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Innate-Adaptive Crosstalk: How Dendritic Cells Shape Immune Responses in the CNS

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Abstract

Dendritic cells (DCs) are a heterogeneous group of professional antigen presenting cells that lie in a nexus between innate and adaptive immunity because they recognize and respond to danger signals and subsequently initiate and regulate effector T-cell responses. Initially thought to be absent from the CNS, both plasmacytoid and conventional DCs as well as DC precursors have recently been detected in several CNS compartments where they are seemingly poised for responding to injury and pathogens. Additionally, monocyte-derived DCs rapidly accumulate in the inflamed CNS where they, along with other DC subsets, may function to locally regulate effector T-cells and/or carry antigens to CNS-draining cervical lymph nodes. In this review we highlight recent research showing that (a) distinct inflammatory stimuli differentially recruit DC subsets to the CNS; (b) DC recruitment across the blood-brain barrier (BBB) is regulated by adhesion molecules, growth factors, and chemokines; and (c) DCs positively or negatively regulate immune responses in the CNS.

Keywords

Dendritic cell (DC); Inflammatory (iDC); Plasmacytoid (pDC); Conventional (cDC); Central nervous system; Chemokines; T-cell responses

1 Introduction to Dendritic Cells and Their Subsets

Since the discovery of dendritic cells (DCs) (Steinman et al. 1975), an extensive body of literature has accumulated showing that these cells are the most efficient stimulators of T-lymphocytes. DCs are hematopoietic cells derived from pre-DCs that migrate from the bone marrow into circulation and eventually give rise to several DC subsets in lymphoid and non-lymphoid tissues (Banchereau and Steinman 1998). From studying skin (Randolph et al. 2005a, b), intestine (Milling et al. 2010; Tezuka and Ohteki 2010), granulomatous tissue (Schreiber et al. 2010), and lung (Hintzen et al. 2006), it is well accepted that in the steady state immature migratory DCs uptake antigen from the local environment, process these antigens, and present them on their surface in the context of major histocompatibility (MHC) molecules. Immature DCs express low levels of MHC and costimulatory molecules (CD80, CD83, CD86) below the threshold for T-cell activation. In the absence of inflammatory signals, immature DCs migrate through lymphatic vessels to regional lymph nodes at basal rates and present self-antigen to naïve T-cells, inducing tolerance by way of

T-cell anergy, apoptosis, and regulatory T (Treg)-cell differentiation (Torres-Aguilar et al. 2010). In contrast, activated maturing DCs upregulate MHC and costimulatory molecules, change their expression of chemokine receptors to promote active migration to the T-cell areas in regional lymph nodes, and there activate naïve antigen-specific T-cells (Steinman et al. 1997; Lanzavecchia 1999; Caux et al. 2002).

Under steady state conditions two types of DCs can be distinguished: plasmacytoid (pDCs) and conventional (cDCs). Both pDCs and cDCs are derived from a committed DC progenitor (CDP) present in the bone marrow (Naik et al. 2007). PDCs are notable for their plasmoid morphology (Corcoran et al. 2003) and their ability to secrete copious amounts of interferon (IFN)- α upon activation (Cella et al. 1999; Siegal et al. 1999; Ito et al. 2004). CDPs give rise to pre-DCs that enter the blood and develop into cDCs in lymphoid and non-lymphoid tissue. Pre-DCs can enter lymphoid organs and become “lymphoid tissue resident DCs”, or they can give rise to migratory antigen-collecting DCs that continuously survey most non-lymphoid tissues, pick up antigens and carry them to lymph nodes (Randolph et al. 2005a, b). Pre-DCs that enter non-lymphoid tissue give rise to all CD103⁺ and some CD103⁻ migratory cDCs. The fact that these cells need to function in radically different tissue environments might itself provide a reason why there are several distinct subsets of migratory DCs (Steinman and Idoyaga 2010). Even in the same lymphoid organ, different DC subsets can be detected amongst non-migratory DCs including CD8⁺ and CD8⁻ cDCs (Shortman and Heath 2010). Compared to their counterparts, CD8⁺ lymphoid tissue resident cDCs and CD103⁺ migratory cDCs have been shown to be efficient cross-presenters capable of priming cytotoxic lymphocyte responses (Liu and Nussenzweig 2010). Additionally, TNF- α and iNOS producing (TIP)-DCs also referred to as “inflammatory” (iDCs) have also been reported to accumulate in target tissues during immune responses (Serbina et al. 2003; Lowes et al. 2005; Engel et al. 2006). The origin of these DCs is still debated, but research has indicated that they may be derived from tissue infiltrating monocytes as well as blood pre-DCs (Romani et al. 1996; Randolph et al. 1998, 2002; Geissmann et al. 2003; Tacke and Randolph 2006; Shortman and Naik 2007; King et al. 2009). We will review recent work investigating recruitment of pDC, cDC, and iDC subsets to the CNS in response to different inflammatory stimuli, how these cells are recruited, and how these DC subsets function to positively or negatively regulate CNS immunity.

2 Dendritic Cell Subsets Accumulate in the CNS During Neuroinflammation

2.1 Dendritic Cells in the Healthy CNS

The CNS is considered an immune privileged tissue due to the relative dearth of resident DCs and the presence of the blood-brain barrier (BBB), which limits immune cell immigration. While healthy CNS parenchyma is essentially devoid of MHC II⁺ DCs, recent studies have demonstrated that DCs do populate several CNS compartments under steady state conditions. Based on morphology and surface expression MHC II⁺ cDCs have been identified in human choroid plexus (Serot et al. 1997) as well as the meninges and choroid plexus of rats (McMenamin 1999, 2003; Chinnery et al. 2010). Both the meninges and the choroid plexus are highly vascularized, suggesting that DC accumulation in these organs might be due to increased recruitment from blood. Indeed, in wild-type mice reconstituted with bone marrow from mice expressing enhanced yellow fluorescent protein under the DC marker CD11c (CD11c-eYFP), DCs were fully repopulated in these tissues 8 weeks after reconstitution (Chinnery et al. 2010), suggesting that these CNS DCs are replenished by continuous recruitment from blood. Other studies employing CD11c-eYFP mice have revealed ovoid, bipolar, and dendriform CD11c⁺ DCs extensively distributed in the layer II of the piriform cortex as well as periventricular tissues including the rostral migratory stream, which extends caudally from the subventricular zone to the anterior commissure of the olfactory bulb (Bullock et al. 2008). More recently, CD11c⁺ DC-like cells have been

detected in the parenchyma surrounding the vasculature of healthy mouse brain tissue (Prodinger et al. 2010). These DC-like cells seem to be integrated into the glial wall that delimits the perivascular space from the CNS parenchyma, and thus these cells may be poised for communication with infiltrating immune cells.

2.2 Dendritic Cell Subset Accumulation in the CNS During Neuroinflammation

It has been proposed that DCs might be part of the immune environment of the CNS, as during neuroinflammation or CNS injury iDCs can be identified in the CNS (Pachter et al. 2003). A large portion of the current literature investigating DC involvement in neuroinflammation comes from experimental autoimmune encephalomyelitis (EAE)—a mouse model of MS. We have shown that DCs rapidly accumulate in the CNS of mice with EAE and that their accumulation precedes clinical onset. Upon histological survey we found CD11c-eYFP DCs accumulating in choroid plexus, periventricular tissue, and subpial spaces prior to clinical onset (Fig. 1). This corresponds well with previous reports showing CD205⁺ CD209⁺ cDCs accumulating in the meninges, choroid plexus, and subpial space of the spinal cord preclinically and in perivascular cuffs circa demyelinating lesions during acute disease (Serafini et al. 2000). While several DC subsets accumulate in the CNS of EAE mice, iDCs expressing the myeloid cell marker CD11b⁺ predominate (Matyszak and Perry 1996; Suter et al. 2000; Bailey et al. 2007). These cells have been suggested to be derived from circulating inflammatory Ly6c^{hi} monocytes that are released from the bone marrow during immune responses. Others have shown that Ly6c^{hi} monocytes are enriched in bone marrow and blood prior to their accumulation in CNS during preclinical EAE and that these CNS infiltrating monocytes differentiate into both macrophage and DC-like CD11c⁺ cells (King et al. 2009). Likewise, using CD11c-eYFP mice we observed that the frequency of CD11b⁺CD11c⁺ cells in bone marrow is increased on days 5–10 following EAE induction (Fig. 2), suggesting that along with monocytes other DC precursors may be released from the bone marrow following EAE induction.

Mature myelin-containing CD209⁺ cDCs (Plumb et al. 2003; Serafini et al. 2006; Cudrici et al. 2007) as well as CD123⁺ pDCs (Lande et al. 2008) have been found in meninges, perivascular cuffs, active demyelinating lesions, and non-lesional gray matter of patients with MS. Both cDCs and pDCs are present at low levels in cerebrospinal fluid (CSF) of patients with non-inflammatory neurological disease and both DC subsets are increased in CSF of patients with neuroinflammatory conditions (Pashenkov et al. 2001). Patients with bacterial meningitis and Lyme neuroborreliosis have elevated levels of DCs in their CSF, with cDCs predominating in bacterial meningitis and pDCs predominating in neuroborreliosis (Pashenkov et al. 2002). Circulating levels of cDC and pDC precursors were reported to transiently decrease following acute stroke, and CD209⁺ cDCs and CD123⁺ pDCs have been detected in postmortem brain tissue from stroke patients (Yilmaz et al. 2010).

DCs have also been shown to accumulate in the CNS in mouse models of prion disease, viral encephalitis, brain ischemia, parasitic CNS infections, and CNS bacterial infections (Fischer et al. 2000; Kostulas et al. 2002; Reichmann et al. 2002; Trifilo and Lane 2004; Rosicarelli et al. 2005; Brehin et al. 2008; Gelderblom et al. 2009; Steel et al. 2009; Savarin et al. 2010). CNS infection with neurotropic mouse hepatitis virus (Trifilo and Lane 2004) or vesicular stomatitis virus (Steel et al. 2009) promotes the accumulation of predominantly CD11b⁺ cDCs, whereas CNS infection with west nile virus promotes the selective recruitment of pDCs to CNS as early as 4 days after infection (Brehin et al. 2008). In models of focal brain ischemia and reperfusion injury CD11b⁺ DCs have been reported to accumulate at the CNS as soon as 1 h after occlusion and persist for weeks, occupying the border zone of the stroke penumbra area (Kostulas et al. 2002; Reichmann et al. 2002; Gelderblom et al. 2009). Chronic CNS infection with *Toxoplasma gondii* promotes

accumulation of 33D1⁺CD8-F4/80⁺ myeloid DC-like cells in CNS though these cells seem to be brain-derived as *T. gondii* infection of primary brain cell culture promoted differentiation of 33D1⁺ “dendriform” cells (Fischer et al. 2000).

In order to further investigate whether different inflammatory challenges alter DC recruitment to the CNS, we compared the absolute number and phenotype of CNS-infiltrating CD11c⁺ cells in mice following intracerebral (i.c.) mycobacterial infection or EAE induction. We demonstrated that CD11c⁺ cells could be detected in higher numbers in the spinal cord of mice with EAE compared to brain tissues; whereas i.c. infection with Bacillus Calmette-Guérin (BCG) induced a higher level of recruitment of CD11c⁺ cells to the brain compared to spinal cord (Fig. 3a). The CD11c⁺ DC population can further be divided into two subtypes based on higher or lower surface expression of CD11c. CD11c^{low} CD11b⁺ DCs were a dominant subset among DCs isolated from mice during EAE. In contrast, BCG infection of the CNS resulted in increased recruitment of the CD11c^{high} CD11b⁺ subpopulation, represented by the increased CD11c^{high}: CD11c^{low} ratio in both meninges and brain parenchyma (Fig. 3b left). Interestingly, we observed that—as in liver—CNS mycobacterial infection promoted the accumulation of DCs highly expressing the coinhibitory molecule B7H2 (Lee et al. 2009). Additionally, we found that mycobacterial infections promoted higher CNS recruitment of the CD8⁺ and CD11c^{high} subpopulations of DCs compared to EAE (Fig. 3b right). On brain cryosections from BCG infected mice we identified dsRed BCG rods residing in CD8⁺CD11c⁺ cells (Fig. 3c), which supports data showing that CD8⁺ DCs play an important role in mediating BCG-specific immune responses.

While CD8⁺ cDCs are usually restricted to lymphoid tissue, it is possible that pre-DCs migrating into non-lymphoid tissues can differentiate into CD8⁺ “lymphoid tissue resident DCs” under conditions of ectopic lymphogenesis—a feature of chronic inflammation that has been shown to be induced by mycobacterium in lung (Maglione et al. 2007). Additionally, it is suggested that the formation of tissue granulomas—a histological hallmark of mycobacterial infections—shares several features with tertiary lymphoid tissue development (Voswinkel et al. 2008; Lamprecht et al. 2009; Day et al. 2010). Our results are consistent with previous reports showing higher accumulation of cDCs during CNS mycobacterial delayed type hypersensitivity responses compared to EAE (Matyszak and Perry 1996).

3 Mechanisms of Dendritic Cell Migration into the CNS

3.1 Regulation of DC Migration across the BBB: Adhesion Molecules

While several studies have documented the accumulation of DCs in the CNS during neuroinflammation, less is known about DC migration into the CNS, mechanistically. Natalizumab, a monoclonal antibody against $\alpha 4$ integrin used to treat MS, was shown to reduce DC accumulation in the CNS (del Pilar Martin et al. 2008). In another recent study, intravital microscopy during ongoing EAE demonstrated that immature blood DCs adhere to inflamed vessel endothelium in a β -1 integrin dependent manner (Jain et al. 2010). The $\alpha 4\beta 1$ complex also known as VLA4 is known to bind VCAM-1, which is upregulated on the surface of endothelial cells during neuroinflammation (Shaftel et al. 2007; Gobel et al. 2011; Li et al. 2011). Indeed, VLA-4 has been widely implicated in leukocyte adhesion to brain microvessels during neuroinflammation; however, directed migration of vessel-associated DCs into demyelinating lesions in the CNS parenchyma is restricted by the presence of the BBB. This barrier is comprised of two layers, the vessel endothelium and the glia limitans. The endothelial cells that comprise the wall of post-capillary venules express occludin and form tight junctions, which restrict transmigration of immune cells and fluid exchange (Pachter et al. 2003). Surrounding this layer is the Virchow-Robin space

(perivascular space), which is continuous with the CSF-filled subarachnoid space. The perivascular space is separated from the neuropil by a second layer called the glia limitans formed by a basement membrane surrounded by tightly interlocking astroglial endfeet (Pachter et al. 2003).

3.2 Regulation of DC Migration across the BBB: Chemokines and Growth Factors

Migration across the endothelium and glia limitans is thought to critically depend upon specific DC growth factors and chemoattractants, which are expressed in the inflamed CNS. Granulocyte-macrophage colony stimulating factor (GM-CSF) and Fms-like tyrosine kinase 3 ligand (Flt3L) are growth factors that bind the DC receptors CD115 and FLT3, respectively. GM-CSF promotes expansion of monocytes and iDCs, whereas Flt3L is considered to be specific for the expansion of DCs and committed DC precursors. I.c. injection of Flt3L promotes the recruitment of pDCs (Curtin et al. 2006), whereas injection of GM-CSF—producing cells promotes the recruitment of monocytes and iDCs (Hesske et al. 2010). GM-CSF can also transform brain-resident microglia into DC-like cells and accumulation of DC-like cells in CNS tissues during chronic *T. gondii* infections has been shown to be GM-CSF dependent (Fischer et al. 2000). Additionally it has been shown that human blood monocytes can transform into mature DC-like cells following migration across activated human BBB endothelial cells. These newly transformed cells upregulate markers of cDCs (CD209) and pDCs (CD123) as well as DC activation markers (HLA-DR, CD80, CD86, and CD83) and induce stronger effector T-cell responses than untransformed blood monocytes. Importantly, this transformation was dependent upon BBB-derived TGF- β and GM-CSF (Iferrgan et al. 2008). More recently, it was shown that Ly6c^{hi} inflammatory monocytes emigrate from bone marrow and accumulate in blood prior to their accumulation in the CNS during EAE (King et al. 2009), and that this accumulation was promoted by GM-CSF which acted to promote accumulation of monocytes in blood and promoted production of the DC chemoattractant CCL2 (Serbina and Pamer 2006; Hesske et al. 2010).

The receptor for CCL2, CCR2, has been shown to be required for emigration of inflammatory monocytes from bone marrow during bacterial infection in mice (Serbina and Pamer 2006). CCL2^{-/-} mice have impaired monocyte recruitment to the perivascular space during CNS viral infection (Savarin et al. 2010) and experiments in bone marrow chimera mice have shown that glia-derived CCL2 is required for recruitment of iDCs during EAE (Dogan et al. 2008), demonstrating that CCL2 is also involved in monocyte and DC immigration into CNS tissues. Indeed, CNS-specific overexpression of CCL2 leads to spontaneous asymptomatic accumulation of perivascular monocytes in the brain with little infiltration into the CNS-parenchyma (Toft-Hansen et al. 2006). Interestingly, conditional expression of Flt3L in these mice leads to the expansion of monocytes and DCs in the CNS and the onset of ascending hind-limb paralysis within 9 days of gene induction (Furtado et al. 2006). In summary, these data suggest that Flt3L promotes pDC recruitment to the CNS whereas the GM-CSF–CCL2 axis is involved in recruitment of monocyte-derived DCs. Indeed, CCL2^{-/-}, CCR2^{-/-}, and GM-CSF^{-/-} mice as well as mice treated with CCL2 neutralizing antibodies are markedly resistant to EAE (Fife et al. 2000; Izikson et al. 2000; Zaheer et al. 2007).

Importantly, CCL2 does not seem to promote cell progression across the glia limitans, which is thought to be a rate-limiting step in the induction of neuroinflammation. Pertussis toxin can be used in the induction of EAE, and pertussis toxin-induced encephalitis is associated with disruption of the glia limitans (Toft-Hansen et al. 2006). However, the mechanism by which DCs migrate across the glia limitans and into CNS parenchyma during neuroinflammation is poorly understood. In epithelial tissues, circulating DCs are recruited across the vascular endothelium by CCL2 and subsequently directed to the site of injury via a CCL20 gradient (Caux et al. 2002). It is believed that these chemokines are produced by

stromal cells and resident macrophages present in the inflammatory site. Likewise, it is postulated that astrocytes and microglia produce chemoattractants that recruit DCs into the CNS parenchyma. Both cDCs and pDCs express CXCR3 and CCR6 which promote directional migration in response to the injury-associated chemokines CXCL10 and CCL20, respectively (Cravens and Lipsky 2002; Kohrgruber et al. 2004; Charles et al. 2010). CXCL10 is also known as interferon inducible protein 10 owing to the fact that it is highly upregulated by interferon signaling and accordingly highly expressed during viral infection. During CNS herpes simplex virus-1 infection, CXCL10^{-/-} and CXCR3^{-/-} mice had similar deficiencies in the recruitment of pDCs, while cDC recruitment to the CNS was unaffected (Wuest and Carr 2008). CXCL10 is also elevated in the CSF of patients with MS and optic neuritis (Sorensen et al. 2001, 2004) and in MS lesions (Simpson et al. 2000) but has mainly been correlated with recruitment of CXCR3⁺ T-cells in CNS autoimmunity. CCL20 mRNA has been detected in the CNS by day 13 of EAE, and protein has been detected in mouse brain tissue sections in cells with astrocytic morphology (Serafini et al. 2000). Astrocytes stimulated with IL-1 β and TNF- α secrete CCL2 (CCR2 ligand); CCL3, CCL4, CCL5 (shared ligands for CCR1 and CCR5); CCL20 (CCR6 ligand) and CXCL12 (CXCR4 ligand) and promote the transmigration of immature DCs across an artificial BBB *in vitro* (Ambrosini et al. 2005). Additionally, CCR1⁺ and CCR5⁺ mononuclear cells have been identified in MS lesions (Trebst et al. 2001) along with their ligands (Boven et al. 2000).

We have demonstrated that CCL3 (a shared ligand for CCR1 and CCR5, also known as macrophage inflammatory protein (MIP-1 α)) increases the transmigration of bone marrow-derived GFP-labeled DCs across brain microvessel endothelial cell monolayers. We have also shown that the tight junction protein occludin is reorganized when DCs migrate across brain capillary endothelial cell monolayers, and DCs produce matrix metalloproteinases (MMPs) 2 and 9 when cultured with brain endothelial cells. CCL3 is also required for upregulation of costimulatory markers on CNS-infiltrating DCs during murine viral encephalitis (Trifilo and Lane 2004) and both CCL5 and CCL3 promote secretion of matrix metalloproteinase (MMP9) by monocytes (Robinson et al. 2002) and pDCs (Hu and Ivashkiv 2006) *in vitro*. Interestingly, in our experiments DCs showed an activated phenotype upon migration, and migration could be abrogated by treatment with an MMP inhibitor (Zozulya et al. 2007). Recently others have shown that dystroglycan—a glycoprotein that anchors astrocytic endfeet to the basement membrane of the glia limitans—is a cleavable substrate of MMP-2 and MMP-9, suggesting that MMP expression may be required for DCs to gain access to the CNS parenchyma. Indeed, it has been shown that MMP2^{-/-} MMP9^{-/-} double knockout mice are resistant to EAE (Agrawal et al. 2006).

3.3 Regulation of DC Migration across the Blood-CSF Barrier

Other studies have begun to describe an alternative route by which DCs and DC precursors could migrate into the CNS. In this route, cells cross the more permissive endothelium of the choroid plexus and meninges. These cells could then enter CSF or migrate through choroid plexus stroma into periventricular tissue. Indeed, both monocytes and DCs have been identified on electron micrographs of choroid plexus mounts from naïve mice (Serot et al. 1997, 1998; McMenamin et al. 2003). Monocytes detected within the stroma of the choroid plexus have increased proliferative capacity compared to blood monocytes and are capable of giving rise to DCs upon treatment with GM-CSF and IL-4 (Nataf et al. 2006). Interestingly, monocytes were more or less restricted to the internal stromal compartment of the choroid plexus, whereas DCs were found on the surface of the choroid plexus epithelium. This suggests that during steady state conditions, DCs and DC precursors are recruited to the choroid plexus where they are poised for migration into the CSF. Indeed, as mentioned above DCs are elevated in CSF of patients with neuroinflammatory diseases (Pashenkov et al. 2001, 2002). CXCL10 and CXCL12 are highly upregulated in the CSF of

patients with bacterial meningitis and Lyme neuroborreliosis. Moreover, DC accumulation in CSF of these patients could be partially blocked by neutralizing antibodies to CXCL12 (Pashenkov et al. 2002). In mice, DCs injected into the CSF during EAE were recruited to periventricular demyelinating lesions, meninges and the parenchyma of the cerebellum and brain stem (Hatterer et al. 2008), suggesting that DCs in the cerebroventricular system may actively contribute to neuroinflammation. Both CCL20 and CCL2 are highly expressed in the healthy choroid plexus epithelium (Mitchell et al. 2009; Reboldi et al. 2009) and the choroid plexus has been shown to be an important route of entry for T-cells and neutrophils during EAE and traumatic brain injury models, respectively (Mitchell et al. 2009; Reboldi et al. 2009; Szymdynger-Chodobska et al. 2009); however, the extent to which this migratory route is used by DCs *in vivo* during neuroinflammatory conditions is unknown.

4 Dendritic Cell Functions in the CNS

4.1 Dendritic Cells can be Positive or Negative Regulators of CNS Immunity

Monophasic EAE can be induced in C57BL/6 mice by subcutaneous immunization with myelin oligodendrocyte glycoprotein peptide (MOG₃₅₋₅₅). We have shown that i.c. injection of mature MOG-loaded DCs prior to induction of EAE decreases the proportion of regulatory T-cells infiltrating the brain and increased activation of CNS infiltrating T-cells. This effect was associated with acceleration of clinical disease, indicating that CNS DC accumulation might represent a limiting factor during acute EAE. In contrast, when we i.c. injected TNF- α treated “semi-mature” DCs we observed diminished production of the encephalitogenic cytokine IL-17 by CNS-infiltrating lymphocytes, which substantially delayed or prevented EAE (Zozulya et al. 2009). Surprisingly, injection of DCs lacking the coinhibitory molecule B7H1 also ameliorated EAE clinical disease. This effect was associated with the recruitment of CD122⁺ CD8⁺ regulatory cytotoxic T-lymphocytes (Zozulya et al. 2009). While these studies demonstrate that CNS DCs can be both positive and negative regulators of CNS immunity, the mechanism of their action is still poorly understood. For example, it is not known whether these DCs function principally by locally restimulating and directing T-cell differentiation or by capturing antigens and transporting them to draining lymph nodes for the priming of naïve T-cells.

4.2 Dendritic Cells Migrate Out of the CNS to Deep Cervical Lymph Nodes

The CNS is considered immune privileged owing to reduced immune surveillance in these tissues. As such, little is known about the mechanisms of DC migration out of the CNS during neuroinflammation. The CNS does not have typical lymphatic vessels that drain directly to regional lymph nodes. Nevertheless, mice injected intracerebroventricularly with exogenous antigen develop humoral and cell-mediated immune responses in cervical lymph nodes (Cserr et al. 1992). Indeed, in chronic relapsing EAE excising the deep cervical lymph nodes during acute disease partially ameliorates relapse (van Zwam et al. 2009). We have shown that in mice injected i.c. with OVA protein, CD205⁺ CD11c⁺ DCs accumulate in the CNS and uptake this antigen. Following this, CD8⁺ OVA-specific T-cells accumulate first in the cervical lymph nodes and subsequently in the CNS, demonstrating that afferent immunity is intact in the CNS and that antigens drain or are trafficked to the cervical lymph nodes (Ling et al. 2003). Chronic relapsing EAE can be induced in SJL mice by subcutaneous immunization with peptide sequences from proteolipid protein (PLP). It is thought that relapse is the consequence of epitope spreading, whereby secondary immune responses develop against antigens released during the course of neuroinflammation. Myelin antigen-containing cells have been detected in the cervical lymph nodes of monkeys with EAE as well as patients with MS. In both cases, these cells were increased in frequency compared to healthy controls and predominantly expressed DC surface markers (de Vos et al. 2002). Recently we have shown that i.c. injected mature OVA-loaded DCs migrate from

CNS tissue to the cervical lymph nodes and prime OVA-specific T-cell responses (Karman et al. 2004). This migration could be blocked by pretreatment of DCs with pertussis toxin, which suggests that this migration is not passive and is mediated by G protein-dependent motility—strongly implicating DC maturation-associated chemokine receptors.

CXCR4 and CCR7 chemokine receptors are both important in mature DC migration. CXCR4 binds CXCL12 and mediates directional migration into tissue and lymphatic vessels under steady state conditions, whereas CCR7 binds CCL19/CLL21 and directs activated mature DCs to T-cell areas in draining lymph nodes under inflammatory conditions. Both cDCs and pDCs upregulate CCR7 upon maturation; however, whereas mature cDCs express high levels of CXCR4, activated maturing pDCs downregulate CXCR4 expression and lose responsiveness to CXCL12 (Cravens and Lipsky 2002). It is thought that mature DCs migrate through interstitial fluid along arterial walls of cerebral vessels and through CSF which drains across the cribriform plate into the nasal mucosa and cervical lymph nodes (Furukawa et al. 2008; Weller et al. 2009, 2010). Both CXCL12 and CCL19 are elevated in the CSF of patients with neuroinflammatory diseases (Pashenkov et al. 2003; Krumbholz et al. 2007) and have been correlated with total CSF cell number (Pashenkov et al. 2002, 2003), suggesting these chemokines may direct DC migration out of the CNS. Indeed, DCs expressing CCR7 have been detected in the CSF of MS patients (Kivisakk et al. 2004). These data suggest that CCR7 and possibly CXCR4 mediate DC migration from CNS to cervical lymph nodes; however, it is also possible that these chemokine receptors promote retention of DCs in the CNS. CCL19 and CCL21 have been detected perivascularly in the CNS during EAE (Alt et al. 2002) and have been implicated in the generation of tertiary lymphoid tissues in the CNS, which have been associated disease progression in both MS and EAE (Magliozzi et al. 2004, 2007; Serafini et al. 2004).

4.3 Dendritic Cells might Locally Restimulate Effector T-cells

In T-cell stimulation assays using antigen presenting cells (APCs) isolated from the CNS of mice with relapsing EAE, DCs were superior to monocyte and macrophages in restimulating effector T-cells *ex vivo*. Additionally, only DCs were capable of priming naïve T-cells specific for a non-immunizing antigen (Miller et al. 2007), suggesting that DCs may play an important role in T-cell restimulation and epitope spreading locally in the CNS. EAE can also be induced by adoptive transfer of preactivated myelin specific CD4⁺ T-cells. This model allows the dissection of afferent and efferent immune components as the activated effector T-cells no longer require secondary lymph tissue to induce disease; though they still require professional MHC II⁺ APCs in the target tissue in order “see” their antigen and carry out effector functions. Importantly, MHC II expression on CNS-resident APCs (such as microglia and astrocytes) has been shown to be dispensable for induction of adoptive transfer EAE, whereas MHC II expression on CNS-infiltrating APCs is required. Moreover, selective expression of MHC II only on CD11c⁺ DCs is sufficient for local restimulation of adoptively transferred encephalitogenic T-cells and EAE disease progression (Greter et al. 2005). These data provide strong evidence that CNS-infiltrating DCs are crucial for local restimulation of encephalitogenic effector T-cells.

We have previously shown that DCs may act as platforms for cooperative interactions between infiltrating T-cells. In our model system, *i.e.* injection of DCs loaded with multiple antigens synergistically promoted recruitment of antigen-specific TCR transgenic T-cells restricted to different MHC II molecules. This suggested that T-cell–T-cell cooperation occurs when antigens are presented on the same DC—a hypothesis that was corroborated in experiments where we showed *i.c.* injection of antigen fusion proteins promoted more antigen-specific T-cell recruitment than injection of mixtures of multiple antigens. Furthermore, following *i.c.* injection of two antigens into TCR transgenic mice with T-cells specific for one of the antigens, transfer of T-cells specific for the other antigen promoted

recruitment of host T-cells to the CNS—an effect not observed when antigens were injected in spatially distinct compartments. This could be blocked by retrovirally inhibiting CD40L or IL-2 expression in the donor T-cells, suggesting that perhaps paracrine IL-2 signaling between closely associated T-cells as well as CD40L-induced DC activation may be important for T-cell–T-cell cooperation (Karman et al. 2006). Recently, we generated transgenic mice that express OVA_{257–264}–OVA_{323–339}–PCC_{88–104} T-cell epitopes in oligodendrocytes (manuscript in preparation). We demonstrated that upon T-cell epitope upregulation in the CNS, DCs and adoptively transferred antigen-specific T-cells localize around oligodendrocytes in the inflamed brain and spinal cord of these mice (Fig. 4). These data suggest that initial neuroinflammation may induce oligodendrocyte death leading to the release of cytoplasmic antigens and recruitment of DCs, which capture and present antigen to T-cells locally or in regional lymph nodes—thus amplifying the neuroinflammatory processes.

4.4 Dendritic Cells Subsets Skew Effector T-cell Responses

According to the current view, at least four well-characterized helper T-cell lineages can be defined: Th1 cells predominantly secrete IFN- γ and are protective against intracellular pathogens; Th2 cells migrate to B cell areas within lymph nodes and promote humoral immunity by secreting IL-4, IL-5, and IL-13; Th17 promote mucosal immunity by secreting IL-17, IL-22, and IL-21 and have also been implicated in organ-specific autoimmunity; while Treg predominantly secrete IL-10 and are indispensable for maintenance of peripheral tolerance (reviewed in Weaver et al. 2007). DC-derived IL-12 promotes Th1 skewing. In its absence, Th2 differentiation is promoted by T-cell autocrine IL-4 signaling. TGF- β promotes the differentiation of Th17 and regulatory T-cells. In the presence of DC-derived IL-23/IL-6 responding T-cells differentiate into Th17 effector cells, whereas regulatory T-cell differentiation is promoted by incomplete T-cell activation due to increased expression of coinhibitory molecules relative to costimulatory molecules (reviewed in Romagnani 2006).

DC subsets differ in their propensity to skew responding effector T-cell phenotypes. For example, cDCs and pDCs can both induce Th1 or Th2 responses from responding T-cells; however, CD8⁺ cDCs are strongly Th1 skewing. Evidence from this comes from Id2^{−/−} mice, which are both selectively deficient in CD8⁺ DCs and strongly Th2 skewed despite the fact that Id2^{−/−} T-cells could be differentiated into Th1 cells *in vitro* (Kusunoki et al. 2003). As mentioned above, cDCs induce Th1 phenotype by IL-12 secretion. In contrast, pDCs promote Th1 differentiation with IFN- α (Ito et al. 2002). Additionally, pDCs are the only DC subset expressing IL-3R (CD123) and pDCs matured with IL-3 upregulate OX40L, which promotes secretion of Th2 cytokines from responding T-cells. Furthermore, TLR-matured pDCs but not cDCs upregulate ICOS-L as part of their maturation process and induce IL-10-producing Tregs (Ito et al. 2007). DC subsets also differ in their ability to promote Th17 differentiation. In mice, resistance to experimental asthma is associated with diminished Th17 responses, reduced IL-23 secretion, and lower cDC to pDC ratios in lung infiltrate. Interestingly, disease susceptibility could be restored by transferring antigen-loaded cDCs to lung tissue (Lewkowich et al. 2008). This is consistent with previous reports of deficient IL-23 translation in TLR-stimulated pDCs compared to cDCs (Waibler et al. 2007).

It has been demonstrated that CD11b⁺ DCs isolated from mouse CNS during ongoing EAE are better activators of naïve and effector T-cells than CNS pDCs or CD8⁺ cDCs (Miller et al. 2007). As mentioned above, we have previously shown that i.c. mycobacterial infection promoted differential recruitment of DC subsets to the CNS, with increased recruitment of CD11c^{high} CD8⁺ cDCs to the CNS during mycobacterial infection. We have also shown that this differential recruitment was associated with differences in effector T-cell responses.

Specifically, mycobacterial infection promoted strongly Th1 skewed effector T-cell responses, whereas EAE was associated with both Th1 and Th17 responses. We also investigated whether pre-existing CNS mycobacterial infection could modulate T-cell responses during subsequent EAE. We observed that concomitant mycobacterial infection locally suppressed Th17 responses and partially ameliorated EAE in mice (Lee et al. 2008). This is in agreement with data showing that CD8⁺ cDCs are strongly Th1 polarizing (see above).

pDCs are a major source of type-1 IFNs *in vivo*. IFN- β is a widely used therapy for the treatment of relapsing remitting MS; however, its mechanism of action is unknown. Interestingly, IFN- β treatment has recently been shown to decrease the frequency of circulating cDCs relative to pDCs (Lopez et al. 2006). In addition, circulating pDCs isolated from MS patients have an immature phenotype and show deficient maturation in response to both IL-3 and CpG (Stasiolek et al. 2006). These data suggest that in the context of CNS autoimmunity pDCs may be tolerogenic. Indeed, IFN- β treatment has been shown to further suppress MHC II expression and increase expression of the coinhibitory molecule B7H1 on circulating pDCs in MS patients (Lande et al. 2008). Transient depletion of pDCs has been shown to exacerbate both acute EAE and relapse and is associated with increased CNS T-cell activation (Bailey-Bucktrout et al. 2008). Moreover, specifically suppressing MHC II surface expression on pDCs worsens disease (Irla et al. 2010), suggesting that pDCs are directly T-cell suppressive during EAE.

Recent research has revealed that T-cell lineages—once thought to be very rigid effector fates for differentiating CD4 T-cells—are in fact much more plastic, especially during early differentiation (Lee et al. 2009; Afzali et al. 2010; Zhu and Paul 2010). For example, *in vitro* differentiated Th2 effector memory cells can act as IL-10 secreting suppressor T-cells *in vivo* (Xu et al. 2010), and Th17 cells can be transformed into either Th1 or Th2 effector cells given the proper stimulus *ex vivo* (Zhu and Paul 2010). It has also been shown that Tregs can transform into both Th1 (Wei et al. 2009) and Th17 cells *in vitro* (Xu et al. 2007; Yang et al. 2008), in mice (Lochner et al. 2008), and in humans (Voo et al. 2009). This newly discovered plasticity might imply that target tissue factors, such as local DC subset composition and cytokine milieu, may largely determine the effector phenotype of infiltrating T-cells—an implication with far-reaching consequences for immunotherapy. For example, sterile immunity is the Holy Grail for vaccine research targeting latent *M. tuberculosis* infection. Yet it was recently suggested that vaccine-induced systemic immunity may be insufficient for bacterial clearance because the chronic granuloma is replete with tolerogenic DCs that shut down cytokine secretion in infiltrating lymphocytes (Schreiber et al. 2010). Likewise—in light of evidence showing Treg transformation into inflammatory Th1 and Th17—it is important to consider that DCs present locally in the inflamed CNS may represent obstacles for tolerance-based immunotherapies targeting autoimmune or chronic inflammatory diseases of the CNS.

5 Summary

During neuroinflammation cDCs, pDCs, and iDCs accumulate in the CNS; however, it seems that different DC subsets predominate in response to different neuroinflammatory stimuli. For example, while EAE promotes the recruitment of predominantly CD11b⁺ iDCs through GM-CSF, CCL2, and other chemokines; mycobacterial encephalitis promotes granuloma formation and is associated with the accumulation of CD8⁺ cDCs. CD8⁺ cDCs strongly skew T-cell responses toward an IFN- γ secreting Th1 phenotype whereas iDCs secrete IL-12 and IL-23, which promote Th1 and Th17 differentiation. A minor fraction of CNS-infiltrating DCs, pDCs are thought to promote Treg development during EAE, perhaps due to deficiency in IL-23 translation or the lack of viral stimuli, which would promote pDC

maturation. In contrast, during viral encephalitis pDCs can be selectively recruited to the CNS, perhaps through pDC-selective chemoattractants, such as CXCL10, Flt3L, or chemerin. These pDCs respond to viral stimuli by secreting IFN- α , which dominates the immune response and promotes IFN- γ secretion by Th1 cells (Fig. 5). We also outlined evidence indicating that CNS-DCs can restimulate effector T-cells locally and/or migrate to cervical lymph nodes where they prime naïve T-cells, both of which might shape immune responses in the CNS. Despite these advances in our understanding, many questions remain unanswered. What chemokines govern DC recruitment across the glia limitans? To what extent do DCs migrate into the inflamed CNS via the choroid plexus? How do CNS danger- and pathogen-associated molecular patterns dictate the chemokine expression patterns that govern selective DC migration? How do DCs migrate out of the CNS to cervical lymph nodes? Answers to these questions might lead to better therapies for CNS autoimmune and chronic inflammatory diseases that target subset-specific modulation of DC migration and activation status.

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References

- Afzali B, Mitchell P, Lechler RI, John S, Lombardi G. Translational mini-review series on Th17 cells: induction of interleukin-17 production by regulatory T cells. *Clin Exp Immunol.* 2010; 159:120–130. [PubMed: 19912251]
- Agrawal S, Anderson P, Durbeek J, van Rooijen N, Ivars F, Opdenakker G, Sorokin LM. Dystroglycan is selectively cleaved at the parenchymal basement membrane at sites of leukocyte extravasation in experimental autoimmune encephalomyelitis. *J Exp Med.* 2006; 203:1007–1019. [PubMed: 16585265]
- Alt C, Laschinger M, Engelhardt B. Functional expression of the lymphoid chemokines CCL19 (ELC) and CCL 21 (SLC) at the blood-brain barrier suggests their involvement in G-protein-dependent lymphocyte recruitment into the central nervous system during experimental autoimmune encephalomyelitis. *Eur J Immunol.* 2002; 32:2133–2144. [PubMed: 12209625]
- Ambrosini E, Remoli ME, Giacomini E, Rosicarelli B, Serafini B, Lande R, Aloisi F, Coccia EM. Astrocytes produce dendritic cell-attracting chemokines in vitro and in multiple sclerosis lesions. *J Neuropathol Exp Neurol.* 2005; 64:706–715. [PubMed: 16106219]
- Bailey-Bucktrout SL, Caulkins SC, Goings G, Fischer JA, Dzionek A, Miller SD. Cutting edge: central nervous system plasmacytoid dendritic cells regulate the severity of relapsing experimental autoimmune encephalomyelitis. *J Immunol.* 2008; 180:6457–6461. [PubMed: 18453561]
- Bailey SL, Schreiner B, McMahon EJ, Miller SD. CNS myeloid DCs presenting endogenous myelin peptides ‘preferentially’ polarize CD4+ T(H)-17 cells in relapsing EAE. *Nat Immunol.* 2007; 8:172–180. [PubMed: 17206145]
- Banchereau J, Steinman RM. Dendritic cells and the control of immunity. *Nature.* 1998; 392:245–252. [PubMed: 9521319]
- Boven LA, Montagne L, Nottet HS, De Groot CJ. Macrophage inflammatory protein-1 α (MIP-1 α), MIP-1 β , and RANTES mRNA semiquantification and protein expression in active demyelinating multiple sclerosis (MS) lesions. *Clin Exp Immunol.* 2000; 122:257–263. [PubMed: 11091283]
- Brehin AC, Mouries J, Frenkiel MP, Dadaglio G, Despres P, Lafon M, Couderc T. Dynamics of immune cell recruitment during West Nile encephalitis and identification of a new CD19+B220-BST-2+ leukocyte population. *J Immunol.* 2008; 180:6760–6767. [PubMed: 18453596]
- Bullock K, Miller MM, Gal-Toth J, Milner TA, Gottfried-Blackmore A, Waters EM, Kaunzner UW, Liu K, Lindquist R, Nussenzweig MC, Steinman RM, McEwen BS. CD11c/EYFP transgene illuminates a discrete network of dendritic cells within the embryonic, neonatal, adult, and injured mouse brain. *J Comp Neurol.* 2008; 508:687–710. [PubMed: 18386786]

- Caux C, Vanbervliet B, Massacrier C, Ait-Yahia S, Vaure C, Chemin K, Dieu N Mc, Vicari A. Regulation of dendritic cell recruitment by chemokines. *Transplantation*. 2002; 73:S7–11. [PubMed: 11810053]
- Cella M, Jarrossay D, Facchetti F, Alebardi O, Nakajima H, Lanzavecchia A, Colonna M. Plasmacytoid monocytes migrate to inflamed lymph nodes and produce large amounts of type I interferon. *Nat Med*. 1999; 5:919–923. [PubMed: 10426316]
- Charles J, Di Domizio J, Salameire D, Bendriss-Vermare N, Asford C, Muhammad R, Lefebvre C, Plumas J, Leccia MT, Chaperot L. Characterization of circulating dendritic cells in melanoma: role of CCR6 in plasmacytoid dendritic cell recruitment to the tumor. *J Invest Dermatol*. 2010; 130:1646–1656. [PubMed: 20220766]
- Chinnery HR, Ruitenberg MJ, McMenamin PG. Novel characterization of monocyte-derived cell populations in the meninges and choroid plexus and their rates of replenishment in bone marrow chimeric mice. *J Neuropathol Exp Neurol*. 2010; 69:896–909. [PubMed: 20720507]
- Corcoran L, Ferrero I, Vremec D, Lucas K, Waithman J, O’Keeffe M, Wu L, Wilson A, Shortman K. The lymphoid past of mouse plasmacytoid cells and thymic dendritic cells. *J Immunol*. 2003; 170:4926–4932. [PubMed: 12734335]
- Cravens PD, Lipsky PE. Dendritic cells, chemokine receptors and autoimmune inflammatory diseases. *Immunol Cell Biol*. 2002; 80:497–505. [PubMed: 12225387]
- Cserr HF, Harling-Berg CJ, Knopf PM. Drainage of brain extracellular fluid into blood and deep cervical lymph and its immunological significance. *Brain Pathol*. 1992; 2:269–276. [PubMed: 1341962]
- Cudrici C, Ito T, Zafranskaia E, Niculescu F, Mullen KM, Vlaicu S, Judge SI, Calabresi PA, Rus H. Dendritic cells are abundant in non-lesional gray matter in multiple sclerosis. *Exp Mol Pathol*. 2007; 83:198–206. [PubMed: 17662270]
- Curtin JF, King GD, Barcia C, Liu C, Hubert FX, Guillonneau C, Josien R, Anegon I, Lowenstein PR, Castro MG. Fms-like tyrosine kinase 3 ligand recruits plasmacytoid dendritic cells to the brain. *J Immunol*. 2006; 176:3566–3577. [PubMed: 16517725]
- Day TA, Koch M, Nouailles G, Jacobsen M, Kosmiadi GA, Miekley D, Kuhlmann S, Jorg S, Gamradt P, Mollenkopf HJ, Hurwitz R, Reece ST, Kaufmann SH, Kursar M. Secondary lymphoid organs are dispensable for the development of T-cell-mediated immunity during tuberculosis. *Eur J Immunol*. 2010; 40:1663–1673. [PubMed: 20222088]
- de Vos AF, van Meurs M, Brok HP, Boven LA, Hintzen RQ, van der Valk P, Ravid R, Rensing S, Boon L, Hart BA, Laman JD. Transfer of central nervous system autoantigens and presentation in secondary lymphoid organs. *J Immunol*. 2002; 169:5415–5423. [PubMed: 12421916]
- del Pilar Martin M, Cravens PD, Winger R, Frohman EM, Racke MK, Eagar TN, Zamvil SS, Weber MS, Hemmer B, Karandikar NJ, Kleinschmidt-DeMasters BK, Stuve O. Decrease in the numbers of dendritic cells and CD4+ T cells in cerebral perivascular spaces due to natalizumab. *Arch Neurol*. 2008; 65:1596–1603. [PubMed: 18852339]
- Dogan RN, Elhofy A, Karpus WJ. Production of CCL2 by central nervous system cells regulates development of murine experimental autoimmune encephalomyelitis through the recruitment of TNF- and iNOS-expressing macrophages and myeloid dendritic cells. *J Immunol*. 2008; 180:7376–7384. [PubMed: 18490737]
- Engel D, Dobrindt U, Tittel A, Peters P, Maurer J, Gutgemann I, Kaissling B, Kuziel W, Jung S, Kurts C. Tumor necrosis factor alpha- and inducible nitric oxide synthase-producing dendritic cells are rapidly recruited to the bladder in urinary tract infection but are dispensable for bacterial clearance. *Infect Immun*. 2006; 74:6100–6107. [PubMed: 16966414]
- Fife BT, Huffnagle GB, Kuziel WA, Karpus WJ. CC chemokine receptor 2 is critical for induction of experimental autoimmune encephalomyelitis. *J Exp Med*. 2000; 192:899–905. [PubMed: 10993920]
- Fischer HG, Bonifas U, Reichmann G. Phenotype and functions of brain dendritic cells emerging during chronic infection of mice with *Toxoplasma gondii*. *J Immunol*. 2000; 164:4826–4834. [PubMed: 10779791]

- Furtado GC, Pina B, Tacke F, Gaupp S, van Rooijen N, Moran TM, Randolph GJ, Ransohoff RM, Chensue SW, Raine CS, Lira SA. A novel model of demyelinating encephalomyelitis induced by monocytes and dendritic cells. *J Immunol.* 2006; 177:6871–6879. [PubMed: 17082601]
- Furukawa M, Shimoda H, Kajiura T, Kato S, Yanagisawa S. Topographic study on nerve-associated lymphatic vessels in the murine craniofacial region by immunohistochemistry and electron microscopy. *Biomed Res.* 2008; 29:289–296. [PubMed: 19129672]
- Geissmann F, Jung S, Littman DR. Blood monocytes consist of two principal subsets with distinct migratory properties. *Immunity.* 2003; 19:71–82. [PubMed: 12871640]
- Gelderblom M, Leypoldt F, Steinbach K, Behrens D, Choe CU, Siler DA, Arumugam TV, Orthey E, Gerloff C, Tolosa E, Magnus T. Temporal and spatial dynamics of cerebral immune cell accumulation in stroke. *Stroke.* 2009; 40:1849–1857. [PubMed: 19265055]
- Gobel K, Pankratz S, Schneider-Hohendorf T, Bittner S, Schuhmann MK, Langer HF, Stoll G, Wiendl H, Kleinschmitt C, Meuth SG. Blockade of the kinin receptor B1 protects from autoimmune CNS disease by reducing leukocyte trafficking. *J Autoimmun.* 2011
- Greter M, Heppner FL, Lemos MP, Odermatt BM, Goebels N, Laufer T, Noelle RJ, Becher B. Dendritic cells permit immune invasion of the CNS in an animal model of multiple sclerosis. *Nat Med.* 2005; 11:328–334. [PubMed: 15735653]
- Hatterer E, Touret M, Belin MF, Honnorat J, Nataf S. Cerebrospinal fluid dendritic cells infiltrate the brain parenchyma and target the cervical lymph nodes under neuroinflammatory conditions. *PLoS ONE.* 2008; 3:e3321. [PubMed: 18830405]
- Hesske L, Vincenzetti C, Heikenwalder M, Prinz M, Reith W, Fontana A, Suter T. Induction of inhibitory central nervous system-derived and stimulatory blood-derived dendritic cells suggests a dual role for granulocyte-macrophage colony-stimulating factor in central nervous system inflammation. *Brain.* 2010; 133:1637–1654. [PubMed: 20424288]
- Hintzen G, Ohl L, del Rio ML, Rodriguez-Barbosa JI, Pabst O, Kocks JR, Kregge J, Hardtke S, Forster R. Induction of tolerance to innocuous inhaled antigen relies on a CCR7-dependent dendritic cell-mediated antigen transport to the bronchial lymph node. *J Immunol.* 2006; 177:7346–7354. [PubMed: 17082654]
- Hu Y, Ivashkiv LB. Costimulation of chemokine receptor signaling by matrix metalloproteinase-9 mediates enhanced migration of IFN- α dendritic cells. *J Immunol.* 2006; 176:6022–6033. [PubMed: 16670311]
- Ifergan I, Kebir H, Bernard M, Wosik K, Dodelet-Devillers A, Cayrol R, Arbour N, Prat A. The blood-brain barrier induces differentiation of migrating monocytes into Th17-polarizing dendritic cells. *Brain.* 2008; 131:785–799. [PubMed: 18156156]
- Irla M, Kupfer N, Suter T, Lissilaa R, Benkhoucha M, Skupsky J, Lalive PH, Fontana A, Reith W, Hugues S. MHC class II-restricted antigen presentation by plasmacytoid dendritic cells inhibits T cell-mediated autoimmunity. *J Exp Med.* 2010; 207:1891–1905. [PubMed: 20696698]
- Ito T, Amakawa R, Inaba M, Hori T, Ota M, Nakamura K, Takebayashi M, Miyaji M, Yoshimura T, Inaba K, Fukuhara S. Plasmacytoid dendritic cells regulate Th cell responses through OX40 ligand and type I IFNs. *J Immunol.* 2004; 172:4253–4259. [PubMed: 15034038]
- Ito T, Amakawa R, Kaisho T, Hemmi H, Tajima K, Uehira K, Ozaki Y, Tomizawa H, Akira S, Fukuhara S. Interferon- α and interleukin-12 are induced differentially by Toll-like receptor 7 ligands in human blood dendritic cell subsets. *J Exp Med.* 2002; 195:1507–1512. [PubMed: 12045249]
- Ito T, Yang M, Wang YH, Lande R, Gregorio J, Perng OA, Qin XF, Liu YJ, Gilliet M. Plasmacytoid dendritic cells prime IL-10-producing T regulatory cells by inducible costimulator ligand. *J Exp Med.* 2007; 204:105–115. [PubMed: 17200410]
- Izikson L, Klein RS, Charo IF, Weiner HL, Luster AD. Resistance to experimental autoimmune encephalomyelitis in mice lacking the CC chemokine receptor (CCR)2. *J Exp Med.* 2000; 192:1075–1080. [PubMed: 11015448]
- Jain P, Coisne C, Enzmann G, Rottapel R, Engelhardt B. $\alpha 4 \beta 1$ integrin mediates the recruitment of immature dendritic cells across the blood-brain barrier during experimental autoimmune encephalomyelitis. *J Immunol.* 2010; 184:7196–7206. [PubMed: 20483748]

- Karman J, Chu HH, Co DO, Seroogy CM, Sandor M, Fabry Z. Dendritic cells amplify T cell-mediated immune responses in the central nervous system. *J Immunol.* 2006; 177:7750–7760. [PubMed: 17114446]
- Karman J, Ling C, Sandor M, Fabry Z. Dendritic cells in the initiation of immune responses against central nervous system-derived antigens. *Immunol Lett.* 2004; 92:107–115. [PubMed: 15081534]
- King IL, Dickendesher TL, Segal BM. Circulating Ly-6C⁺ myeloid precursors migrate to the CNS and play a pathogenic role during autoimmune demyelinating disease. *Blood.* 2009; 113:3190–3197. [PubMed: 19196868]
- Kivisakk P, Mahad DJ, Callahan MK, Sikora K, Trebst C, Tucky B, Wujek J, Ravid R, Staugaitis SM, Lassmann H, Ransohoff RM. Expression of CCR7 in multiple sclerosis: implications for CNS immunity. *Ann Neurol.* 2004; 55:627–638. [PubMed: 15122702]
- Kohrgruber N, Groger M, Meraner P, Kriehuber E, Petzelbauer P, Brandt S, Stingl G, Rot A, Maurer D. Plasmacytoid dendritic cell recruitment by immobilized CXCR3 ligands. *J Immunol.* 2004; 173:6592–6602. [PubMed: 15557149]
- Kostulas N, Li HL, Xiao BG, Huang YM, Kostulas V, Link H. Dendritic cells are present in ischemic brain after permanent middle cerebral artery occlusion in the rat. *Stroke.* 2002; 33:1129–1134. [PubMed: 11935071]
- Krumbholz M, Theil D, Steinmeyer F, Cepok S, Hemmer B, Hofbauer M, Farina C, Derfuss T, Junker A, Arzberger T, Sinicina I, Hartle C, Newcombe J, Hohlfeld R, Meinl E. CCL19 is constitutively expressed in the CNS, up-regulated in neuroinflammation, active and also inactive multiple sclerosis lesions. *J Neuroimmunol.* 2007; 190:72–79. [PubMed: 17825430]
- Kusunoki T, Sugai M, Katakai T, Omatsu Y, Iyoda T, Inaba K, Nakahata T, Shimizu A, Yokota Y. TH2 dominance and defective development of a CD8⁺ dendritic cell subset in Id2-deficient mice. *J Allergy Clin Immunol.* 2003; 111:136–142. [PubMed: 12532109]
- Lamprecht P, Wieczorek S, Epplen JT, Ambrosch P, Kallenberg CG. Granuloma formation in ANCA-associated vasculitides. *APMIS Suppl.* 2009;32–36. [PubMed: 19515137]
- Lande R, Gafa V, Serafini B, Giacomini E, Visconti A, Remoli ME, Severa M, Parmentier M, Ristori G, Salvetti M, Aloisi F, Coccia EM. Plasmacytoid dendritic cells in multiple sclerosis: intracerebral recruitment and impaired maturation in response to interferon-beta. *J Neuropathol Exp Neurol.* 2008; 67:388–401. [PubMed: 18431257]
- Lanzavecchia A. Dendritic cell maturation and generation of immune responses. *Haematologica.* 1999; 84(Suppl EHA-4):23–25. [PubMed: 10907459]
- Lee J, Ling C, Kosmowski MM, Hulseberg P, Schreiber HA, Sandor M, Fabry Z. Intracerebral *Mycobacterium bovis* bacilli Calmette-Guerin infection-induced immune responses in the CNS. *J Neuroimmunol.* 2009a; 213:112–122. [PubMed: 19535154]
- Lee YK, Mukasa R, Hatton RD, Weaver CT. Developmental plasticity of Th17 and Treg cells. *Curr Opin Immunol.* 2009b; 21:274–280. [PubMed: 19524429]
- Lee J, Reinke EK, Zozulya AL, Sandor M, Fabry Z. *Mycobacterium bovis* bacille Calmette-Guerin infection in the CNS suppresses experimental autoimmune encephalomyelitis and Th17 responses in an IFN-gamma-independent manner. *J Immunol.* 2008; 181:6201–6212. [PubMed: 18941210]
- Lewkowich IP, Lajoie S, Clark JR, Herman NS, Sproles AA, Wills-Karp M. Allergen uptake, activation, and IL-23 production by pulmonary myeloid DCs drives airway hyperresponsiveness in asthma-susceptible mice. *PLoS ONE.* 2008; 3:e3879. [PubMed: 19060952]
- Li Q, Powell N, Zhang H, Belevych N, Ching S, Chen Q, Sheridan J, Whitacre C, Quan N. Endothelial IL-1R1 is a critical mediator of EAE pathogenesis. *Brain, Behavior, and Immunity.* 2011; 25:160–167.
- Ling C, Sandor M, Fabry Z. In situ processing and distribution of intracerebrally injected OVA in the CNS. *J Neuroimmunol.* 2003; 141:90–98. [PubMed: 12965258]
- Liu K, Nussenzweig MC. Origin and development of dendritic cells. *Immunol Rev.* 2010; 234:45–54. [PubMed: 20193011]
- Lochner M, Peduto L, Cherrier M, Sawa S, Langa F, Varona R, Riethmacher D, Si-Tahar M, Di Santo JP, Eberl G. In vivo equilibrium of proinflammatory IL-17⁺ and regulatory IL-10⁺ Foxp3⁺ RORgamma t⁺ T cells. *J Exp Med.* 2008; 205:1381–1393. [PubMed: 18504307]

- Lopez C, Comabella M, Al-zayat H, Tintore M, Montalban X. Altered maturation of circulating dendritic cells in primary progressive MS patients. *J Neuroimmunol.* 2006; 175:183–191. [PubMed: 16698091]
- Lowes MA, Chamian F, Abello MV, Fuentes-Duculan J, Lin SL, Nussbaum R, Novitskaya I, Carbonaro H, Cardinale I, Kikuchi T, Gilleaudeau P, Sullivan-Whalen M, Wittkowski KM, Papp K, Garovoy M, Dummer W, Steinman RM, Krueger JG. Increase in TNF-alpha and inducible nitric oxide synthase-expressing dendritic cells in psoriasis and reduction with efalizumab (anti-CD11a). *Proc Natl Acad Sci U S A.* 2005; 102:19057–19062. [PubMed: 16380428]
- Maglione PJ, Xu J, Chan J. B cells moderate inflammatory progression and enhance bacterial containment upon pulmonary challenge with *Mycobacterium tuberculosis*. *J Immunol.* 2007; 178:7222–7234. [PubMed: 17513771]
- Maglinozzi R, Columba-Cabezas S, Serafini B, Aloisi F. Intracerebral expression of CXCL13 and BAFF is accompanied by formation of lymphoid follicle-like structures in the meninges of mice with relapsing experimental autoimmune encephalomyelitis. *J Neuroimmunol.* 2004; 148:11–23. [PubMed: 14975582]
- Maglinozzi R, Howell O, Vora A, Serafini B, Nicholas R, Puopolo M, Reynolds R, Aloisi F. Meningeal B-cell follicles in secondary progressive multiple sclerosis associate with early onset of disease and severe cortical pathology. *Brain.* 2007; 130:1089–1104. [PubMed: 17438020]
- Matyszak MK, Perry VH. The potential role of dendritic cells in immune-mediated inflammatory diseases in the central nervous system. *Neuroscience.* 1996; 74:599–608. [PubMed: 8865208]
- McMenamin PG. Distribution and phenotype of dendritic cells and resident tissue macrophages in the dura mater, leptomeninges, and choroid plexus of the rat brain as demonstrated in wholemount preparations. *J Comp Neurol.* 1999; 405:553–562. [PubMed: 10098945]
- McMenamin PG, Wealhall RJ, Deverall M, Cooper SJ, Griffin B. Macrophages and dendritic cells in the rat meninges and choroid plexus: three-dimensional localisation by environmental scanning electron microscopy and confocal microscopy. *Cell Tissue Res.* 2003; 313:259–269. [PubMed: 12920643]
- Miller SD, McMahon EJ, Schreiner B, Bailey SL. Antigen presentation in the CNS by myeloid dendritic cells drives progression of relapsing experimental autoimmune encephalomyelitis. *Ann N Y Acad Sci.* 2007; 1103:179–191. [PubMed: 17376826]
- Milling S, Yrlid U, Cerovic V, MacPherson G. Subsets of migrating intestinal dendritic cells. *Immunol Rev.* 2010; 234:259–267. [PubMed: 20193024]
- Mitchell K, Yang HY, Berk JD, Tran JH, Iadarola MJ. Monocyte chemoattractant protein-1 in the choroid plexus: a potential link between vascular pro-inflammatory mediators and the CNS during peripheral tissue inflammation. *Neuroscience.* 2009; 158:885–895. [PubMed: 19032979]
- Naik SH, Sathe P, Park HY, Metcalf D, Proietto AI, Dakic A, Carotta S, O'Keeffe M, Bahlo M, Papenfuss A, Kwak JY, Wu L, Shortman K. Development of plasmacytoid and conventional dendritic cell subtypes from single precursor cells derived in vitro and in vivo. *Nat Immunol.* 2007; 8:1217–1226. [PubMed: 17922015]
- Nataf S, Strazielle N, Hatterer E, Mouchiroud G, Belin MF, Gherzi-Egea JF. Rat choroid plexuses contain myeloid progenitors capable of differentiation toward macrophage or dendritic cell phenotypes. *Glia.* 2006; 54:160–171. [PubMed: 16817190]
- Pachter JS, de Vries HE, Fabry Z. The blood-brain barrier and its role in immune privilege in the central nervous system. *J Neuropathol Exp Neurol.* 2003; 62:593–604. [PubMed: 12834104]
- Pashenkov M, Huang YM, Kostulas V, Haglund M, Soderstrom M, Link H. Two subsets of dendritic cells are present in human cerebrospinal fluid. *Brain.* 2001; 124:480–492. [PubMed: 11222448]
- Pashenkov M, Soderstrom M, Huang YM, Link H. Cerebrospinal fluid affects phenotype and functions of myeloid dendritic cells. *Clin Exp Immunol.* 2002; 128:379–387. [PubMed: 11985531]
- Pashenkov M, Soderstrom M, Link H. Secondary lymphoid organ chemokines are elevated in the cerebrospinal fluid during central nervous system inflammation. *J Neuroimmunol.* 2003; 135:154–160. [PubMed: 12576236]
- Plumb J, Armstrong MA, Duddy M, Mirakhor M, McQuaid S. CD83-positive dendritic cells are present in occasional perivascular cuffs in multiple sclerosis lesions. *Mult Scler.* 2003; 9:142–147. [PubMed: 12708809]

- Prodinger C, Bunse J, Kruger M, Schiefenhovel F, Brandt C, Laman JD, Greter M, Immig K, Heppner F, Becher B, Bechmann I. CD11c-expressing cells reside in the juxtavascular parenchyma and extend processes into the glia limitans of the mouse nervous system. *Acta Neuropathol.* 2010
- Randolph GJ, Angeli V, Swartz MA. Dendritic-cell trafficking to lymph nodes through lymphatic vessels. *Nat Rev Immunol.* 2005a; 5:617–628. [PubMed: 16056255]
- Randolph GJ, Beaulieu S, Lebecque S, Steinman RM, Muller WA. Differentiation of monocytes into dendritic cells in a model of transendothelial trafficking. *Science.* 1998; 282:480–483. [PubMed: 9774276]
- Randolph GJ, Sanchez-Schmitz G, Angeli V. Factors and signals that govern the migration of dendritic cells via lymphatics: recent advances. *Springer Semin Immunopathol.* 2005b; 26:273–287. [PubMed: 15338191]
- Randolph GJ, Sanchez-Schmitz G, Liebman RM, Schakel K. The CD16(+) (Fc-gammaRIII(+)) subset of human monocytes preferentially becomes migratory dendritic cells in a model tissue setting. *J Exp Med.* 2002; 196:517–527. [PubMed: 12186843]
- Reboldi A, Coisne C, Baumjohann D, Benvenuto F, Bottinelli D, Lira S, Uccelli A, Lanzavecchia A, Engelhardt B, Sallusto F. C-C chemokine receptor 6-regulated entry of TH-17 cells into the CNS through the choroid plexus is required for the initiation of EAE. *Nat Immunol.* 2009; 10:514–523. [PubMed: 19305396]
- Reichmann G, Schroeter M, Jander S, Fischer HG. Dendritic cells and dendritic-like microglia in focal cortical ischemia of the mouse brain. *J Neuroimmunol.* 2002; 129:125–132. [PubMed: 12161028]
- Robinson SC, Scott KA, Balkwill FR. Chemokine stimulation of monocyte matrix metalloproteinase-9 requires endogenous TNF-alpha. *Eur J Immunol.* 2002; 32:404–412. [PubMed: 11813159]
- Romagnani S. Regulation of the T cell response. *Clin Exp Allergy.* 2006; 36:1357–1366. [PubMed: 17083345]
- Romani N, Reider D, Heuer M, Ebner S, Kampgen E, Eibl B, Niederwieser D, Schuler G. Generation of mature dendritic cells from human blood. An improved method with special regard to clinical applicability. *J Immunol Methods.* 1996; 196:137–151. [PubMed: 8841452]
- Rosicarelli B, Serafini B, Sbriccoli M, Lu M, Cardone F, Pocchiari M, Aloisi F. Migration of dendritic cells into the brain in a mouse model of prion disease. *J Neuroimmunol.* 2005; 165:114–120. [PubMed: 15949848]
- Savarin C, Stohlman SA, Atkinson R, Ransohoff RM, Bergmann CC. Monocytes regulate T cell migration through the glia limitans during acute viral encephalitis. *J Virol.* 2010; 84:4878–4888. [PubMed: 20200240]
- Schreiber HA, Hulseberg PD, Lee J, Prechl J, Barta P, Szlavik N, Harding JS, Fabry Z, Sandor M. Dendritic cells in chronic mycobacterial granulomas restrict local anti-bacterial T cell response in a murine model. *PLoS ONE.* 2010; 5:e11453. [PubMed: 20625513]
- Serafini B, Columba-Cabezas S, Di Rosa F, Aloisi F. Intracerebral recruitment and maturation of dendritic cells in the onset and progression of experimental autoimmune encephalomyelitis. *Am J Pathol.* 2000; 157:1991–2002. [PubMed: 11106572]
- Serafini B, Rosicarelli B, Magliozzi R, Stigliano E, Aloisi F. Detection of ectopic B-cell follicles with germinal centers in the meninges of patients with secondary progressive multiple sclerosis. *Brain Pathol.* 2004; 14:164–174. [PubMed: 15193029]
- Serafini B, Rosicarelli B, Magliozzi R, Stigliano E, Capello E, Mancardi GL, Aloisi F. Dendritic cells in multiple sclerosis lesions: maturation stage, myelin uptake, and interaction with proliferating T cells. *J Neuropathol Exp Neurol.* 2006; 65:124–141. [PubMed: 16462204]
- Serbina NV, Pamer EG. Monocyte emigration from bone marrow during bacterial infection requires signals mediated by chemokine receptor CCR2. *Nat Immunol.* 2006; 7:311–317. [PubMed: 16462739]
- Serbina NV, Salazar-Mather TP, Biron CA, Kuziel WA, Pamer EG. TNF/ iNOS-producing dendritic cells mediate innate immune defense against bacterial infection. *Immunity.* 2003; 19:59–70. [PubMed: 12871639]
- Serot JM, Foliguet B, Bene MC, Faure GC. Ultrastructural and immunohistological evidence for dendritic-like cells within human choroid plexus epithelium. *Neuroreport.* 1997; 8:1995–1998. [PubMed: 9223091]

- Serot JM, Foliguet B, Bene MC, Faure GC. Intraepithelial and stromal dendritic cells in human choroid plexus. *Hum Pathol.* 1998; 29:1174–1175. [PubMed: 9781663]
- Shafteel SS, Carlson TJ, Olschowka JA, Kyrkanides S, Matousek SB, O'Banion MK. Chronic interleukin-1 β expression in mouse brain leads to leukocyte infiltration and neutrophil-independent blood brain barrier permeability without overt neurodegeneration. *J Neurosci.* 2007; 27:9301–9309. [PubMed: 17728444]
- Shortman K, Heath WR. The CD8⁺ dendritic cell subset. *Immunol Rev.* 2010; 234:18–31. [PubMed: 20193009]
- Shortman K, Naik SH. Steady-state and inflammatory dendritic-cell development. *Nat Rev Immunol.* 2007; 7:19–30. [PubMed: 17170756]
- Siegal FP, Kadowaki N, Shodell M, Fitzgerald-Bocarsly PA, Shah K, Ho S, Antonenko S, Liu YJ. The nature of the principal type 1 interferon-producing cells in human blood. *Science.* 1999; 284:1835–1837. [PubMed: 10364556]
- Simpson JE, Newcombe J, Cuzner ML, Woodroffe MN. Expression of the interferon-gamma-inducible chemokines IP-10 and Mig and their receptor, CXCR3, in multiple sclerosis lesions. *Neuropathol Appl Neurobiol.* 2000; 26:133–142. [PubMed: 10840276]
- Sorensen TL, Roed H, Sellebjerg F. Optic neuritis: chemokine receptor CXCR3 and its ligands. *Br J Ophthalmol.* 2004; 88:1146–1148. [PubMed: 15317705]
- Sorensen TL, Sellebjerg F, Jensen CV, Strieter RM, Ransohoff RM. Chemokines CXCL10 and CCL2: differential involvement in intrathecal inflammation in multiple sclerosis. *Eur J Neurol.* 2001; 8:665–672. [PubMed: 11784351]
- Stasiolek M, Bayas A, Kruse N, Wiczarkowicz A, Toyka KV, Gold R, Selmaj K. Impaired maturation and altered regulatory function of plasmacytoid dendritic cells in multiple sclerosis. *Brain.* 2006; 129:1293–1305. [PubMed: 16513684]
- Steel CD, Hahto SM, Ciavarra RP. Peripheral dendritic cells are essential for both the innate and adaptive antiviral immune responses in the central nervous system. *Virology.* 2009; 387:117–126. [PubMed: 19264338]
- Steinman RM, Adams JC, Cohn ZA. Identification of a novel cell type in peripheral lymphoid organs of mice. IV Identification and distribution in mouse spleen. *J Exp Med.* 1975; 141:804–820. [PubMed: 1127378]
- Steinman RM, Idoyaga J. Features of the dendritic cell lineage. *Immunol Rev.* 2010; 234:5–17. [PubMed: 20193008]
- Steinman RM, Pack M, Inaba K. Dendritic cell development and maturation. *Adv Exp Med Biol.* 1997; 417:1–6. [PubMed: 9286329]
- Suter T, Malipiero U, Otten L, Ludewig B, Muehlethaler-Mottet A, Mach B, Reith W, Fontana A. Dendritic cells and differential usage of the MHC class II transactivator promoters in the central nervous system in experimental autoimmune encephalitis. *Eur J Immunol.* 2000; 30:794–802. [PubMed: 10741394]
- Szmydynger-Chodobska J, Strazielle N, Zink BJ, Ghersi-Egea JF, Chodobski A. The role of the choroid plexus in neutrophil invasion after traumatic brain injury. *J Cereb Blood Flow Metab.* 2009; 29:1503–1516. [PubMed: 19471279]
- Tacke F, Randolph GJ. Migratory fate and differentiation of blood monocyte subsets. *Immunobiology.* 2006; 211:609–618. [PubMed: 16920499]
- Tezuka H, Ohteki T. Regulation of intestinal homeostasis by dendritic cells. *Immunol Rev.* 2010; 234:247–258. [PubMed: 20193023]
- Toft-Hansen H, Buist R, Sun XJ, Schellenberg A, Peeling J, Owens T. Metalloproteinases control brain inflammation induced by pertussis toxin in mice overexpressing the chemokine CCL2 in the central nervous system. *J Immunol.* 2006; 177:7242–7249. [PubMed: 17082642]
- Torres-Aguilar H, Aguilar-Ruiz SR, Gonzalez-Perez G, Munguia R, Bajana S, Meraz-Rios MA, Sanchez-Torres C. Tolerogenic dendritic cells generated with different immunosuppressive cytokines induce antigen-specific anergy and regulatory properties in memory CD4⁺ T cells. *J Immunol.* 2010; 184:1765–1775. [PubMed: 20083662]
- Trebst C, Sorensen TL, Kivisakk P, Cathcart MK, Hesselgesser J, Horuk R, Sellebjerg F, Lassmann H, Ransohoff RM. CCR1⁺/CCR5⁺ mononuclear phagocytes accumulate in the central nervous

- system of patients with multiple sclerosis. *Am J Pathol.* 2001; 159:1701–1710. [PubMed: 11696431]
- Trifilo MJ, Lane TE. The CC chemokine ligand 3 regulates CD11c+CD11b+CD8alpha-dendritic cell maturation and activation following viral infection of the central nervous system: implications for a role in T cell activation. *Virology.* 2004; 327:8–15. [PubMed: 15327893]
- van Zwam M, Huizinga R, Heijmans N, van Meurs M, Wierenga-Wolf AF, Melief MJ, Hintzen RQ, t Hart BA, Amor S, Boven LA, Laman JD. Surgical excision of CNS-draining lymph nodes reduces relapse severity in chronic-relapsing experimental autoimmune encephalomyelitis. *J Pathol.* 2009; 217:543–551. [PubMed: 19023878]
- Voo KS, Wang YH, Santori FR, Boggiano C, Arima K, Bover L, Hanabuchi S, Khalili J, Marinova E, Zheng B, Littman DR, Liu YJ. Identification of IL-17-producing FOXP3+ regulatory T cells in humans. *Proc Natl Acad Sci U S A.* 2009; 106:4793–4798. [PubMed: 19273860]
- Voswinkel J, Assmann G, Held G, Pitann S, Gross WL, Holl-Ulrich K, Herlyn K, Mueller A. Single cell analysis of B lymphocytes from Wegener's granulomatosis: B cell receptors display affinity maturation within the granulomatous lesions. *Clin Exp Immunol.* 2008; 154:339–345. [PubMed: 18826496]
- Waibler Z, Kalinke U, Will J, Juan MH, Pfeilschifter JM, Radeke HH. TLR-ligand stimulated interleukin-23 subunit expression and assembly is regulated differentially in murine plasmacytoid and myeloid dendritic cells. *Mol Immunol.* 2007; 44:1483–1489. [PubMed: 17052755]
- Weaver CT, Hatton RD, Mangan PR, Harrington LE. IL-17 family cytokines and the expanding diversity of effector T cell lineages. *Annu Rev Immunol.* 2007; 25:821–852. [PubMed: 17201677]
- Wei G, Wei L, Zhu J, Zang C, Hu-Li J, Yao Z, Cui K, Kanno Y, Roh TY, Watford WT, Schones DE, Peng W, Sun HW, Paul WE, O'Shea JJ, Zhao K. Global mapping of H3K4me3 and H3K27me3 reveals specificity and plasticity in lineage fate determination of differentiating CD4+ T cells. *Immunity.* 2009; 30:155–167. [PubMed: 19144320]
- Weller RO, Djuanda E, Yow HY, Carare RO. Lymphatic drainage of the brain and the pathophysiology of neurological disease. *Acta Neuropathol.* 2009; 117:1–14. [PubMed: 19002474]
- Weller RO, Galea I, Carare RO, Minagar A. Pathophysiology of the lymphatic drainage of the central nervous system: Implications for pathogenesis and therapy of multiple sclerosis. *Pathophysiology.* 2010; 17:295–306. [PubMed: 19954936]
- Wuest TR, Carr DJ. Dysregulation of CXCR3 signaling due to CXCL10 deficiency impairs the antiviral response to herpes simplex virus 1 infection. *J Immunol.* 2008; 181:7985–7993. [PubMed: 19017990]
- Xu L, Kitani A, Fuss I, Strober W. Cutting edge: regulatory T cells induce CD4+CD25-Foxp3- T cells or are self-induced to become Th17 cells in the absence of exogenous TGF-beta. *J Immunol.* 2007; 178:6725–6729. [PubMed: 17513718]
- Xu X, Guo Z, Jiang X, Yao Y, Gao Q, Ding Y, Cao X. Regulatory dendritic cells program generation of IL-4-producing alternative memory CD4 T cells with suppressive activity. *Blood.* 2010
- Yang XO, Nurieva R, Martinez GJ, Kang HS, Chung Y, Pappu BP, Shah B, Chang SH, Schluns KS, Watowich SS, Feng XH, Jetten AM, Dong C. Molecular antagonism and plasticity of regulatory and inflammatory T cell programs. *Immunity.* 2008; 29:44–56. [PubMed: 18585065]
- Yilmaz A, Fuchs T, Dietel B, Altendorf R, Cicha I, Stumpf C, Schellinger PD, Blumcke I, Schwab S, Daniel WG, Garlich CD, Kollmar R. Transient decrease in circulating dendritic cell precursors after acute stroke: potential recruitment into the brain. *Clin Sci.* 2010; 118:147–157. [PubMed: 19508233]
- Zaheer A, Sahu SK, Wu Y, Haas J, Lee K, Yang B. Diminished cytokine and chemokine expression in the central nervous system of GMF-deficient mice with experimental autoimmune encephalomyelitis. *Brain Res.* 2007; 1144:239–247. [PubMed: 17316572]
- Zhu J, Paul WE. Heterogeneity and plasticity of T helper cells. *Cell Res.* 2010; 20:4–12. [PubMed: 20010916]

- Zozulya AL, Ortler S, Fabry Z, Sandor M, Wiendl H. The level of B7 homologue 1 expression on brain DC is decisive for CD8 Treg cell recruitment into the CNS during EAE. *Eur J Immunol.* 2009a; 39:1536–1543. [PubMed: 19424967]
- Zozulya AL, Ortler S, Lee J, Weidenfeller C, Sandor M, Wiendl H, Fabry Z. Intracerebral dendritic cells critically modulate encephalitogenic versus regulatory immune responses in the CNS. *J Neurosci.* 2009b; 29:140–152. [PubMed: 19129392]
- Zozulya AL, Reinke E, Baiu DC, Karman J, Sandor M, Fabry Z. Dendritic cell transmigration through brain microvessel endothelium is regulated by MIP-1alpha chemokine and matrix metalloproteinases. *J Immunol.* 2007; 178:520–529. [PubMed: 17182592]

Fig. 1. CD11c-eYFP cells accumulate in the CNS following MOG immunization prior to clinical onset of EAE. CD11c-eYFP mice were immunized s.c. with MOG in complete Freund's adjuvant (CFA) and 5–12 days later their brains were isolated for analysis by fluorescent microscopy. CD11c-eYFP⁺ cells are shown in choroid plexus and lateral ventricles 5–12 days after immunization (*top*). CD11c-eYFP cells were first detected in the subpial space on day 12 after immunization (*bottom*)

Fig. 2. CD11c⁺ CD11b⁺ cells transiently accumulate in bone marrow following MOG immunization. CD11c-eYFP mice were immunized s.c. with MOG in CFA and 5–12 days later their bone marrow was isolated for analysis by flow cytometry using mAb for CD11b, CD8, and B220. Histograms show fraction of total mononuclear cell population expressing eYFP. *Dot plots* show representative surface expression of DC markers on bone marrow cells

Fig. 3. EAE and intracerebral (i.c.) BCG infection lead to differential recruitment of CD11c⁺ subsets. More CD8⁺, CD11c^{high} cells accumulate in brain tissue following BCG infection compared to EAE. 14 days after MOG immunization or 35 days after i.c. BCG infection mice were sacrificed and perfused transcardially. Single cell suspensions were prepared from brain, spinal cord, spleen, and cervical lymph nodes and stained with anti-CD11c mAb. **a** Graphs show total number of CD11c⁺ cells/gram tissue. CD11c⁺ cells accumulate in the brain of mice i.c. infected with BCG and the spinal cord of mice s.c. immunized with MOG. **b** Expression of CD11b and CD11c among total mononuclear cells in meninges and brain parenchyma is displayed. Boxes and numbers show percentage of CD11c^{low}CD11b⁺ and CD11c^{high}CD11b⁺ DC subsets infiltrating the brain (*left 2 columns*) and CD8 expression among total CD11c⁺ brain-infiltrating DCs (*right 2 columns*). **c** Brain cryosection from BCG infected mouse stained with anti-CD11c (green), anti-CD8 (red), and DAPI (blue). On upper image, *arrows* point at double positive CD8⁺CD11c⁺DCs at 1000× total magnification.

Dashed area is digitally magnified 4× and shown in lower image. *Arrowheads* on lower image point at dsRed BCG rods residing in a CD8⁺CD11c⁺DC

Fig. 4. Neuroinflammation leads to recruitment of DCs and effector T-cells specific for exogenously expressed CNS antigens. EAE was induced in transgenic mice expressing OVA_{257–264}-OVA_{323–339}-PCC_{88–104} epitopes in oligodendrocytes and 9 days later 5×10^5 Thy1.1 OVA_{257–264}-specific OT-I (MHC-I restricted) and Thy1.1 OVA_{323–339}-specific OT-II (MHC II restricted) T-cells were adoptively transferred into the Thy1.2 transgenic hosts. Six days after transfer brains were isolated for analysis by fluorescent microscopy using anti-CD11c APC, anti-Thy1.1 PE, anti-CD8 APC, rabbit anti-CNPase, and goat anti-rabbit. **a** Dotted lines indicate CD11c⁺ DCs (red) juxtaposed to CNPase⁺ oligodendrocytes (green). **b** Adoptively transferred Thy1.1⁺ (red) CD8⁺ (green) OT-I T-cells are detected in brain parenchyma. Blue staining represents DAPI

Fig. 5.

Different DC subsets dominate CNS immune responses during bacterial, viral, and autoimmune neuroinflammation. CNS BCG infections promote recruitment of CD11b⁺ and CD8⁺ CD11c^{hi} cDCs, the latter of which strongly skew T-cells toward a Th1 phenotype. In contrast, EAE predominantly favors recruitment of CD11b⁺CD11c⁺ iDCs, which secrete IL-12/IL-23, skewing T-cells toward Th1 and Th17 phenotypes. During viral encephalitis, CNS-infiltrating B220⁺ CD11c⁺ pDCs secrete copious amounts of IFN- α and thus skew T-cells toward Th1 phenotype