. Nonetheless, beneficial effects of granulocyte-colony stimulating factor (G-CSF) in experimental brain ischemia were attributed, at least in part, to inhibition of DC activation and maturation [134].

3.3 DCs in human stroke

The presence of $HLA\text{-}DR^+$ cells was reported in the post-mortem brain of ischemic and hemorrhagic stroke patients, but the numbers of cDCs ($CD209^+$) and pDCs ($CD123^+$) were considerably lower that the numbers of $HLA\text{-}DR^+$ cells suggesting that resident microglia could acquire antigen presentation capacity after stroke [135]. APCs were seen for several months after stroke in the grey matter and persisted for even longer in the white matter within the degenerating corticospinal tract, where these cells expressed MHC II but not co-stimulatory molecules, suggesting that they may prevent a T cell response [136]. Another study on the accumulation of APCs in the perivascular spaces of the spinal cord of stroke patients suggested that these cells could release myelin products to the CSF [137]. Stroke patients also showed higher numbers of $HLA\text{-}DR^+$ APCs carrying brain antigen in T cell-rich

zones of the draining lymphoid tissue compared to controls, suggesting the possibility that brain antigen could reach the lymphoid tissue after stroke or that brain antigen was carried from the brain to the lymph nodes by APCs [138] (Fig. 2).

In the circulation, monocytes show a reduction of HLA-DR very early after stroke onset contributing to post-stroke immunodepression and predictive of infectious complications [139]. Lower levels of HLA-DR expression are also found in patients with subarachnoid haemorrhage [140], which also show decreased numbers of monocytes with DC features and pDCs in the circulation. Furthermore, the function of these cells, as assessed by TLR stimulation, is impaired [141]. Such peripheral reactions set an environment unfavourable for the development of T cell autoreactivity.

4. DCs and brain tumors

Brain tumors comprise an extended variety of different neoplasms, classified by site and histology in the World Health Organization (WHO) International Classification of Diseases for Oncology (ICD-O-3). Overall malignant CNS tumors represent 1.7% of newly diagnosed cancers and account for 2.1% of cancer deaths worldwide [142]. Glioma is the most represented subtype: in the United States 81% of newly diagnosed malignant CNS cancers belong to this classification. [143]. As a consequence, the majority of studies on the immune reaction to brain tumors refer to this subtype and in this chapter we will limit the analysis to this category.

4.1 Brain tumor-induced alterations in antigen presentation capacity

Tumor cells have the potential to elicit adaptive immune responses by their expression of altered antigens that can be recognized as non-self by APCs [144], but the peculiar immunological condition of the CNS [55] may hamper the generation of an effective immune reaction. Brain parenchymal cells, such as neurons and astrocytes, are the cells most commonly mutated in brain tumors, and have a low basal expression of MHC molecules, limiting their ability to present antigens (reviewed in [145]). Furthermore the malignant transformation of these cells appears to worsen the situation provoking defects in their antigen-presenting machinery [146]. Despite the limiting conditions, it has long been known that together with innate responses, adaptive immune reactions are elicited in the

neoplastic brain with a central, but not exclusive, role apparently played by cytotoxic T lymphocytes as demonstrated both in animal models [147] and humans [148, 149].

Reactive microglia and macrophages, the first innate immune cells that can respond to brain tumors, have been observed in human gliomas [150] and are considered the dominant inflammatory populations in brain tumors [151]. The number of tumor-infiltrating microglia/macrophages positively correlates with malignancy [152], but the role of these cells remains elusive; it is to date debatable whether microglia/macrophages take part in the immune response against the tumors or can lead to immunosuppression and glioma evasion [153]. Having all the necessary molecular machinery, microglia/macrophages could act as APCs but, possibly because of deficits in the expression of CD80, CD86 and CD40 in glioma condition [150], they could instead lead to T-cell anergy, a proposed mechanism for immune evasion by the tumor [154].

Further evidence support a possible detrimental role for these cells, with microglia secreting metalloproteases (MMPs) that degrade the extracellular matrix, possibly enhancing the invasiveness of tumors [155], and proliferating factors such as vascular endothelial growth factor (VEGF) that can contribute to the expansion of tumors [156]. Also the expression on tumor-associated microglia of immunosuppressing factors, such as B7-H1 [157] and Fas ligand [158], could contribute to the limited efficacy of the immune response against glioma cells.

4.2 DCs in brain tumors do they fight against cancer cells or promote immune evasion?

As pointed out in the introduction, DCs represent the professional population of APCs also in the brain. Although not specifically in the brain, the involvement of DCs in generating anti-tumor immunologic reactions was demonstrated long ago (reviewed in [159]). It was then further investigated using Batf3$^{-/-}$ mice, lacking CD8α^{+} cross-presenting DCs: these mice do not develop an effective cytotoxic CD8^{+} T-cell-mediated response against syngeneic fibrosarcomas, ultimately leading to an unhindered growth of the tumor [160]. It was also demonstrated that type I IFN signaling on CD8α^{+} DCs is required for the cross-priming of CD8^{+} T cells specifically reactive to tumor antigens [161]. More recently also pDCs, normally considered immunosuppressive in the cancer environment (reviewed in [162]), were demonstrated to have the potential to aid these tumor rejection mechanisms. When properly activated these cells could contribute to stimulate a Th17 response, ultimately

resulting in an increased cytotoxic anti-tumor immunity [163]. Their role is to date debated, since their number is increased in patients with glioma and may be related to specific symptoms [164].

Either *in situ* in the brain or in the draining cervical lymph nodes, DCs could recognize tumor antigens and orchestrate these T cell-mediated immune mechanisms (Fig. 2), as they do in many other pathological situations of the CNS (reviewed in [165] and discussed in the other sections of this review). The microenvironment that DCs, and immune cells in general, have to face in brain tumor condition is peculiar because of the presence of a strong immunomodulating milieu generated through the expression of many different cytokines directly by tumor cells and by the resident and infiltrating cells that interact with them (extensively reviewed in [166]). Tumor cells can express for example TGF-β [167] and IL-10 [168], both long known for their ability to suppress DC maturation [169, 170] and to exert other complex and interdependent immunoregulatory functions, such as induction of tolerance (reviewed in [171]). Immune evasion is a major factor in glioma development and DCs themselves could participate in these processes. Exposure to PGE_2 from glioma cells overexpressing COX-2 was found to increase the expression of IL-10 by DCs, in turn leading to the induction of a regulatory response in CD4 T cells mediated by IL-10 and TGF-β production, and a reduced stimulation of effector lymphocytes [172]. The recruitment and expansion of regulatory T cells and the inefficient activation of immune cells may indeed play a dominant role in the immune escape by gliomas (extensively reviewed in Rolle et al., 2012).

5. Dendritic cells in neurodegenerative disorders

In recent years the role of the immune response in neurodegenerative disorders has come to the fore in the scientific community because of the increasing aging world population. With an average life expectancy late into the eighth decade the WHO predicts that the prevalence of neurodegenerative disorders, mainly dementia, will increase up to 75.6 million people by 2030 with 7.7 million new cases every year [173]. By 2040 neurodegenerative disorders such as Alzheimer's or Parkinson's disease will become the second leading cause of death after cardiovascular diseases [174].

In most neurological diseases, there is a great need for disease-modifying therapies since no curative therapeutic approaches exist for most of these diseases, especially

neurodegenerative disorders. Both the innate and adaptive immune response are associated with the damage and repair processes in neurodegenerative conditions [175]. The immune response is essential for the CNS development e.g. pruning of dendritic spines of neurons, removal of debris and apoptotic cells, which is essential for normal homeostasis within the CNS. These processes need to be balanced precisely to prevent bystander damage.

5.1 Dendritic cells in Alzheimer disease.

Pathological hallmarks of Alzheimer´s disease (AD) are the non-reversible alterations of brain tissues, such as intracellular deposition of degenerate filaments (neurofibrillary tangles) and extracellular amyloid deposits, called amyloid or senile plaques, associated with synaptic and neuronal loss caused by a progressive withering and dying of brain cells. The amyloid plaques are primarily made of amyloid β (Aβ) peptides resulting from a dysregulated proteolytic cleavage of the ubiquitously expressed amyloid precursor protein (APP), leading to an abnormal accumulation of two Aβ peptide species: $A\beta_{40}$ and $A\beta_{42}$. Neurofibrillary tangles are formed when defective Tau proteins accumulate within a neuron. Due to the degradation of the cytoskeleton and the abnormal aggregation of dissociated Tau protein from microtubules into paired helical filaments, the neuron itself degenerates and connections between the neurons are progressively lost. These cellular and molecular modifications in AD brains are paralleled by chronic neuroinflammatory processes. Accordingly, a consequence of this age-related neurodegenerative illness is a gradually progressive decline in short-term memory, orientation problems and word-finding difficulties. To date, AD is the most common type of dementia (50-80%) and hitherto there is no cure.

The neuroinflammation of AD brains is accompanied by recruitment and/or development of DC-like APCs [176]. The local inflammation response in AD is triggered by abnormal Aβ deposits and mainly orchestrated by resident cells surrounding the senile plaque, such as activated microglia. Until now it is not well known whether blood-derived DCs matured from previously migrated monocytes [177] or DC-like cells (subset of microglia) play the major role in maintaining the neuroinflammatory state of the AD brain [178]. Several venues for the presence of DCs are conceivable: I) Small numbers of DCs identified in the choroid plexus (see above) could directly migrate to the pathological brain tissue. II) Monocyte precursors can differentiate into DCs and, therefore, it is possible that some of the monocytes acquire DC-like properties under the influence of inflammatory stimuli in neurodegenerative

conditions [179]. III) Microglial cells, which can be experimentally driven to express the cell surface DC markers CD11c and CD205 by adding GM-CSF to the cell culture [180], could participate in the antigen processing and presenting. IV) Since the recent description of a lymphatic drainage system in the CNS [56], it is easier to imagine that directed DC trafficking from the systemic immune system could take place. Even though the number of naturally occurring brain-resident DCs is small, in AD they undergo a rapid expansion suggesting that all above-mentioned mechanisms are involved and particularly microglial cells play an important role.

Immune cell migration across the BBB exists at low levels in the healthy physiological conditions and is needed for the immune surveillance of the CNS [181]. A general feature of aging, which is accelerated in AD, is an increase in the BBB permeability. For example Preston [182] and Farrall et al. [183] showed a higher protein leakage from the blood into the CSF. Enhanced migration of monocytes across the human BBB can take place via RAGE and platelet endothelial cell adhesion molecule 1 [184]. This increase in penetrability of the BBB enables the immune system to sense more brain antigens and to elicit local or systemic immune responses. Indeed, blood-derived DCs even get in contact with brain-excreted Aβ peptides at the BBB [185], subsequently mature and migrate to the deep cervical lymph nodes [176] to activate naïve T lymphocytes (Fig. 2). The role in antigen delivery or presentation of the neurovascular unit is further demonstrated in diseases such as cerebral amyloid angiopathy (CAA) or Aβ-related angiitis (ABRA) in which a local inflammatory response is triggered by Aβ in cerebral endothelial cells. The leakiness of the BBB is additionally increased by systemic inflammatory events such as infection or trauma and can enhance the response of pre-activated DC-like cells in the brain and the dysfunction of neurons [186]. Clinically, these phenomena are often observed in demented patients who show a significant cognitive decline, for example after a urinary tract infection.

It is not fully known if DCs cultured *in vitro* show the same behavior or capability as DCs isolated *ex vivo* but due to the absence of genuine brain-derived immune cells, it is accepted as a kind of cellular model to investigate at least the morphology, phenotype, and behavior of DC-like cells in neurodegenerative diseases. These kinds of generated DCs acquire an inflammatory phenotype and a reduced ability to present antigen when differentiated in the presence of the more predominant toxic species $A\beta_{42}$. In experiments, in which MDDCs obtained from AD patients were compared against MDDCs obtained from healthy, age-matched donors, Ciaramella et al. [187] were able to show that these cells have a

comparable antigen internalization ability, but subsequently MDDCs from AD patients showed decreased APC capability. Additionally, a decreased expression of the co-stimulatory CD40 molecule leads to a concomitant impaired ability to induce T cell proliferation. The DCs of AD affected patients also show a consistent increase in the expression of the pro-inflammatory protein ICAM-1, accompanied by increased IL-6 production indicating a general increase in pro-inflammatory cell features. DCs triggered with $A\beta_{42}$ exhibit phenotypic features consistent with DCs at an immature stage, lacking CD14, showing high levels of CD1a and CD11c, moderate expression of CD80 and CD86, and CD40, and additionally low levels of CD83 and high expression of the presentation molecules of class I (HLA-ABC) and class II (HLA-DR) [188]. Furthermore, mature MDDCs were still CD14 negative, maintained the expression of CD1a and CD11c, and up-regulated the co-stimulatory molecules CD80, CD86 and CD38, and interestingly, a reduction of MHC class I and II at the membrane surface was observed. Thus, suggesting that Aβ peptides may escape immune recognition by inhibiting MHC class II surface expression on DCs thereby suppressing their antigen presentation capacity.

Monocytes, microglia, and DCs respond through a variety of receptors such as TLRs, Fc receptors, G protein-linked 7-transmembrane receptors (e.g. FPR1), CD14, and cytokine receptors. To counteract this activation, other receptors need to be upregulated and stimulated. One important member particularly in the case of neurodegenerative diseases is TREM2 (triggering receptor expressed on myeloid cells 2). TREM2 is expressed on macrophages, DCs, osteoclasts, and microglia. In the case of DCs, TREM2 interacts with the adapter molecule DAP12 to initiate a selective activation of the ERK pathway and upregulation of the chemokine receptor CCR7 [189] for an enhanced ability to activate B and T lymphocytes. Controversially, TREM2 is also known to suppress inflammatory responses by repression of microglia-mediated cytokine production and secretion [190] and participate in the regulation of phagocytic pathways that are responsible for the removal of neuronal debris [191]. Recently, a rare variant in TREM2 (R47H substitution) was found which causes susceptibility to late-onset AD [192]. Microglia expressing this TREM2 R47H variant showed a defective activation resulting in reduced clearance of Aβ-plaques due to impaired detection of damage-associated lipid patterns, and TREM2 deficiency in the 5XFAD mouse model of AD exacerbated Aβ accumulation [193]. In contrast, TREM2 deficiency in the APP/PS1 mice ameliorated amyloid and tau pathologies [194]. Therefore, further studies are clearly needed to underscore the role of TREM2 in AD. In the APP/PS1 and 5XFAD mouse models of AD, TREM2+ cells increased around Congo red–positive plaques, but these cells

were identified as $CD45^{hi}Ly6C^{+}$ monocytes rather than resident microglia [194]. Therefore in the disease situation there is some controversy on the actual cellular types expressing TREM2 and the cell-type specific function of TREM2. The expression of TREM2 was found to correlate positively with the ability of microglia to stimulate CD4(+) T-cell proliferation suggesting that TREM2+ cells can present self-antigens to infiltrating lymphocytes, potentially inducing neuroprotective immune responses [195]. Furthermore, TREM2 was identified in DCs as one of the markers of antigen presentation after stimulation [196]. Whether TREM2 is involved in the process of antigen presentation in AD remains to be investigated.

Even if inflammation is detected in the diseased brain at post-mortem, we still have to resolve whether it contributes to or causes the disease. Systemic infections can also enhance cytokine synthesis in the brain leading to an increased production of cytokines and other inflammatory molecules by already primed immune cells in the brain, as suggested in AD.

Although the complex interactions between the CNS and the (innate) immune system are not fully understood, accumulating evidence suggests that immune cells play important roles in the pathogenesis and progression of neurodegenerative diseases.

5.2 Dendritic cells in Parkinson's disease

Parkinson's disease (PD) is a progressive neurodegenerative disorder mainly impairing the locomotor system but also causing non-motor disturbances such as dementia. Symptoms are probably caused by destruction of neurons through intracellular accumulation of the protein α-synuclein forming so-called Lewy bodies. In early stages of the disease, Lewy bodies appear in neurons in the olfactory bulb, medulla oblongata, and pontine tegmentum. As the disease progresses, neurons are destroyed in the substantia nigra, the basal forebrain, and the neocortex. Genetic as well as environmental risk factors have been identified in PD. These triggering factors (age-related immune alterations; bacterial or viral infections, environmental toxins) may lead to a dysregulation of inflammatory pathways. In the last years, immunological changes have been related to PD pathogenesis. Already 20 years ago, microglial activation was described as a neuropathological hallmark in PD. McGeer et al. observed activated microglia and complement components in affected brain regions of PD patients [197] and enhanced levels of TNF-α, IL-1β, IL-6 and IFN-γ have been found in CSF and the striatum of PD patients [198]. The genetic TREM2 R47H variant is also

associated with the development of PD [199]. Also deletions and point mutations of the DJ-1 gene are associated to autosomal recessive PD [200], and microglia deficient in DJ-1 showed reduced expression of TREM2 [201], again pointing to an important role of TREM-2 in PD.

These inflammatory events could be initiated by primary neurodegenerative processes and, in turn, exacerbate the progression of neuronal loss. Nevertheless, it is not clear whether inflammation could also be the primary event leading to neurodegenerative processes. In different animal models of PD, microglial activation was shown to be a prior event before neuronal cell death. Mutation of α–synuclein [202] or nigral injection of LPS [203] resulted in a progressive degeneration of dopaminergic neurons over a long period. Additionally human PET-studies demonstrated microglia activation early in disease progress [204]. Furthermore, microglial cells in aged brains exhibit a more neurotoxic and proinflammatory phenotype compared to younger cohorts by expressing higher levels of TLR4 and CD86.

This chronic neuroinflammation in PD could attract DCs. Interestingly, recently Ciaramella et al. observed reduced levels of blood DCs in PD patients compared to healthy controls [205]. Since different infectious diseases result in a decline of blood DCs but increased DCs at the sites of inflammation, they hypothesized that the low blood DC levels are a result of recruitment of DCs to the site of neurodegeneration. Fitting this hypothesis, DC levels also correlated with disease progression showing lower amounts of DCs in patients with higher impairment.

Attraction of DCs to sites of neurodegenerative inflammation in PD might be followed by maturation and migration to the cervical lymph nodes for autoantigen presentation to T and B cells, thereby triggering an autoimmune response. Several autoantibodies against targets associated with PD pathogenesis have been identified, including antibodies directed at α-synuclein [206], melanin [207], and GM1 ganglioside. Special emphasis has been put on neuromelanin, which accumulates in nigral dopaminergic neurons as a byproduct of the metabolism of these cells. As a requirement to initiate an autoimmune response, Oberländer et al. demonstrated phagocytosis of neuromelanin by DCs *in vitro*. They hypothesized that neuromelanin triggers DC maturation and, after presenting neuromelanin to lymphocytes in cervical lymph nodes, neuromelanin specific autoantibodies are generated [207] (Fig. 2). The presence of these antibodies has been described in PD patients [207, 208].

Additionally, the high affinity of neuromelanin to other proteins might lead to presentation of other neuronal proteins to the adaptive immune system. In addition, Chen et al. demonstrated that these autoantibodies might be clinically relevant for disease progression [209]. Plasma autoantibodies isolated from PD patients induced loss of dopaminergic cells in the substantia nigra of rats compared to control animals treated with autoantibodies from non-PD patients. Moreover, post-mortem analysis of PD patients revealed autoantibodies bound to dopaminergic neurons not only in the plasma but also in the brain [210]. These autoantibodies were opsonized with complement C1q, indicating that they are recognized by the classical complement pathway as a target structure and show the capacity to cause neuroinflammation

6. Dendritic cells in epilepsy

Many different inflammatory settings like infectious or autoimmune disease can cause recurrent epileptic seizures indicating the relevance of inflammation in the pathophysiology of epilepsy [211]. Prolonged seizures not only activate glial cells but also lead to upregulation of adhesion molecules on endothelial cells facilitating the extravasation of leukocytes [212]. This inflammatory response is not an epiphenomenon of the affected tissue since blockage of cell infiltration can prevent the induction of seizures [213] and there are already attempts to treat epilepsy with immunomodulatory strategies [214]. Inflammatory cells increase neuronal excitability and lower seizure thresholds by secretion of cytokines, alterations of neurotransmitter release or uptake, increasing BBB permeability, and damaging neuronal cells [211]. While possible mechanisms of astrocytes [215], microglia [216], lymphocytes [217, 218], macrophages [219, 220] and granulocytes [220] have been investigated more frequently, the role of DCs in the content of epileptogenesis remains elusive. In a Li-pilocarpine induced status epilepticus model in adult rats, Li et al. recently showed that DCs could be detected 24 hours after induction of seizures [221] . Negative staining for Iba1 and radiation experiments proved that these $CD11c^+$ cells were recruited from the periphery and did not derive from microglia. In addition, kainic acid-induced seizures in the *Cd11c/eyfp* Tg mouse also revealed EYFP-expressing cells in the damaged hippocampus [42].

Interesting data has been gained from focal cortical dysplasia (FCD), where sporadic malformations of the cerebral cortex cause chronic epilepsy in children [222, 223]. Despite an imbalance of the neurotransmitter system, recent data strongly suggest an involvement

of inflammatory processes in this non-infectious epilepsy. Activation of microglia and macrophages has been described in the tissue of FCD patients [224]. Whether this inflammatory response is triggered by recurrent seizures or represents an intrinsic feature of FCD is unclear. In the specimen of FCD type II [222] and chronic epileptic encephalopathy [223] patients, DCs has been described around blood vessels associated with perivascular T-lymphocytes. Studies indicate an involvement of PI3K-mTOR (phosphatidylinositol 3 kinase - the mammalian target of rapamycin) pathways [225]. Interestingly, the mTOR pathway also regulates DC function and maturation [226]. This indicates that DCs might be involved in the pathogenesis of epilepsy by maintaining a chronic inflammatory response, probably even causing chronic autoimmune processes, since autoantibodies haven been described in various forms of epilepsy, e.g. Rasmussen encephalitis [227]. On the contrary, in FCD type I only minor tissue extravasation of DCs was observed [222] showing that more data on inflammatory mechanisms particularly concerning the role of DCs in epilepsy is necessary. Otherwise, it might be challenging to develop an immunomodulatory therapy for seizure prevention.

7. DC-based therapies

The role of DCs linking innate and adaptive immunity and modulating immune responses makes them as potential tools for cell therapy. DC-based therapies have been investigated in different diseases, mainly related to cancer and immune diseases. In the CNS, DC-based therapies have been studied for the treatment of brain tumors with the objective of activating T-cell responses against tumor while suppressing Tregs. Currently, DC-based therapies are also under investigation in the context of a variety of neurological diseases, where experimental studies and several small clinical trials are being conducted. The rationale behind the use of DCs in neurodegenerative diseases is that DCs sensitized against proteins with a pathogenic role should promote immune recognition and clearance of toxic products. In contrast, DC therapies in autoimmune-related diseases have the objective of favoring Treg responses inducing tolerization against certain brain antigens. In spite of a great potential, DC-based therapies have not yet been translated to effective treatments and more investigation is needed due to the complexity of DC modulation.

7.1 DC therapies in brain tumors

The described unique ability of DCs in harnessing anti-tumor immune reactions has for a long time been driving the investigation on DC-based vaccine therapies. These vaccines are prepared after exposing *ex vivo* DCs to tumor-associated antigens [228, 229] or by directly targeting antigens *in vivo* to the cells [230]. Cancer immunotherapy aims to produce efficient and durable immune responses against tumors and to inhibit tumor evasion: the main purpose is to generate a strong cytotoxic immune reaction by CD8+ T cells. To achieve this goal it is substantial to be able to produce a proper T helper response by $CD4^+$ T cells and at the same time to limit the immunosuppressive functions of regulatory T cells that develop in tumor conditions [229].

DC vaccines were observed to stimulate the production of Th1 cytokines in patients with different types of cancer [231] and some studies found them to be associated with longer survival in patients with glioblastoma multiforme compared to conventional treatments [232]. However, the number of patients studied up to now is still low and larger trials with rigorous designs are required to prove efficacy and durability of the response [232]. The choice of the antigen to be presented by DCs may also have a profound effect on the efficacy of the treatment, and the extreme variability of tumor-associated proteins can represent a challenge to be overcome with a rigorous personalization of the therapy [229]. Although DC vaccines have shown limited benefit in advanced stages of cancer [229], recent studies in mice and humans with newly diagnosed glioblastoma found that preconditioning the vaccine site with a potent recall antigen, such as tetanus toxoid, increases the migratory capacity of DCs and lymph node homing, and may increase the efficacy of tumor antigen-specific DCs [233].

7.2 DC Therapies in EAE

After showing the feasibility and excellent tolerability of treating refractory Crohn's disease using *ex vivo*-generated autologous tolerogenic DCs [234], this approach is being tested now in a phase I trial in patients with MS or neuromyelitis optica (clinicaltrials.gov identifier NCT02283671). Tolerogenic DCs loaded with myelin peptides are administered every two weeks with a total of three administrations using increasing doses of cells in absence of limiting toxicity in the previous dosage. In this pilot study, the primary outcome will be the safety of the treatment, although a number of secondary measures will focus on functional

outcome, quality of life and immunological changes induced by the treatment. The trial plans to include a total of 12 patients and results are expected for mid-2017.

7.3 DC Therapies in stroke

In experimental ischemia, *ex vivo*-derived DCs have been used to deliver proteins such as the soluble tumor necrosis factor receptor 1 [235] or intracellular-acting anti-apoptotic protein Tat-BH4 [236] that can limit local inflammation and suppress neuronal death and reduce infarct size. However, these strategies have not reached yet clinical trials in humans.

7.4 DC Therapies in AD

Following promising vaccine studies in experimental AD models, the first AD vaccine phase II clinical trial was suspended after a few patients developed meningoencephalitis. However, the fact that some plaque clearance and modest clinical improvements were observed in patients following immunization gives still some hope for immunotherapy in the prevention of progression in earlier stages of AD [237]. The role of DCs in immunotherapy has been confirmed in recent experimental studies showing that the administration of DCs sensitized with Aβ peptide was able to slow the rate of cognitive decline in mice [238], and that the combined treatment of $A\beta_{1-42}$-BMDCs with intraperitoneal injection of splenocytes from young mice elevated the level of anti-Aβ antibodies, reduced amyloid plaques in brain, and attenuated deterioration of spatial learning and memory in APP/PS1 mice [239].

7.5 DC Therapies in PD

While DCs contribute to PD progression, DCs could also be used as a therapy in PD. Given that pathogenesis in PD is driven by α-synuclein accumulation, vaccination against this protein might be beneficial. Ugen et al. showed that intravenous injection of DCs after *ex vivo* sensitization against α-synuclein resulted in the generation of anti-α-synuclein antibodies in mice with improved locomotor functions and no overwhelming inflammatory response. A phase 1 study with an antibody against α-synuclein started in 2014 [240].

Final Remarks

In this review we have described the involvement of DCs and DC-like cells in neuroinflammatory conditions. However, the actual role of these cells in the various brain diseases is not completely understood. One main handicap is the difficulty in the identification of cells with the capacity to present antigen. DCs, microglia, and macrophages in the brain can become virtually indistinguishable under disease conditions. Potentially, the use of novel genetically modified reporter animals for tracing and targeting cells expressing specific molecules will help in the future to better identify the nature and features of the DC-like group of cells. For now, DCs seem to be unique in their greater capacity to sense antigen, migrate to the draining lymph nodes and prime T cells, but their action in the brain tissue often remains elusive. Microglia, macrophages, and DCs seem to contribute to disease onset and progression, but it is not sufficiently clear how their dynamic response is orchestrated in the various disease conditions as well as their potential contribution in regeneration processes. Certain experimental studies have provided controversial results suggesting that some of the findings could be, to some extent, model-dependent. Also, it is possible that the functions of the cells might change during the course of the diseases. All these aspects must be known to find therapeutic drugs and the best dosing regimens, and to exploit the potential of DC-based therapies. Targeting specific immune cells or molecules can trigger unexpected or unwanted side effects that need to be carefully identified in the animal studies. Finally, most of the current knowledge relies on animal work while it is known that many immune molecules are different in humans, and the experimental animal models only reproduce certain aspects of the human diseases, particularly brain diseases. Non-invasive imaging techniques can provide the means to validate some of the animal findings in humans by allowing the study of molecules and cells, and following up disease progression. The remarkable advances made in recent years in the field of neuroinflammation have indicated potential involvement of immune responses in multiple brain diseases. Hopefully, a better understanding of the fascinating group of cells that includes microglia, macrophages and DCs will eventually result in effective treatments for human brain diseases.

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Figure Legends

Figure 1: Schematic illustration of representative DC subtypes and their key function. Lymphoid tissue and non-lymphoid tissue resident DCs as well as migratory DCs are represented. Representative cDCs, pDCs, and MDDCs are illustrated. See Table 1 for further information on specific DC surface markers.

Figure 2: Immunomodulatory capacities of dendritic cells in neuroinflammation. This figure illustrates the various functions of DCs in neuroinflammatory disease pathogenesis and highlights possible starting points to drive neuroinflammation towards neuroprotection and not neurodegeneration. Under acute (e.g. ischemic stroke) or chronic inflammatory (e.g. Parkinson's disease) conditions several steps could be identified as follows: (1) Microglial cells become activated, and also have the capacity to differentiate into effective APCs. (2) The inflammatory response attracts peripheral DCs to the regions of cellular stress. (3) During cell death in CNS injury, brain antigens may be taken up and presented by DCs. (4) This is then followed by DC maturation and migration to secondary lymphoid organs. Presentation of these antigens to T-cells can activate neuroprotective or neurodestructive pathways. (5) Development of tolerogenic dendritic cells followed by elimination of self-destructive T-effector cells and generation of Tregs is crucial to promote tolerance against autoantigens and suppress immunological reactions that can slow down disease progression. (6) However, DC-mediated antigen presentation can also result in priming and massive expansion of T-effector cells (e.g. Th1 and Th17 cells). (7) Production of autoantibodies against the presented antigens after B-cell activation, T-cell mediated cytotoxicity and cytokine production, as well as autoantibodies, then contribute to neuronal dysfunction and cell death and may exacerbate disease progression (8). Understanding the balance and manipulation of these pathways could be a new treatment option in these diseases.

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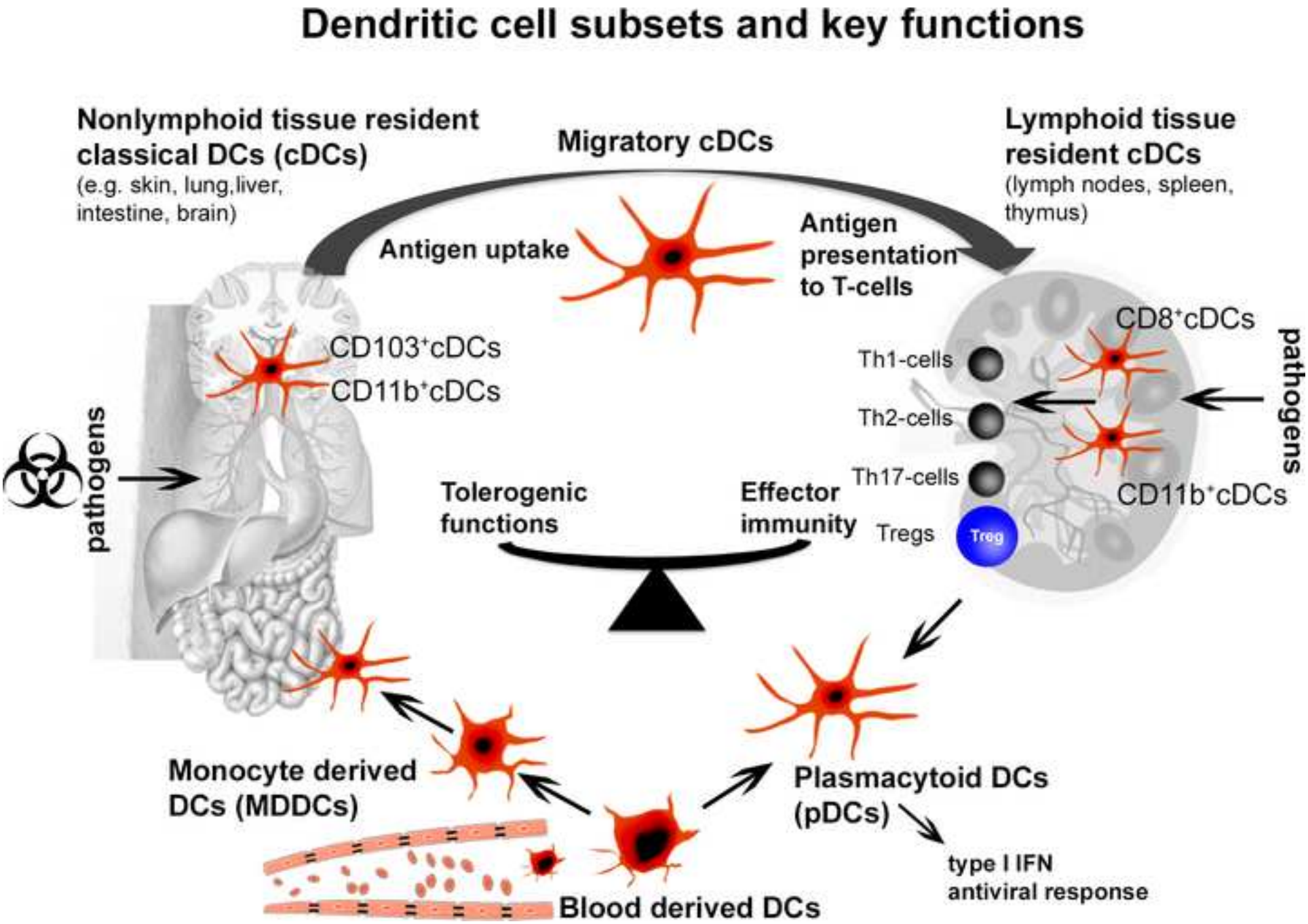

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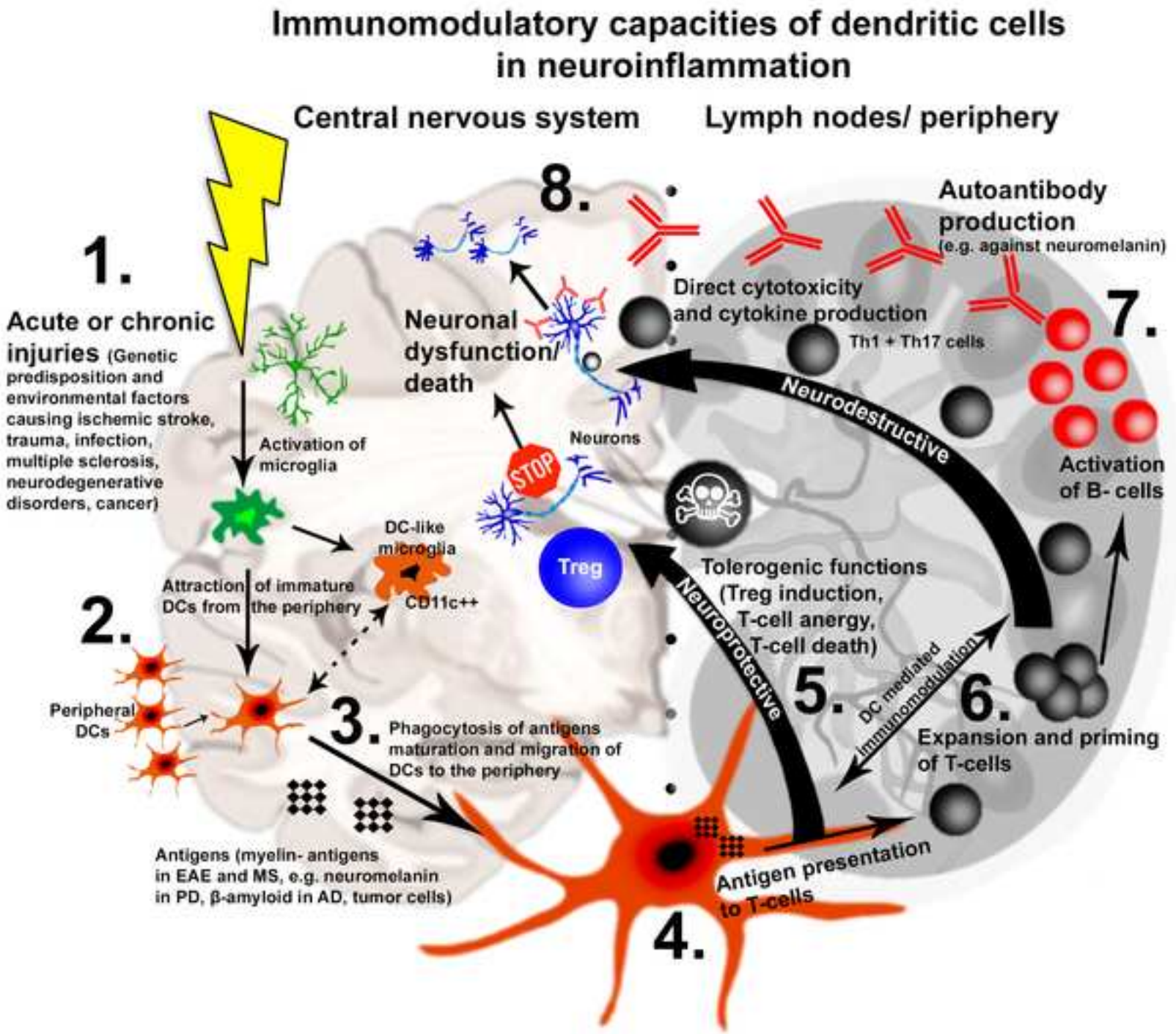

Table

Table 1.

Type	subtypes	mouse surface markers	human surface markers	main functions	references
Cells of the DC lineage					
cDCs Myeloid	general	$CD11c^+$ $MHCII^+$	$CD11c^{+/lo/-}$ $HLA\text{-}DR^+$	T cell priming / protective immunity	ref. 14, 18, 21
	resident in LT, conventional	$CD11b^{lo}CD8\alpha^+DNGR1^+XCR1^+$ $CD11b^+CD4^+$ $DEC\text{-}205^{+/-}$ $CD11b^{-/+}$	$BDCA3^+$/ $DNGR1^+$ / $XCR1^+$ $CD11b^+CD4^+$	crosspresentation / induction of Th1 induction of $CD4^+$ T cell immunity production of IL-12	ref. 2, 19, 22 ref. 12, 14
	tissue resident & migratory tissues	$CD103^+CD11b^-$ $DNGR1^+XCR1^+$ $CD103^-CD11b^+DEC\text{-}205^+$ $CD103^+CD11b^+CD24^+$ $SIRP\alpha$	$BDCA3^+$ / $DNGR1^+$ $CD1a^+$ $BCDA\text{-}1^+$ $SIRP\alpha$	crosspresentation / induction of Th1cells induction of Treg and Th2 cells promote Th17 responses	ref. 2, 16, 28 ref. 13, 15 ref. 12
	blood	$CD11c^+$	$BDCA1^+/BDCA3^+CD103^{+/-}$		ref. 20
pDCs Plasmacytoid	blood /LT	$CD11c^{-/+}$/ $B220^+$ $CCR9^+$ $SCA1^+$ $LY49Q^+$ $CD123^+$	$BDCA2^+$ $BDCA4^+$ $ILT7^+$ $CD11c^-$ $CD123^{hi}$ / $B220^+$	anti viral responses produce type I IFNs, ICOS-L Ag presentation (poorer than cDCs) Treg induction	ref. 23-27, 29 ref. 14 ref. 79
Cells capable of expressing some DC surface markers					
MDDCs (moDCs) Monocyte-derived Dcs	general	$CD11b^+Ly6C^{lo}CD11c^+$ some $CD103^+$	$CD14^+$ $CD16^{+/-}$ $CD11c^+$	generated in vitro from monocytes with GM-CSF (+/- IL4, Flt3)	ref. 30, 31
	infDCs (iDCs)	$CD11b^+CD11c^+MHCII^+$ $CD64^+$ $Fc\varepsilon RI^+$ $DEC\text{-}205^{+/-}$ / $CD209^+$	$CD11b^+$ $CD64^+$ $CD209^+$	induced in inflammatory conditions innate immune defence can upregulate CD11c and MHC II	ref. 12, 32 ref. 33, 58 ref. 33
	Tip-DCs	$CD115^+Gr1^+$		Produce TNF-α and express iNOS	ref. 34, 59
MDSCs Myeloid-derived sup-pressor cells	general	$Gr1^+CD11b^+$ $Arg1^+iNOS^+$	$CD33^+CD11b^+$ $CD15^+CD66b^+$	induced in disease conditions immune suppressive activity represent a functional cell status can upregulate CD11c	ref. 77
	granulocytic	$CD11b^+Ly6G^+Ly6C^{lo}$			
	monocytic	$CD11b^+Ly6G^-$ $Ly6C^{hi}$			
Microglia	several phenotypes	$CD45^{lo}$ $CD11b^{dim}$ / $Iba1^+$	$P2Y12^+$ / $Iba1^+$	CNS resident innate immune cells can upregulate CD11c and MHC II	ref. 45-51, 82, 88 ref. 61-62

LT: lymphoid tissue; LN: lymph node

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