REVIEW ARTICLE

Inflammation in the Central Nervous System: the Role for Dendritic Cells

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Dendritic cells (DCs) are a subclass of antigen-presenting cells critical in the initiation and regulation of adaptive immunity against pathogens and tumors, as well as in the triggering of autoimmunity. Recent studies have provided important knowledge regarding distribution of DCs in the central nervous system (CNS) and their role in intrathecal immune responses. DCs are present in normal meninges, choroid plexus, and cerebrospinal fluid, but absent from the normal brain parenchyma. Inflammation is accompanied by recruitment and/or development of DCs in the affected brain tissue. DCs present in different compartments of the CNS are likely to play a role in the defence against CNS infections, and also may contribute to relapses/chronicity of CNS inflammation and to break-down of tolerance to CNS autoantigens. CNS DCs can therefore be viewed as a future therapeutic target in chronic inflammatory diseases such as multiple sclerosis.

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Dendritic Cells and the Central Nervous System: Old Concept Challenged

The brain is traditionally termed "an immunoprivileged site." The basis for this has been laid by observations that many foreign antigens inoculated into the parenchyma of the brain, such as allografts or heat-killed BCG, escape recognition by the adaptive immune system (48, 53). However, brain pathogens and even autoantigens can be targeted by T-cell responses, which play a pivotal role in a variety of inflammatory diseases of the central nervous system (CNS), ranging from infections to multiple sclerosis (MS) (23, 39). To cross the blood-brain barrier (BBB), T-cells directed against foreign or self antigens residing in the brain must be activated in the periphery (30). In experimental animals, this is often achieved by peripheral immunization with respective antigens emulsified in complete Freund's adjuvant (45, 90). In real human diseases, however, the mechanisms behind activation of the brain-directed T-cells are far less clear.

The key role in the initiation and regulation of T-cell responses belongs to dendritic cells (DCs) (1). Immature DCs, located in non-lymphoid tissues, efficiently endocytose antigens, but cannot yet present them. Additional immature DCs are recruited from surrounding tissues and blood to the sites of inflammation, microbial invasion, and/or tumor growth (52, 68). Microbial products, inflammatory cytokines, and products of tissue necrosis trigger maturation of DCs, which then lose the ability to take up antigens, but upregulate the expression of MHC and costimulatory molecules, becoming the most potent antigen presenting cells (APCs) in the body (22, 70). Mature DCs migrate via afferent lymphatics to T-cell areas of draining lymph nodes and activate naive T-cells (81). Mature DCs are virtually the only type of APCs that can induce a primary T-cell response (1). Aimed at elimination of pathogens and tumors, DCs can also trigger responses against autoantigens released in tissues during inflammation; one prerequisite for this is sustained presentation of the autoantigens by a sufficiently large number of mature DCs (15, 42, 43).

Until recently, this concept did not appear to apply to the CNS, which was presumed to be devoid of DCs. However, the pathway connecting the CNS with the lymphatic system, in particular with deep cervical lymph nodes (DCLNs), had been studied in detail (29). In rodents, soluble antigens injected into either brain ventricles or brain parenchyma reach the DCLNs equally well (via perineural and periarterial spaces of the olfactory nerves—submucosa of the upper nasal ducts—conventional afferent lymphatics) (5, 12, 29, 41). In both cases, T-cell-dependent antibody production is induced in the DCLNs, indicating that the immune privilege of the brain is not absolute (25, 29). However, this antibody response is much stronger when the proteins are injected into the cerebrospinal fluid (CSF), as compared to injections into the brain parenchyma (25, 29). The difference is even more striking with particulate antigens, less prone to passive diffusion: when carefully inoculated into the brain parenchyma, they are not recognized by the adaptive immune system; when inoculated

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	CSF compartment*	Brain parenchyma
Drainage of soluble proteins to DCLNs	Yes	Yes
Immune responses to antigens injected into respective compartments		
Soluble proteins	Ab production in DCLNs	More delayed and weak Ab response
Heat-killed BCG	DTH response at the site of injection	No systemic response
Live influenza virus	Proliferation in DCLNs, cytotoxic T-cell response	No systemic response initially, very delayed T-cell response in a proportion of animals
DCs	Present	Absent

* Including meninges and choroid plexus

Table 1. Comparison of immune responses induced from different compartments of normal CNS and their relationship with DCs (5, 28, 29, 46, 47, 50, 60, 74, 82, 84, 87).

into the CSF, they induce primary T-cell responses as in conventional sites (47, 82; Table 1).

Studies of DCs provide a clue to this dissociation. Vass and colleagues were probably the first to report that normal rat leptomeninges contain a distinct population of dendriform cells located predominantly around blood vessels, constitutively expressing of MHC class II molecules, and apparently capable of taking up protein antigens, such as horseradish peroxidase, injected into the CSF (87). Using a panel of double immunostainings, McMenamin subsequently confirmed these MHC class II-positive cells to be DCs and also demonstrated their presence in the rat dura mater (50). Several groups detected DCs in normal choroid plexus in the rat and in man (28, 46, 50). Thus, DCs are normally present in all non-neural structures that are in direct contact with the CSF (Table 1). Notably, no other cells in rat meninges and choroid plexus express MHC class II under normal conditions (50). Furthermore, mature DCs injected into the subarachnoid space of the rat migrate via the above-mentioned olfactory pathway to T-cell areas of the DCLNs (7), suggesting that meningeal and choroid plexus DCs, after maturation, can travel by the same route and induce a primary T-cell response against pathogens invading the CSF compartment.

In contrast, normal brain parenchyma contains no DCs (46, 74, 84) and thus no vehicle to transport antigens to DCLNs in the form recognizable by T-cells. Antigens reaching the DCLNs on their own would encounter lymph node DCs, most of which are mature and hence have low capacity of antigen uptake (70, 81). The reason for the absence of DCs from normal brain parenchyma is unclear: either DCs are not recruited or do not survive there (40).

However, in case of infection or autoimmune inflammation, DCs do appear in the affected brain parenchyma. In acute models, such as acute experimental autoimmune encephalomyelitis (EAE) in Lewis rats, DC infiltration in the brain is relatively minor and restricted to perivascular cuffs (46). In models of chronic CNS inflammation, such as delayed-type hypersensitivity (DTH) reaction against BCG sequestered in the brain parenchyma (46), chronic EAE (19, 74, 84), and experimental toxoplasmic encephalitis (18), brain DCs are numerous and located not only perivascularly, but also throughout the brain parenchyma. During the initial phase of chronic EAE, DCs appear in the brain relatively late, after infiltration of macrophages and simultaneously with the onset of clinical disease (84), suggesting their involvement in chronicity of inflammation, rather than in its initiation.

The source of brain DCs, particularly in mouse EAE, may be diverse. Based on perivascular localization of these cells, their phenotype ($CD11b^{-}CD11c^{+}DEC\text{-}205^{+}$) and co-localization with cells expressing the chemokine CCL20 (MIP-3α), Serafini et al suggested that brain DCs are recruited from the systemic circulation (74). On the other hand, Fischer and colleagues described $CD11b^{+}CD11c^{low/+}$ $DEC\text{-}205^{-}$ myeloid brain DCs (18, 19) and generated similar cells from glial cultures (17),

	Myeloid DCs	Plasmacytoid DCs
Morphological features	Pseudopods, vacuolized cytoplasm	Smooth plasma membrane, abundant endoplasmic reticulum
Surface markers		
CD11c	++	–
CD123 (α-chain of IL-3 receptor)	+/–	++
Growth factor	GM-CSF	IL-3
Differentiation into macrophages	Yes	No
Phagocytosis	Yes	No
HLA-DR (MHC class II)	++	+
Co-stimulatory molecules (CD80, CD86)	+	+/–
Ability to activate naive T-cells	++	– †
Production of IFN-α/β	+/–	+++

* Data refer to DC subsets freshly isolated from blood of healthy donors.
† Become potent T-cell stimulators after a maturation process, which can be induced by enveloped viruses, TNF-α or CD40L and takes a few days.

Table 2. Comparison of myeloid and plasmacytoid blood DCs (34, 37, 69, 77)*.

suggesting DC development from microglia or another co-isolated CNS-resident DC precursor; the latter possibility has been confirmed by another group (73). Different types of DCs may have been described in these reports, and their proportions in the total pool of brain DCs remain to be determined. DCs isolated from the chronically inflamed brain are mature, as they produce IL-12 and can activate naive T-cells (18, 19); intrathecal maturation of brain DCs is likely to be driven by inflammatory cytokines and infiltrating T-cells expressing CD40 ligand (9, 24). Importantly, as noted by Fischer and Reichmann (19), DCs do not require IFN-γ for their optimal function, which distinguishes them from other types of brain APCs. A key question to be answered is whether mature brain DCs can migrate to DCLNs or other secondary lymphoid organs.

DCs in Human CSF

The interior of human body is surveyed by two DC types: myeloid DCs and plasmacytoid DCs. Both of them circulate in blood (37, 69, 77; Table 2). Circulating myeloid DCs are potent APCs, while plasmacytoid DCs, when freshly isolated from blood, essentially lack this capacity (37). At the same time, circulating plasmacytoid DCs produce large amounts of type I interferons (IFNs) upon infection with enveloped viruses and certain bacteria (77), which makes them important effector cells of the anti-infectious immunity. Plasmacytoid DCs become strong APCs after a several-day maturation process, which can be induced in vitro by enveloped viruses, cytokines such as TNF-α, or CD40 ligand (26, 34, 37).

We have recently reported that human CSF contains both myeloid and plasmacytoid DCs, phenotypically similar to those circulating in blood (60-62). A few cells of each subset are present in the CSF in non-inflammatory neurological diseases (NIND; Figure 1). This is expected, as DCs are normally present in tissues that surround the CSF space. Numbers of CSF DCs are elevated in clinically definite MS, early MS in the form of acute monosymptomatic optic neuritis (ON) with oligoclonal IgG in the CSF, acute bacterial meningitis (BM), and Lyme meningoencephalitis (LM) (60-62). Highest DC numbers are observed in LM CSF, with striking accumulation of plasmacytoid DCs.

Further experiments showed that in BM and LM, CSF supernatants are strongly chemotactic for immature monocyte-derived DCs (myeloid DCs generated in vitro from blood monocytes [70]), suggesting that recruitment of DCs to the CSF in these diseases is mediated by chemoattractants present in the CSF (61). Myeloid blood DCs respond to an array of chemoattractants, including CXCL12 (SDF-1), CCL2 (MCP-1), CCL4 (MIP-1β), CCL5 (RANTES) and C5a; plasmacytoid DCs respond strongly to CXCL12, much weaker to CCL2, and do not respond to other chemoattractants studied (8, 61, 65). CXCL12 is elevated in the CSF in both BM and LM; in addition, C5a, CCL2, CCL4, and CCL5 are elevated in BM CSF (61). These chemoattractants, acting in concert, appear to play a key role in recruitment of respective DC subsets to the CSF in BM

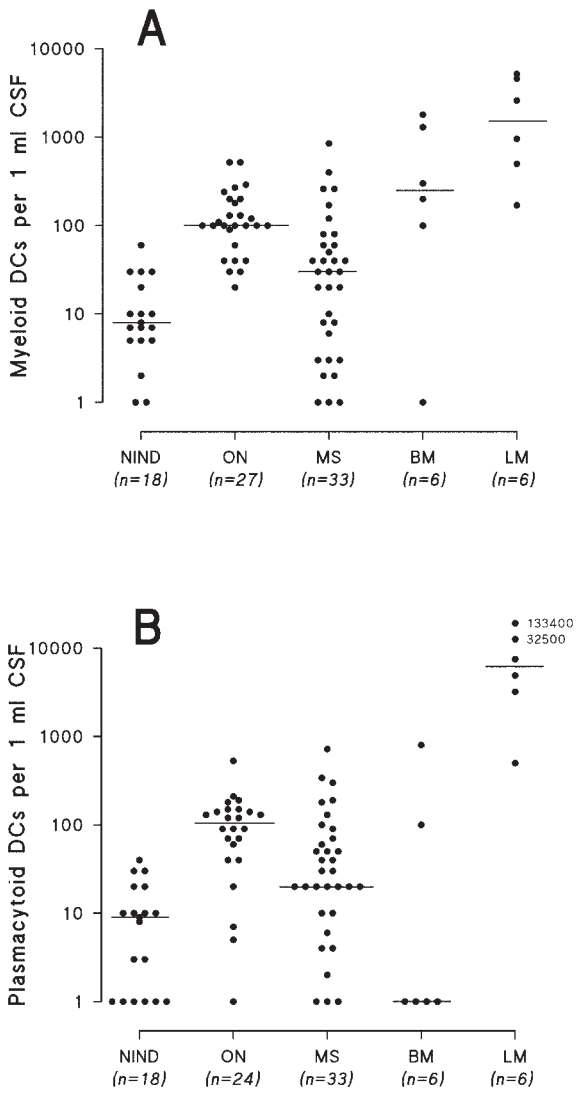

Figure 1. Numbers of myeloid (**A**) and plasmacytoid (**B**) DCs in CSF from patients with non-inflammatory neurological diseases (NIND); acute monosymptomatic optic neuritis (ON) with oligoclonal IgG in the CSF, regarded as early multiple sclerosis (MS); clinically definite MS; acute bacterial meningitis (BM); Lyme meningoencephalitis (LM). Patients with ON were sampled within 4 weeks after disease onset. Patients with BM were sampled within 3 days and those with LM within 10 weeks after disease onset, and before antibiotic treatment. None of the MS or ON patients had been treated with any kind of immunomodulatory drugs, including steroids. DCs were detected by 3-color flow cytometry as cells negative for CD3, CD14, CD16, CD19, CD20 and CD56, positive for HLA-DR, and positive for either CD11c (myeloid DCs) or CD123/IL-3Rα (plasmacytoid DCs) (60-62). Bars = medians.

and LM. In ON and MS, chemotactic activity of the CSF does not differ from that in NIND (62). Accumulation of DCs in CSF of these patients is more likely due to shedding from juxta-CSF brain lesions, where CCL2, CCL4, and CCL5 are expressed (4, 49, 79), or from leptomeningeal mononuclear infiltrates typically observed in MS (27). Expression of the chemokine receptor CCR5 by blood myeloid DCs is elevated in MS and ON, which may facilitate recruitment of myeloid DCs to the CNS in response to CCR5 ligands CCL4 and CCL5 (62).

Thus, accumulation of DCs in human CSF is directly related to the CNS inflammation. Plasmacytoid DCs in CSF have the same immature phenotype as in blood (60), suggesting their low antigen-presenting capacity and strong ability to produce type I IFNs. This awaits further investigation. Considering a beneficial effect of type I IFNs in MS, one may wonder whether plasmacytoid DCs play any anti-inflammatory role in this disease. Myeloid DCs, on the other hand, are potent APCs and may activate T-cells either within the CNS, or in the regional lymph nodes. The next section of this review will focus mainly on the myeloid DCs.

CNS DCs in Human Diseases: a Model

The primary task of DCs during the immune response is to activate T helper (Th) cells. It has been known for a long time that naive Th cells, after priming, can differentiate either into Th1 cells, which produce IFN-γ, IL-2 and TNF-β and promote cell-mediated immunity, or into Th2 cells, which produce IL-4, IL-5 and IL-13 and promote humoral immunity (56). More recently, it was shown that naive T-cells can also develop into anergic regulatory T-cells, which constitutively express the α-chain of the IL-2 receptor (CD25), do not proliferate and suppress activation of other naive Th cells (32, 33). Myeloid DCs, depending on their maturity, numbers, cytokine profiles and other conditions, can drive naive Th cells towards any of these subsets (32, 35, 38, 85; Table 3), which should be taken into account when estimating the role of DCs in different CNS diseases. Based on the findings by Carson et al, who showed that mature DCs injected into the CSF migrate to T-cell areas of DCLNs (7), we assume here that the same process occurs during the natural course of inflammation; however, it should be noted that this awaits direct experimental proof. Below, we shall consider 3 situations: *i)* non-inflammatory conditions, *ii)* CNS infections, and *iii)* MS.

	Th1	Th2	Regulatory T-cells or T-cell anergy
DC maturation time	Short	Long	Immature DCs
Antigen concentration	High	Low	?
Cytokines and other molecules present during DC maturation	Molecules that augment production of IL-12 by DCs (IFN-γ, CD40L, viral RNA, pertussis toxin)	Molecules that suppress production of IL-12 by DCs (PGE_2, cholera toxin)	Molecules that inhibit DC maturation (IL-10, glucocorticoids)
DC:T ratio	High	Low	?

Table 3. Conditions favoring the induction of different types of T-cell responses by myeloid DCs (6, 32, 35, 38, 80, 85).

Non-inflammatory conditions. In the non-inflamed CNS, DCs are only present in the CSF compartment (meninges, choroid plexus and CSF) (28, 46, 50, 60, 74, 84). The connection between the CNS and the DCLNs, described earlier in rodents and potentially allowing DCs to migrate out of the CNS, exists in humans as well, although the main pathway of the lymphatic drainage appears to be the one along major brain arteries, rather than along the olfactory nerves (41, 91). In non-inflammatory conditions, however, the absence of DC maturation factors and of significant antigen release in the CSF would not allow DC to mature and migrate to the DCLNs; thus no substantial T-cell activation will occur (Figure 2).

Little is known so far about the influence of the normal CSF on the immunoregulatory properties of DCs. Several reports have suggested that normal CSF environment suppresses Th1 responses, such as DTH, and favors Th2 responses. For instance, macrophages after culture with normal CSF supernatants become able to inhibit DTH; this has been attributed to the presence of TGF-β in the CSF (93). Soluble proteins infused into the CSF, such as ovalbumin, induce stronger Th2 responses than those elicited by the same dose of antigen injected subcutaneously (25). On the other hand, microorganisms inoculated into the CSF of rodents induce same types of primary T-cell responses as when injected outside of the CNS, including DTH (47, 82). Furthermore, culture of monocyte-derived DCs with non-inflammatory CSF does not impair DCs' ability to stimulate IFN-γ production by naive allogeneic T-cells in vitro (63), indicating that the effect of normal CSF on DCs may differ from that on macrophages. In all, the available data suggest that DCs normally present in the CSF compartment can induce both Th1 and Th2 responses, depending on the type of antigen, possibly with an enhanced ability to elicit Th2 responses to soluble proteins.

CNS infections. In CNS infections, such as BM and LM, myeloid DCs are actively recruited from blood to the CSF (61). These DCs, together with resident meningeal and choroid plexus DCs, can be expected to mature and migrate to the DCLNs in order to generate the anti-infectious T-cell response. DC maturation at the sites of infections is usually driven by inflammatory cytokines such as TNF-α and IL-1β, and by microbial products such as LPS (1, 70). In contrast, the anti-inflammatory cytokine IL-10 inhibits maturation of myeloid DCs (6, 80) or even converts them into $CD14^+$ macrophage-like cells with low antigen-presenting capacity (20). In BM, the CSF does contain TNF-α, IL-1β and LPS (88); yet, monocyte-derived myeloid DCs fail to mature when cultured with BM CSF supernatants and are deficient in priming IFN-γ production by naive T-cells (63). In contrast, DCs cultured with LM CSF supernatants undergo maturation and prime a strong IFN-γ production by naive T-cells (63). The opposing effects of the CSF from the 2 patient groups correlate with CSF levels of IL-10, which are high in BM and are several-fold lower in LM (21, 63). These data are consistent with the character of T-cell responses in vivo: relatively minor intrathecal T-cell response in BM and strong Th1 response in LM (16, 23). Although more studies will be required, the data allow us to suggest that the functional status of CNS DCs, modulated by the cytokine milieu, can influence differentiation of naive T-cells in CNS infections (Figure 2).

Multiple sclerosis. MS is characterized by multiple foci of chronic inflammation, demyelination, and axonal loss in the brain and spinal cord (39). The present consensus is that inflammation in MS is initiated by Th1 cells that after activation in the periphery cross the BBB, recognize their target antigen and activate non-specific effectors such as macrophages and microglia, which are

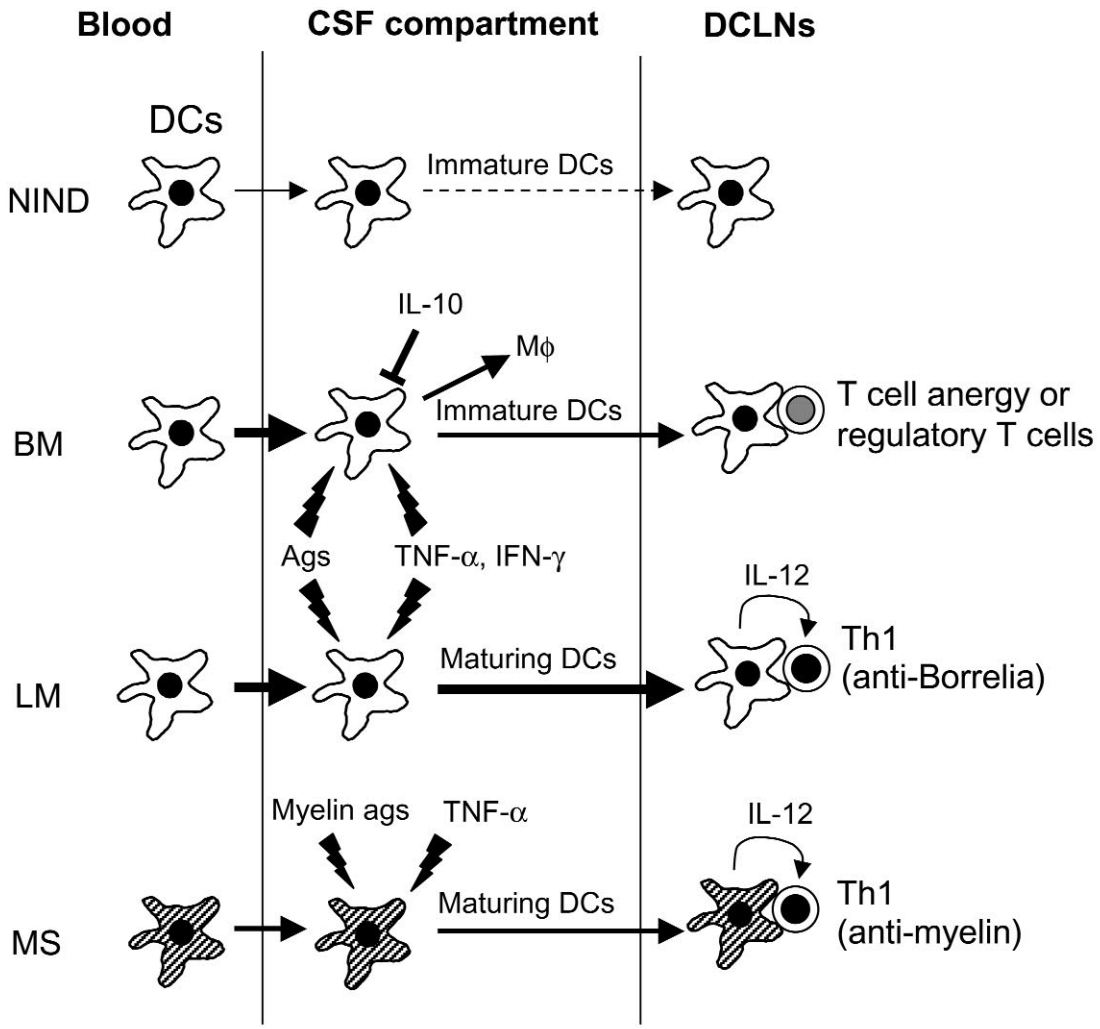

Figure 2. A proposed model of the role of myeloid dendritic cells (DCs) present in the CSF compartment in immune responses in the CNS. DCs are recruited from blood to the CSF compartment in non-inflammatory neurological diseases (NIND), acute bacterial meningitis (BM), Lyme meningoencephalitis (LM) and multiple sclerosis (MS), and may migrate further to the deep cervical lymph nodes (DCLNs) (7). Weight of arrows corresponds to the possible magnitude of migration. In NIND, the DC traffic is minor, no foreign antigens or maturation factors are present, and therefore no significant T-cell activation is induced in the DCLNs. In BM and LM, the DC traffic is increased, and foreign antigens (ags) and maturation factors (such as TNF-α) are present in the CSF, potentially allowing DCs to activate T-cells in the DCLNs . However, IL-10 present at high levels in BM CSF may inhibit maturation of DCs or even convert them into macrophages, thereby limiting their T-cell-stimulatory potential. In MS, recruited DCs take up myelin antigens released into the CSF, migrate to the DCLNs and activate autoreactive myelin antigen-specific T-cells. Hatched pattern of DCs in MS indicates that DCs may bear certain aberrations that contribute to the development of MS, eg, expression of certain HLA molecules or increased ability to produce pro-inflammatory cytokines. Note: this scheme is referred only to DCs in the CSF compartment (meninges, choroid plexus and CSF) and may not be applicable to the DCs infiltrating brain parenchyma upon inflammation.

principal mediators of myelin and axonal damage (11, 30, 39, 54). The dissemination of inflammatory lesions in time indicates that peripheral T-cell activation in MS is repetitive or continuous. The cause of this is still uncertain. Furthermore, it remains unclear why the infiltrating T-cells persist in MS lesions and are not eliminated by apoptosis, unlike in the monophasic T-cell-mediated demyelinating disease acute disseminated encephalomyelitis (2, 64). DCs present in the CSF compartment and, presumably, in MS lesions may represent one of the factors contributing to the chronicity of MS.

DCs in meninges, choroid plexus and CSF. Although the etiology of MS is unknown, subclinical viral infection persisting in the CNS is considered to be a likely

cause of the disease (10). The initial immune attack in MS may be directed against the persisting virus, the myelin damage being a bystander phenomenon at this stage. Indeed, a Th1 response against a microorganism sequestered in the brain (eg, heat-killed BCG) produces all pathological hallmarks of the MS lesion, including edema, demyelination and axonal transsection (45, 57). However, since normal brain parenchyma lacks DCs, the immune response against the putative virus has to be elicited by DCs at other locations. Two scenarios are possible: *i)* systemic reinfection with the same pathogen, involving DCs outside of the CNS and leading to activation of pathogen-specific T-cells in the lymph nodes draining the site of reinfection; and *ii)* release of the virus from the brain parenchyma into the CSF and uptake by meningeal, choroid plexus and/or CSF DCs, which would migrate to, and activate T-cells in the DCLNs. The former scenario is predicted by the above-mentioned experiments with BCG performed by Perry and colleagues (45, 57). The latter scenario gained some support from experiments by Stevenson et al (82), who showed that live influenza virus inoculated into the brain parenchyma in mice and initially ignored by the adaptive immune system, does induce a very delayed systemic T-cell response in some animals, suggesting that the virus is released from the infected cells, taken up by CNS APCs (eg, DCs) and presented to T-cells in the secondary lymphoid organs.

Even if MS is initiated by a T-cell attack against the persisting virus, a true autoimmune Th1 response against myelin antigens develops rather early in the disease (78, 83), possibly due to cross-reactivity between viral and myelin antigens, or due to epitope spreading (55, 58). We propose that DCs in CSF, meninges and choroid plexus participate in systemic activation of these autoreactive T-cells. In early MS, myeloid DCs accumulate in the CSF (Figure 1) and remain elevated over several months; thus, relatively high traffic of DCs to the DCLNs and high DC:T-cell ratios could be expected, favoring a Th1 response (Table 3). Myelin antigens, such as myelin basic protein (MBP)-like material, are released into the CSF during MS exacerbations (92) and could be captured by DCs (Figure 2). After maturation in the presence of inflammatory cytokines and products of tissue destruction, myelin antigen-loaded DCs could migrate to the DCLNs and induce an anti-myelin Th1 response (Figure 2). These Th1 cells, recirculating via blood back to the CNS, would incite new lesions with release of more myelin antigens into the CSF, which may eventually render MS a self-perpetuating disease. This hypothesis is partly supported by data in EAE, the commonly used animal model of MS: *i)* in acute EAE in monkeys, cells with a phenotype of mature DCs and containing myelin antigens are found in the DCLNs (14); and *ii)* aggravation of murine EAE by surface cryotrauma of the brain can be abolished by removal of the DCLNs (66).

In addition, there are indications of some DCs interacting with T-cells directly in the meninges. Mononuclear infiltrates in the leptomeninges are typical features of both MS and EAE (27, 86, 87). Vass et al, who were the first to identify meningeal DCs as a distincT-cell population, noted that these cells constitute a large proportion of MHC class II-positive cells in the meningeal infiltrates in EAE and are closely apposed with the infiltrating T-cells (87). In MBP-induced EAE in Lewis rats, T-cells in the meningeal infiltrates preferentially express TCR Vβ8.2 (ie, are directed against MBP) and proliferate in situ (76), which is consistent with the presence of professional APCs presenting MBP. In MS, the nature of antigens being presented by meningeal APCs is unknown; these could be myelin or viral peptides.

Brain DCs. Evidence for the presence of DCs in MS brain lesions remains indirect (3). In chronic EAE, however, brain DCs are numerous (19, 74). They undergo intrathecal maturation (74) and can be expected to migrate to DCLNs. This process is normally directed by the chemokines CCL19 (ELC, MIP-3β), CCL21 (SLC, 6Ckine) and CXCL12 produced in the afferent lymphatics and lymph nodes (65, 71). However, a recent report indicates that CCL19 is expressed de novo in chronic EAE lesions (75) and is likely to retain mature DCs. Retention of mature DCs in the inflamed tissue has been recently suggested as a critical event in development of chronic inflammation (42, 72). Retained DCs can activate naive T-cells directly in the target organ, drive a Th1 response by producing IL-12 (19), and prevent apoptosis of activated T-cells by secreting cytokines such as IL-15 (89). Mature DCs persisting in the inflamed tissue are proposed to serve as a framework for the formation of ectopic secondary lymphoid structures frequently observed at the sites of chronic inflammation (72), including old MS plaques (67).

Summarizing, this model implies the existence of 2 pools of CNS DCs in chronic EAE and MS: *i)* meningeal, choroid plexus and CSF DCs, which after uptake of myelin antigens migrate to DCLNs, activate naive T-cells outside of the CNS, and contribute to onset of new MS lesions; and *ii)* brain DCs, which are recruited and retained in already existing lesions and activate T-cells intrathecally, contributing to lesion activity. Further characterization of CNS DCs and their migration

will be required to validate this model. A key question to be answered in this context is why chronic inflammation and autoimmunity do not develop in other neurological diseases associated with tissue damage and release of myelin antigens, such as stroke (92). We suggest that immunological aberrations at the level of DCs may contribute to the break-down of immune tolerance to myelin antigens, leading to development of MS. Some of these aberrations could be: *i)* expression of HLA molecules that can present specific myelin antigens; *ii)* increased recruitment and survival of DCs in the CNS, caused by uncontrolled intrathecal expression of DC chemoattractants and growth factors and/or by increased expression of chemokine receptors by DCs (4, 49, 62, 79); *iii)* increased ability of DCs to induce a Th1 response (31); and *iv)* systemic or intrathecal deficiency of DC anti-maturation factors (such as IL-10) and predominance of pro-maturation factors (such as TNF-α) (59), determined genetically or caused by common infections. These factors may enable DCs to drive chronic CNS inflammation resulting in the large demyelinating plaques typical for MS.

Conclusions and Future Prospectives

Recent studies have shed light on the role of DCs in immune responses in the CNS. Available data suggest that depending on the situation, the effect of DCs can be beneficial or deleterios, resulting not only in the elimination of pathogens, but also in chronic inflammation and autoimmunity. The CNS DCs can therefore be considered as a therapeutic target in chronic inflammatory diseases of the CNS, first of all in MS. Possible strategies could interfere with recruitment of DCs to the CNS and with intrathecal DC survival/ maturation. In this context, experimentation with IL-10 appears to be promising. For instance, intrathecal inoculation of a viral vector expressing the IL-10 gene almost totally suppresses relapses of chronic EAE in SJL mice (13); one target of IL-10 in these mice could be brain DCs, which are numerous in this model (74). IFN-β, one of the few drugs with some clinical effect in MS, may also affect DCs, decreasing their ability to produce IL-12 and to prime the Th1 response (36, 51). Recent advances in the fields of chemokine receptor antagonists and matrix metalloproteinase inhibitors open up the prospect of developing drugs that would target migration of specific cell types, including DCs (44). Further studies are warranted to define more efficient approaches to influence CNS DCs, in particular: *i)* detailed characterization of molecular mechanisms of DC recruitment at the BBB, and of DC survival in the brain; *ii)* further search for immunological aberrations at the level of DCs in MS patients vs. controls; *iii)* defining the role of DC migration from the CNS to secondary lymphoid organs in initiation and progression of MS; and *iv)* search for tools to "inactivate" CNS DCs in MS.

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References

1. Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, Pulendran B, Palucka K (2000) Immunobiology of dendritic cells. *Annu Rev Immunol* 18:767-811.
2. Bauer J, Stadelmann C, Bancher C, Jellinger K, Lassmann H (1999) Apoptosis of T lymphocytes in acute disseminated encephalomyelitis. *Acta Neuropathol (Berl)* 97:543-546.
3. Bo L, Mork S, Kong PA, Nyland H, Pardo CA, Trapp BD (1994) Detection of MHC class II-antigens on macrophages and microglia, but not on astrocytes and endothelia in active multiple sclerosis lesions. *J Neuroimmunol* 51:135-146.
4. Boven LA, Montagne L, Nottet HS, De Groot CJ (2000) Macrophage inflammatory protein-1alpha (MIP-1alpha), MIP-1beta, and RANTES mRNA semiquantification and protein expression in active demyelinating multiple sclerosis (MS) lesions. *Clin Exp Immunol* 122:257-263.
5. Bradbury MW, Cserr HF, Westrop RJ (1981) Drainage of cerebral interstitial fluid into deep cervical lymph of the rabbit. *Am J Physiol* 240:F329-F336.
6. Buelens C, Verhasselt V, De Groote D, Thielemans K, Goldman M, Willems F (1997) Human dendritic cell responses to lipopolysaccharide and CD40 ligation are differentially regulated by interleukin-10. *Eur J Immunol* 27:1848-1852.
7. Carson MJ, Reilly CR, Sutcliffe JG, Lo D (1999) Disproportionate recruitment of CD8+ T-cells into the central nervous system by professional antigen-presenting cells. *Am J Pathol* 154:481-494.
8. Caux C, Ait-Yahia S, Chemin K, de Bouteiller O, Dieu-Nosjean MC, Homey B, Massacrier C, Vanbervliet B, Zlotnik A, Vicari A (2000) Dendritic cell biology and regulation of dendritic cell trafficking by chemokines. *Springer Semin Immunopathol* 22:345-369.
9. Cella M, Scheidegger D, Palmer-Lehmann K, Lane P, Lanzavecchia A, Alber G (1996) Ligation of CD40 on dendritic cells triggers production of high levels of interleukin-12 and enhances T-cell stimulatory capacity: T-T help via APC activation. *J Exp Med* 184:747-752.
10. Challoner PB, Smith KT, Parker JD, MacLeod DL, Coulter SN, Rose TM, Schultz ER, Bennett JL, Garber RL, Chang M, Schad PA, Stewart PM, Nowinski RC, Brown JP, Burmer GC (1995) Plaque-associated expression of human herpesvirus 6 in multiple sclerosis. *Proc Natl Acad Sci U S A* 92:7440-7444.

11. Cross AH, Cannella B, Brosnan CF, Raine CS (1991) Hypothesis: antigen-specific T-cells prime central nervous system endothelium for recruitment of nonspecific inflammatory cells to effect autoimmune demyelination. *J Neuroimmunol* 33:237-244.
12. Cserr HF, Harling-Berg CJ, Knopf PM (1992) Drainage of brain extracellular fluid into blood and deep cervical lymph and its immunological significance. *Brain Pathol* 2:269-276.
13. Cua DJ, Hutchins B, LaFace DM, Stohlman SA, Coffman RL (2001) Central nervous system expression of IL-10 inhibits autoimmune encephalomyelitis. *J Immunol* 166:602-608.
14. De Vos AF, von Meurs M, Brok HPM, Boon L, Rensing S, 't Hart BA, Laman JD (2001) Transfer of CNS autoantigens and presentation in secondary lymphoid organs. *J Neuroimmunol* 118:75.
15. Dittel BN, Visintin I, Merchant RM, Janeway CA Jr (1999) Presentation of the self antigen myelin basic protein by dendritic cells leads to experimental autoimmune encephalomyelitis. *J Immunol* 163:32-39.
16. Ekerfelt C, Ernerudh J, Bunikis J, Vrethem M, Aagesen J, Roberg M, Bergstrom S, Forsberg P (1997) Compartmentalization of antigen specific cytokine responses to the central nervous system in CNS borreliosis: secretion of IFN-gamma predominates over IL-4 secretion in response to outer surface proteins of Lyme disease Borrelia spirochetes. *J Neuroimmunol* 79:155-162.
17. Fischer HG, Bielinsky AK (1999) Antigen presentation function of brain-derived dendriform cells depends on astrocyte help. *Int Immunol* 11:1265-1274.
18. Fischer H-G, Bonifas U, Reichmann G (2000) Phenotype and functions of brain dendritic cells emerging during chronic infection of mice with Toxoplasma gondii. *J Immunol* 164:4826-4834.
19. Fischer HG, Reichmann G (2001) Brain dendritic cells and macrophages/microglia in central nervous system inflammation. *J Immunol*166:2717-2726.
20. Fortsch D, Rollinghoff M, Stenger S (2000) IL-10 converts human dendritic cells into macrophage-like cells with increased antibacterial activity against virulent Mycobacterium tuberculosis. *J Immunol* 165:978-987.
21. Frei K, Nadal D, Pfister HW, Fontana A (1993) Listeria meningitis: identification of a cerebrospinal fluid inhibitor of macrophage listericidal function as interleukin 10. *J Exp Med* 178:1255-1261.
22. Gallucci S, Lolkema M, Matzinger P (1999). Natural adjuvants: endogenous activators of dendritic cells. *Nat Med* 5:1249-1255.
23. Garcia-Monco JC, Benach JL (1995) Lyme neuroborreliosis. *Ann Neurol* 37:691-702.
24. Gerritse K, Laman JD, Noelle RJ, Aruffo A, Ledbetter JA, Boersma WJ, Claassen E (1996) CD40-CD40 ligand interactions in experimental allergic encephalomyelitis and multiple sclerosis. *Proc Natl Acad Sci U S A* 93:2499-2504.
25. Gordon LB, Knopf PM, Cserr HF (1992) Ovalbumin is more immunogenic when introduced into brain or cerebrospinal fluid than into extracerebral sites. *J Neuroimmunol* 40:81-87.
26. Grouard G, Rissoan MC, Filgueira L, Durand I, Banchereau J, Liu YJ (1997) The enigmatic plasmacytoid T-cells develop into dendritic cells with interleukin (IL)-3 and CD40-ligand. *J Exp Med* 185:1101-1111.
27. Guseo A, Jellinger K (1975) The significance of perivascular inflitrations in multiple sclerosis. *J Neurol* 211:51-60.
28. Hanly A, Petito CK (1998) HLA-DR-positive dendritic cells of the normal human choroid plexus: a potential reservoir of HIV in the central nervous system. *Hum Pathol* 29:88-93.
29. Harling-Berg CJ, Park JT, Knopf PM (1999) Role of cervical lymphatics in the Th2-type hierarchy of CNS immune regulation. *J Neuroimmunol* 101:111-127.
30. Hickey WF (1991) Migration of hematogenous cells through the blood-brain barrier and the initiation of CNS inflammation. *Brain Pathol* 1:97-105.
31. Huang YM, Stoyanova N, Jin YP, Teleshova N, Hussien Y, Xiao BG, Fredrikson S, Link H (2001) Altered phenotype and function of blood dendritic cells in multiple sclerosis are modulated by IFN-beta and IL-10. *Clin Exp Immunol* 124:306-314.
32. Jonuleit H, Schmitt E, Schuler G, Knop J, Enk AH (2000) Induction of interleukin 10-producing, nonproliferating CD4(+) T-cells with regulatory properties by repetitive stimulation with allogeneic immature human dendritic cells. *J Exp Med* 192:1213-1222.
33. Jonuleit H, Schmitt E, Stassen M, Tuettenberg A, Knop J, Enk AH (2001) Identification and functional characterization of human CD4(+)CD25(+) T-cells with properties isolated from peripheral blood. *J Exp Med* 193:1285-1294.
34. Kadowaki N, Antonenko S, Lau JY, Liu YJ (2000) Natural interferon alpha/beta-producing cells link innate and adaptive immunity. *J Exp Med* 192:219-226.
35. Kalinski P, Hilkens CM, Wierenga EA, Kapsenberg ML (1999) T-cell priming by type-1 and type-2 polarized dendritic cells: the concept of a third signal. *Immunol Today* 20:561-567.
36. Karp CL, Biron CA, Irani DN (2000) Interferon beta in multiple sclerosis: is IL-12 suppression the key? *Immunol Today* 21:24-28.
37. Kohrgruber N, Halanek N, Groger M, Winter D, Rappersberger K, Schmitt-Egenolf M, Stingl G, Maurer D (1999) Survival, maturation, and function of CD11c- and CD11c+ peripheral blood dendritic cells are differentially regulated by cytokines. *J Immunol* 163:3250-3259.
38. Langenkamp A, Messi M, Lanzavecchia A, Sallusto F (2000) Kinetics of dendritic cell activation: impact on priming of TH1, TH2 and nonpolarized T-cells. *Nat Immunol* 1:311-316.
39. Lassmann H, Bruck W, Lucchinetti C (2001) Heterogeneity of multiple sclerosis pathogenesis: implications for diagnosis and therapy. *Trends Mol Med* 7:151-121.
40. Lowenstein PR (2002) Immunology of viral-vector-mediated gene transfer into the brain: an evolutionary and developmental perspective. *Trends Immunol* 23:23-30.

41. Lowhagen P, Johansson BB, Nordborg C (1994) The nasal route of cerebrospinal fluid drainage in man. A light-microscope study. *Neuropathol Appl Neurobiol* 20:543-550.
42. Ludewig B, Odermatt B, Landmann S, Hengartner H, Zinkernagel RM (1998) Dendritic cells induce autoimmune diabetes and maintain disease via de novo formation of local lymphoid tissue. *J Exp Med* 188:1493-1501.
43. Ludewig B, Junt T, Hengartner H, Zinkernagel RM (2001) Dendritic cells in autoimmune diseases. *Curr Opin Immunol* 13:657-662.
44. Mackay CR (2001) Chemokines: immunology's high impact factors. *Nat Immunol* 2:95-101.
45. Matyszak MK, Perry VH (1995) Demyelination in the central nervous system following a delayed-type hypersensitivity response to bacillus Calmette-Guerin. *Neuroscience* 64:967-977.
46. Matyszak MK, Perry VH (1996) The potential role of dendritic cells in immune-mediated inflammatory diseases in the central nervous system. *Neuroscience* 74:599-608.
47. Matyszak MK, Perry VH (1996) A comparison of leucocyte responses to heat-killed bacillus Calmette-Guerin in different CNS compartments. *Neuropathol Appl Neurobiol* 22:44-53.
48. Matyszak MK, Perry VH (1998) Bacillus Calmette-Guerin sequestered in the brain parenchyma escapes immune recognition. *J Neuroimmunol* 82:73-80.
49. McManus C, Berman JW, Brett FM, Staunton H, Farrell M, Brosnan CF (1998) MCP-1, MCP-2 and MCP-3 expression in multiple sclerosis lesions: an immunohistochemical and in situ hybridization study. *J Neuroimmunol* 86:20-29.
50. McMenamin PG (1999) 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* 405:553-562.
51. McRae BL, Semnani RT, Hayes MP, van Seventer GA (1998) Type I IFNs inhibit human dendritic cell IL-12 production and Th1 cell development. *J Immunol* 160:4298-4304.
52. McWilliam AS, Napoli S, Marsh AM, Pemper FL, Nelson DJ, Pimm CL, Stumbles PA, Wells TN, Holt PG (1996) Dendritic cells are recruited into the airway epithelium during the inflammatory response to a broad spectrum of stimuli. *J Exp Med* 184:2429-2432.
53. Medawar PB (1948) Immunity to homologous grafted skin: III. Fate of skin homografts transplanted to the brain, to subcutaneous tissue, and to the anterior chamber of the eye. *Br J Exp Pathol* 29:58-69.
54. Merill J, Benveniste E (1996) Cytokines in inflammatory brain lesions: helpful and harmful. *Trends Neurosci* 19:331-338.
55. Miller SD, Vanderlugt CL, Begolka WS, Pao W, Yauch RL, Neville KL, Katz-Levy Y, Carrizosa A, Kim BS (1997) Persistent infection with Theiler's virus leads to CNS autoimmunity via epitope spreading. *Nat Med* 3:1133-1136.
56. Mosmann TR, Sad S (1996) The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunol Today* 17:138-146.
57. Newman TA, Woolley ST, Hughes PM, Sibson NR, Anthony DC, Perry VH (2001) T-cell- and macrophage-mediated axon damage in the absence of a CNS-specific immune response: involvement of metalloproteinases. *Brain* 124:2203-2214.
58. Olson JK, Croxford JL, Calenoff MA, Dal Canto MC, Miller SD (2001) A virus-induced molecular mimicry model of multiple sclerosis. *J Clin Invest* 108:311-318.
59. Ozenci V, Kouwenhoven M, Huang YM, Kivisakk P, Link H (2000) Multiple sclerosis is associated with an imbalance between tumour necrosis factor-alpha (TNF-alpha)- and IL-10-secreting blood cells that is corrected by interferon-beta (IFN-beta) treatment. *Clin Exp Immunol* 120:147-153.
60. Pashenkov M, Huang Y-M, Kostulas V, Haglund M, Soderstrom M, Link H (2001) Two subsets of dendritic cells are present in human cerebrospinal fluid. *Brain* 124:480-492.
61. Pashenkov M, Teleshova N, Kouwenhoven M, Smirnova T, Jin YP, Kostulas V, Huang YM, Pinegin B, Boiko A, Link H (2002) Recruitment of dendritic cells to the cerebrospinal fluid in bacterial neuroinfections. *J Neuroimmunol* 122:106-116.
62. Pashenkov M, Teleshova N, Kouwenhoven M, Huang YM, Söderström M, Link H (2002) Elevated expression of CCR5 by myeloid (CD11c+) blood dendritic cells in multiple sclerosis and acute optic neuritis. *Clin Exp Immunol* 127:519-527.
63. Pashenkov M, Soderstrom M, Huang Y-M, Link H (2002) Cerebrospinal fluid affects phenotype and functions of myeloid dendritic cells. *Clin Exp Immunol* 128:379-387.
64. Pender MP (1998) Genetically determined failure of activation-induced apoptosis of autoreactive T-cells as a cause of multiple sclerosis. *Lancet* 351:978-981.
65. Penna G, Sozzani S, Adorini L (2001) Cutting edge: selective usage of chemokine receptors by plasmacytoid dendritic cells. *J Immunol* 167:1862-1866.
66. Phillips MJ, Needham M, Weller RO (1997) Role of cervical lymph nodes in autoimmune encephalomyelitis in the Lewis rat. *J Pathol* 182:457-464.
67. Prineas JW (1979). Multiple sclerosis: presence of lymphatic capillaries and lymphoid tissue in the brain and spinal cord. *Science* 203:1123-1125.
68. Robert C, Fuhlbrigge RC, Kieffer JD, Ayehunie S, Hynes RO, Cheng G, Grabbe S, von Andrian UH, Kupper TS (1999) Interaction of dendritic cells with skin endothelium: A new perspective on immunosurveillance. *J Exp Med* 189:627-636.
69. Robinson SP, Patterson S, English N, Davies D, Knight SC, Reid CD (1999) Human peripheral blood contains two distinct lineages of dendritic cells. *Eur J Immunol* 29:2769-2778.
70. Sallusto F, Lanzavecchia A (1994) Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. *J Exp Med* 179:1109-1118.

71. Sallusto F, Schaerli P, Loetscher P, Schaniel C, Lenig D, Mackay CR, Qin S, Lanzavecchia A (1998) Rapid and coordinated switch in chemokine receptor expression during dendritic cell maturation. *Eur J Immunol* 28:2760-2769.

72. Sallusto F, Lanzavecchia A (1999) Mobilizing dendritic cells for tolerance, priming and chronic inflammation. *J Exp Med* 189:611-614.

73. Santambrogio L, Belyanskaya SL, Fischer FR, Cipriani B, Brosnan CF, Ricciardi-Castagnoli P, Stern LJ, Strominger JL, Riese R (2001) Developmental plasticity of CNS microglia. *Proc Natl Acad Sci U S A* 98:6295-6300.

74. Serafini B, Columba-Cabezas S, Di Rosa F, Aloisi F (2000) Intracerebral recruitment and maturation of dendritic cells in the onset and progression of experimental autoimmune encephalomyelitis. *Am J Pathol* 157:1991-2002.

75. Serafini B, Magliozzi R, Columba-Cabezas S, Ambrosini E, Aloisi F (2001) Recruitment of dendritic cells and occurrence of lymphoid-like features in the central nervous system during autoimmune encephalomyelitis. *J Neuroimmunol* 118:5.

76. Shin T, Kojima T, Tanuma N, Ishihara Y, Matsumoto Y (1995) The subarachnoid space as a site for precursor T-cell proliferation and effector T-cell selection in experimental autoimmune encephalomyelitis. *J Neuroimmunol* 56:171-178.

77. Siegal FP, Kadowaki N, Shodell M, Fitzgerald-Bocarsly PA, Shah K, Ho S, Antonenko S, Liu YJ (1999) The nature of the principal type 1 interferon-producing cells in human blood. *Science* 284:1835-1837.

78. Soderstrom M, Link H, Sun JB, Fredrikson S, Wang ZY, Huang WX (1994) Autoimmune T-cell repertoire in optic neuritis and multiple sclerosis: T-cells recognising multiple myelin proteins are accumulated in cerebrospinal fluid. *J Neurol Neurosurg Psychiatry* 57:544-551.

79. Sorensen TL, Tani M, Jensen J, Pierce V, Lucchinetti C, Folcik VA, Qin S, Rottman J, Sellebjerg F, Strieter RM, Frederiksen JL, Ransohoff RM (1999) Expression of specific chemokines and chemokine receptors in the central nervous system of multiple sclerosis patients. *J Clin Invest* 103:807-815.

80. Steinbrink K, Wolfl M, Jonuleit H, Knop J, Enk AH (1997) Induction of tolerance by IL-10-treated dendritic cells. *J Immunol* 159:4772-4780.

81. Steinman RM, Pack M, Inaba K (1997) Dendritic cells in the T-cell areas of lymphoid organs. *Immunol Rev* 156:25-37.

82. Stevenson PG, Hawke S, Sloan DJ, Bangham CR (1997) The immunogenicity of intracerebral virus infection depends on anatomical site. *J Virol* 71:145-151.

83. Sun JB, Link H, Olsson T, Xiao BG, Andersson G, Ekre HP, Linington C, Diener P (1991) T and B cell responses to myelin-oligodendrocyte glycoprotein in multiple sclerosis. *J Immunol* 146:1490-1495.

84. Suter T, Malipiero U, Otten L, Ludewig B, Muelethaler-Mottet A, Mach B, Reith W, Fontana A (2000) Dendritic cells and differential usage of the MHC class II transactivator promoters in the central nervous system in experimental autoimmune encephalitis. *Eur J Immunol* 30:794-802.

85. Tanaka H, Demeure CE, Rubio M, Delespesse G, Sarfati M (2000) Human monocyte-derived dendritic cells induce naive T-cell differentiation into T helper cell type 2 (Th2) or Th1/Th2 effectors. Role of stimulator/responder ratio. *J Exp Med* 192:405-412.

86. Traugott U, Raine CS (1979) Acute experimental allergic encephalomyelitis. Myelin basic protein-reactive T-cells in the circulation and in meningeal infiltrates. *J Neurol Sci* 42:331-336.

87. Vass K, Lassmann H, Wekerle H, Wisniewski HM (1986) The distribution of Ia antigen in the lesions of rat acute experimental allergic encephalomyelitis. *Acta Neuropathol (Berl)* 70:149-160.

88. Waage A, Halstensen A, Shalaby R, Brandtzaeg P, Kierulf P, Espevik T (1989) Local production of tumor necrosis factor alpha, interleukin 1, and interleukin 6 in meningococcal meningitis. Relation to the inflammatory response. *J Exp Med* 170:1859-1867.

89. Waldmann TA, Dubois S, Tagaya Y (2001) Contrasting roles of IL-2 and IL-15 in the life and death of lymphocytes: implications for immunotherapy. *Immunity* 14:105-110.

90. Wekerle H, Kojima K, Lannes-Vieira J, Lassmann H, Linington C (1994) Animal models. *Ann Neurol* 36:S47-S53.

91. Weller RO (1998) Pathology of cerebrospinal fluid and interstitial fluid of the CNS: significance for Alzheimer disease, prion disorders and multiple sclerosis. *J Neuropathol Exp Neurol* 57:885-894.

92. Whitaker J (1998) Myelin basic protein in cerebrospinal fluid and other body fluids. *Mult Scler* 4:16-21.

93. Wilbanks GA, Streilein JW (1992) Fluids from immune privileged sites endow macrophages with the capacity to induce antigen-specific immune deviation via a mechanism involving transforming growth factor-beta. *Eur J Immunol* 22:1031-1036.