PERSPECTIVE

On the road to new treatments for multiple sclerosis: targeting dendritic cell migration into the central nervous system

Distinct migratory pathways and trafficking of dendritic cells to the central nervous system (CNS): The immune system is a host defense mechanism protecting against invaders, such as bacteria and viruses, while maintaining tolerance to self. Nonetheless, a few sites throughout the body are believed to be immunologically inert, such as the testes, the eye and the brain. Indeed, experiments in the mid-20th century gave rise to the concept of the brain as a site of immune privilege. Originally, the immune privilege of the brain was thought to be absolute, attributed by a physical blood-brain barrier (BBB) protecting the CNS from the entry of pathogens and circulating immune cells. These views have changed and currently, the CNS is seen as an immune-specialized site regulated by immunological components into and within the CNS. However, in neuroinflammatory disorders, such as multiple sclerosis (MS), the resident and infiltrating immune cells damage components of the CNS resulting in neurodegeneration. Among the various immune cells that infiltrate the CNS are dendritic cells (DCs), professional antigen-presenting cells capable to initiate both immunity and tolerance. DCs are known to transmigrate into the CNS during neuro-inflammation *via* different routes, one of them is through the activation and breakdown of the BBB. The infiltration of peripheral DCs in the CNS follow a classical multistep model, which are arbitrated by the expression of chemokine receptors and adhesion molecules on the surface of DCs (**Figure 1**). Previous findings from our group have demonstrated aberrant expression of migration markers and increased chemotaxis, besides aberrant expression of maturation markers, by circulating DCs of MS patients as compared to DCs from healthy controls (Thewissen et al., 2014). A better understanding of immune cell infiltration, explicitly DC transmigration into the CNS, can provide a better comprehension of the underlying processes driving neuroinflammation, such as in MS, ultimately moving forward the field by identifying new treatment targets. Indeed, although currently available therapeutics can modulate immune cell migration in general, selective hampering of pathogenic DC recruitment into the CNS in particular, might form the basis for the design of new therapeutic strategies for MS.

DC traffic *via* different migratory routes into the CNS during steady state and inflammation: DCs, professional antigen-presenting cells, serve as the sentinels of the immune system continuously surveying their local environment. Also in the brain, they play a role in the regulation of immune surveillance as well as in the development of neuroinflammation. For instance, selective disruption of the antigen-presenting capacity of DC renders mice completely resistant to neuroinflammation (Greter et al., 2005). In contrast, others found that depletion of DC results in aggravated disease in experimental autoimmune encephalomyelitis (EAE), the mouse model for MS (Yogev et al., 2012). DC populate the CNS in low numbers in the steady state and reside mostly in the perivascular regions suggestive of their peripheral origin. The majority of DCs in the CNS is thought to be derived from circulating bone marrow-derived precursor cells that have passed the barriers protecting the CNS. During neuroinflammation, DCs annihilate these barriers to different levels and overpopulate the CNS, resulting in a highly increased accumulation of DCs in cerebrospinal fluid (CSF) including the perivascular lesions. This augmented trafficking of DCs to the CNS is thought to be a result of increased activation of the barriers normally protecting the brain although their derivation and function during neuroinflammation still remains unclear.

Immune cells can invade the CNS *via* different routes (De Laere et al., 2018), the choroid plexus, the meninges and the BBB. The choroid plexus is considered to play an important role in leukocyte trafficking in the initial phases of neuroinflammatory disease. It consists of an epithelial layer, which constitutes the actual blood-CSF barrier, and expresses several tight junction proteins. Conventional DCs (cDCs) found in the CSF of MS patients display a higher expression of CD80, CD86 and CD40 suggesting a more mature phenotype than their blood counterparts (Pashenkov et al., 2001). Although several findings underscore the role of the choroid plexus in DC trafficking, the precise mechanisms involved in the process remain elusive.

Other than the choroid plexus, the meninges are also known to be suitable for entry of DCs into the CNS due to its constitutive high expression levels of adhesion molecules, in particular intercellular adhesion molecule 1 (ICAM-1). DCs are known to be present at the meningeal sites in healthy human CNS. Also, in EAE, DCs were found in the meninges before they further accumulate in the spinal cord parenchyma. Nonetheless, the specific homeostatic recruitment of DCs to the meninges still remains indefinable and requires further studies focusing on the mechanisms involved.

Among these routes of entry, the BBB is considered to be the most ideal and the best-studied site of immune cell trafficking to the CNS. The BBB plays both a role in immune cell recruitment to the CNS under normal conditions but is activated and broken down during neuroinflammation, being even more permissive for immune cell migration into the CNS. The BBB has a specialized structure which typically constitutes of tight junctions and low basal expression of adhesion molecules including ICAM-1, vascular cell adhesion protein-1 (VCAM-1) and P- and E-selectin, whereas ICAM-2 and platelet endothelial cell adhesion molecule are expressed at high levels in cerebral microvessels under non-inflammatory conditions. The expression of adhesion molecules ICAM-1 and VCAM-1 is upregulated following inflammation while ICAM-2 expression seems to be significantly downregulated upon pro-inflammatory inflammation as previously presented by our group (De Laere et al., 2017). Also, the expression of P- and E-selectin is upregulated in MS patients. The occurrence of an increase in immune cell infiltration across the BBB, uncontrolled activation and antigen presentation in MS is indicative of a compromised BBB during neuroinflammation. In a healthy CNS, both the mature and immature monocyte-derived DCs adhere to the human brain endothelial cells, albeit that immature DCs adhere with a greater efficacy when compared with the mature population as demonstrated by intravital fluorescence video microscopy (Jain et al., 2010). The adhesion molecules ICAM-1, ICAM-2, VCAM-1 and platelet endothelial cell adhesion molecule on the endothelial cell layer of the BBB facilitate this adherence by immature DCs, whereas only ICAM-1 plays a role in the adherence of mature DCs. In relapsing-remitting (RR) MS and chronic progressive MS patients, higher numbers of CCR5-expressing cDCs and pDCs are seen when compared to their healthy counterparts, in addition to upregulated expression of CCR7 on pDC of RRMS patients. Similarly, also monocyte-derived DCs from MS patients expressed significantly higher levels of CCR7 when compared with healthy controls (Nuyts et al., 2014).

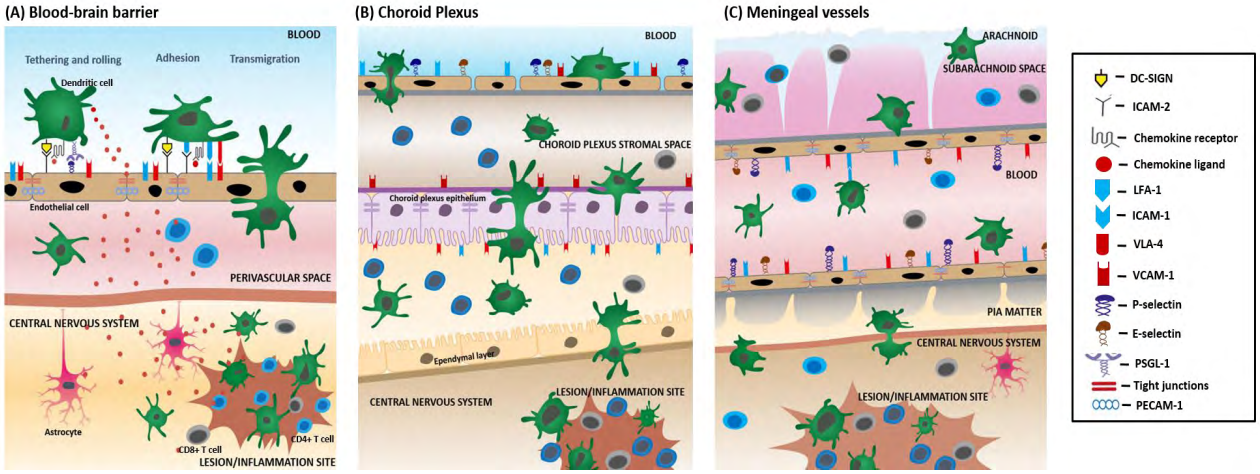

Figure 1 Different routes of entry of DCs to the CNS parenchyma following neuroinflammation.
(A) **Blood-brain barrier:** DC undergo the migration process through the BBB in different steps. In the steady state DCs are normally circulating in the bloodstream and crosstalk with the brain endothelium layer *via* several factors. DC interaction with the endothelial cell of the brain proceeds in a step-wise manner both in steady state and during inflammation. These cells interact with the ICAM-2/3 expressed on the EC which binds to the DC-SIGN expressed on the DCs. Additionally the chemokine receptors binding to their respective ligand leads to the integrin activation resulting in the rolling of DCs on the endothelium. DCs also express PSGL-1 which interacts with P/E-selectins on the endothelial cell layer. Further DC interact with ICAM-1 on the EC *via* LFA-1 expressed on the DC surface leading to firm adhesion to the EC layer. In normal conditions, very low number of DCs are observed in the perivascular region with almost none in the CNS. While in the inflamed state the EC layer is highly activated with a highly increased expression of the adhesion molecules including ICAM-1 and VCAM-1. This results in a higher DC interaction and adhesion to the EC and hence a greater migration to the MS lesion sites. Along with DC different subsets of T cells ($CD4^+$ and $CD8^+$) also infiltrate the CNS and are present in the inflammation sites. (B) **Choroid plexus:** In a healthy state, low number of DC migrate through the stromal space *via* the CP epithelium but no DC invade the CNS parenchyma. Whereas, in the inflammatory conditions the different layers of the choroid plexus are again activated with an increased number of selectins and activation molecule expression leading to a much higher invasion of DC towards the lesion sites in MS. (C) **Meningeal vessels:** Similar to the CP, under normal conditions DC remain circulating in the subarachnoid spaces although a drastically high number is observed during inflammation where DC interact with the highly expressed adhesion molecules and proceed to move towards the CNS along with T cells. Despite several ligands involved in the process of attachment and transmigration of DC to the epithelium in choroid plexus and meninges, their involvement and salient role in the different steps of DC migration still remains to be evaluated. Adapted from De Laere et al. (2018). BBB: Blood-brain barrier; CNS: central nervous system; EC: endothelial cells; DC-SIGN: dendritic cell-specific ICAM-grabbing nonintegrin; ICAM-1: intercellular adhesion molecule-1; CCL: chemokine ligand; CCR: chemokine receptor; LFA-1: lymphocyte function-associated antigen-1; VLA-4: very late antigen-4; VCAM: vascular cell adhesion molecule; CP: choroid plexus; CSF: cerebrospinal fluid; DC: dendritic cell; ICAM-2: intercellular adhesion molecule-2; MS: multiple sclerosis; PECAM-1: platelet and endothelial cell adhesion molecule-1; PSGL: P-selectin glycoprotein ligand.

The development of realistic *in vitro* BBB models that imitate the *in vivo* expression of enzymes, transporters, receptors, and structural proteins on the BBB will demonstrate to be an invaluable tool for understanding the pathological factors involved in the development of various CNS disorders. For instance, using a static *in vitro* BBB model, we previously showed that the inflammatory chemokine CCL3 alone was unable to drive the transmigration of the immune cell *via* the BBB under inflammatory conditions *in vitro* (De Laere et al., 2017).

Current therapeutic strategies for MS amend the migration of DCs into the CNS: Although a cure for MS is still lacking, the medical arsenal for the treatment of MS expands continuously. These disease-modifying therapies have been shown to slow disease progression and prevent disability symptoms, thereby improving the course of MS. Whereas most of the currently available treatments function in an anti-inflammatory fashion, pharmacological targeting of immune cell trafficking to the CNS during pathogenesis also presents an attractive treatment strategy for MS. One of the first biological treatments specifically developed to intervene with migration of immune cells was natalizumab. It is a humanized monoclonal antibody that selectively binds to the α4-chain of $\alpha_4\beta_1$- and $\alpha_4\beta_7$-integrins expressed on the surface of human leukocytes. In doing so, natalizumab directly interferes with the adhesion of immune cells to endothelial cell layers including the BBB. It was observed that treatment of MS patients with natalizumab reduces the proportion of $\alpha_4\beta_1$-expressing circulating pDCs and cDCs after 48 hours of initiating therapy and consequently the coagulation of DCs in the perivascular space of RRMS patients (Andrés et al., 2012). Fingolimod, a sphingosine-1-phosphate receptor, has also been demonstrated to affect the migratory capacity of immune cells. In particular, fingolimod traps the lymphocytes in the lymph nodes thereby preventing their migration to the inflamed CNS. DCs, among other immune cells, express the sphingosine-1-phosphate receptor isoforms 1–4 that are mainly targeted by fingolimod. In EAE, treatment with fingolimod resulted in a significant decrease in CCR7 expression by circulating DC and *in vitro* generated bone marrow-derived DC. This observation was further reflected by a reduced *in vitro* migratory capacity by DCs towards the CCL19 chemokine (Lan et al., 2005). Moreover, *in vitro* treatment of monocyte-derived DCs with therapeutic doses of fingolimod resulted in a dose-dependent reduction in chemotaxis, albeit without altering the chemokine receptor expression by DCs. Also, interferon-β treatment is shown to ameliorate the symptoms in patients with MS *via* modulation of the expression of migration-associated molecules by various immune cells, besides the general immune-modulatory function of interferon-β. In doing so, interferon-β reduces the amassing of inflammatory cells in the BBB. For instance, IFN-β treatment in MS patients resulted in downregulation of the expression of CCR7 in MS-derived pDCs upon TLR9 stimulation similar to the expression level on pDCs from healthy controls (Aung et al., 2010).

Despite the increased efficacy of these drugs in the treatment of MS, they also pose some downsides and sometimes life-threatening adverse events. For instance, natalizumab therapy was seen to be associated with an increased risk of progressive multifocal leukoencephalopathy, caused by the JC virus (Sadiq et al., 2010). This is thought to be a consequence of broad-spectrum targeting of immune cell trafficking across the blood-brain barrier to the CNS thereby impairing regular immune surveillance of the brain. Hence, selective interference with the recruitment of inflammatory DCs towards the CNS and consequently of their accumulation at strategic locations within the CNS will aid in the design of novel therapeutic strategies to tackle the neuro-inflammation in MS. In this context, a variety of molecules, known to be involved in DC migration, could be key including adhesion molecules, matrix metalloproteinase, chemokine receptors and/or the signaling molecules including NF-κB and extracellular signal-regulated kinases. These specialized molecules can be specifically exploited to indirectly or directly interfere with DC recruitment to the CNS. For instance, neutralizing antibodies for CCR1 and CCR2 have been tested in several clinical trials in MS but with rather disappointing results where these agents failed to meet the therapeutic expectations showing almost no efficacy. Antagonists against CCR5 are also being tested in clinical trials for the treatment of autoimmune disorders other than MS and show positive results on the blocking of DC trafficking in the human immunodeficiency virus infection. Moreover, G protein-coupled receptors, which are also known to play a vital role in the control of DC migration, are being explored for their therapeutic potential as antibody target. Additionally, MMP inhibitors are being clinically explored and tested in an early phase of development as therapeutic compounds in inflammatory diseases such as MS, and are shown to display a higher specificity and efficacy (De Laere et al., 2018). Nonetheless, caution needs to be paid while targeting these specialized migratory-associated molecules as their expression is likely not to be restricted to disease-causing DCs, but also on tolerance-inducing DC subsets and other lymphocytes resulting in aggravation of the disease instead of the anticipated amelioration. Hence, there is still an increased need for additional studies evaluating the effectiveness and the possibility to use DC-specific approaches as a treatment for autoimmune diseases. Addressing DC-targeting strategies, alone or in combination with established therapies, will improve our ability to limit disease activity and progression in patients with MS.

Conclusion and future perspective: Migration of immune cells, among other immune effector cell functions, serves as a vital therapeutic target of several of the approved drugs for MS. Future studies explicating the mechanisms and forces specifically driving DC accumulation in the meninges, choroid plexus and in the CSF, will provide a better understanding of the underlying disease processes. In this context, accurate three-dimensional models of the BBB will aid in moving the study forward towards the direction of developing new therapies for various neuroinflammatory disorders.

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References

Aung LL, Fitzgerald-Bocarsly P, Dhib-Jalbut S, Balashov K (2010) Plasmacytoid dendritic cells in multiple sclerosis: Chemokine and chemokine receptor modulation by interferon-beta. J Neuroimmunol 226:158-164.

De Andrés C, Teijeiro R, Alonso B, Sánchez-Madrid F, Martínez ML, de Villoria JG, Fernández-Cruz E, Sánchez-Ramón S (2012) Long-term decrease in VLA-4 expression and functional impairment of dendritic cells during natalizumab therapy in patients with multiple sclerosis. PLoS One 7:e34103.

De Laere M, Berneman ZN, Cools N (2018) To the brain and back: migratory paths of dendritic cells in multiple sclerosis. J Neuropathol Exp Neurol 77:178-192.

De Laere M, Sousa C, Meena M, Buckinx R, Timmermans JP, Berneman Z, Cools N (2017) Increased transendothelial transport of CCL3 is insufficient to drive immune cell transmigration through the blood-brain barrier under inflammatory conditions in vitro. Mediators Inflamm 2017:6752756.

Greter M, Heppner FL, Lemos MP, Odermatt BM, Goebels N, Laufer T, Noelle RJ, Becher B (2005) Dendritic cells permit immune invasion of the CNS in an animal model of multiple sclerosis. Nat Med 11:328-334.

Jain P, Coisne C, Enzmann G, Rottapel R, Engelhardt B (2010) α4β1 integrin mediates the recruitment of immature dendritic cells across the blood-brain barrier during experimental autoimmune encephalomyelitis. J Immunol 184:7196-7206.

Lan YY, De Creus A, Colvin BL, Abe M, Brinkmann V, Coates PT, Thomson AW (2005) The sphingosine-1-phosphate receptor agonist FTY720 modulates dendritic cell trafficking in vivo. Am J Transplant 5:2649-2659.

Nuyts AH, Ponsaerts P, Van Tendeloo VF, Lee WP, Stein B, Nagels G, D'hooghe MB, Willekens B, Cras P, Wouters K, Goossens H, Berneman ZN, Cools N (2014) Except for CC chemokine receptor 7 expression, monocyte-derived dendritic cells from patients with multiple sclerosis are functionally comparable to those of healthy controls. Cytotherapy 16:1024-1030.

Pashenkov M, Huang YM, Kostulas V, Haglund M, Söderström M, Link H (2001) Two subsets of dendritic cells are present in human cerebrospinal fluid. Brain 124:480-492.

Sadiq SA, Puccio LM, Brydon EW (2010) JCV detection in multiple sclerosis patients treated with natalizumab. J Neurol 257:954-958.

Thewissen K, Nuyts AH, Deckx N, Wijmeersch BV, Nagels G, D'hooghe M, Willekens B, Cras P, Eijnde BO, Goossens H, Van Tendeloo VF, Stinissen P, Berneman ZN, Hellings N, Cools N (2014) Circulating dendritic cells of multiple sclerosis patients are proinflammatory and their frequency is correlated with MS-associated genetic risk factors. Mult Scler 20:548-557.

Yogev N, Frommer F, Lukas D, Kautz-Neu K, Karram K, Ielo D, von Stebut E, Probst HC, van den Broek M, Riethmacher D, Birnberg T (2012) Dendritic cells ameliorate autoimmunity in the CNS by controlling the homeostasis of PD-1 receptor+ regulatory T cells. Immunity 37:264-275.

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