The Role of Dendritic Cells in Neurodegenerative Diseases

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Abstract. Dendritic cells (DCs) are the most potent antigen-presenting cells (APCs) involved in the induction of adaptive immune responses. The presence of DCs in the central nervous system (CNS) and the active participation of the immune system in a variety of neurodegenerative diseases have been demonstrated. This review will discuss recent findings pertinent to DCs and other antigen-presenting cells in the CNS in health and disease states.

Key words: dendritic cells; neurodegenerative diseases; CNS.

Introduction

Evolutionary pressure has led to the development of adaptive immunity in vertebrate species. This highly sophisticated and potent system of host defense needs to be activated and regulated by antigen-presenting cells (APCs)5, 80, which are heterogeneous and comprise several cell types, including macrophages, B lymphocytes and a variety of professional APCs, namely dendritic cells (DCs)80. DCs are unique because they are the only APCs that are able to induce primary immune responses, thus resulting in the activation of naive immune cells and permitting the establishment of immunological memory38, 49. DC progenitors in the bone marrow give rise to circulating precursors that home to tissues, where they reside as immature DCs (iDCs) with high phagocytic capacity25. Particulated antigens, cell debris or microbes can be taken-up by iDCs through phagocytosis25, and enclosed in macro-pinoctytic vesicles containing extracellular fluid and solutes70. In addition to typical cell surface markers, iDCs also express C-type lectin receptors as well as Fcγ and Fcε receptors which are crucial for antigen (Ag) uptake42, 43, 71. Macro-pinoctytosis and receptor-mediated Ag uptake by iDC is so efficient that picomolar and nanomolar concentrations of Ag are sufficient for antigen processing and presentation22. This is in sharp contrast to other types of APCs, which typically require micromolar levels of antigens.

Following Ag capture at inflammatory sites, iDCs differentiate in response to inflammatory stimuli into mature state (mDCs) and migrate to the lymphoid organs, where they interact with Ag-specific T cells and initiate immune responses69, 79. Numerous factors can induce DC maturation, including exogenous pathogen-related molecules, such as lipopolysaccharide (LPS)68, bacterial DNA39, and double-stranded RNA16; endogenous host-derived pro- and anti-inflammatory signals in
the local microenvironment, including TNF, IL-1, IL-6, IL-10, TGF-β, and prostaglandins, and T cell-derived signals.

Migration of DCs involves differential regulation of chemoattractant receptors on the cell surface during the cell maturation process. For DCs derived from peripheral blood monocytes, there is a "switch" in the pattern of chemoattractant receptor expression, which occurs at both the transcriptional and post-transcriptional levels. Immature DCs express many chemokine receptors, such as CXCR1, CCR1, CCR2, CCR5, and CCR6, as well as the receptors for the classical chemoattractants, such as N-formyl-methionyl-leucyl-phenylalanine (fMLF) and C5a. Immediately after exposure to maturation signals, the majority of the chemokine receptors CCR1, CCR5, and CCR6 on iDCs are down-regulated from the cell surface by internalization. Maturing DCs express high levels of the chemokine receptor CCR7, which is essential for cell migration through lymphatics to the T cell zone of the draining lymph nodes, where the cognate CCR7 ligands CCL19/ECL and CCL21/SLC are constitutively produced. Mature DCs also upregulated the expression of CCR4 and CXCR4, but the physiological significance of these receptors in DC trafficking is less clear.

In peripheral tissues and organs where a lymphatic drainage system is present, the paradigm of chemokine-directed DC trafficking has become widely accepted. However, since such a system is apparently absent in the CNS, the process of DC differentiation, trafficking and Ag presentation in this unique anatomic compartment has become the focus of investigation by many laboratories. This review aims at discussing some recent progress in the studies of DCs in patho-physiologic states of the central nervous system (CNS).

Antigen-Presenting Cells in the CNS

The CNS was thought to be isolated from the immune system by the blood-brain barrier (BBB) and the apparent absence of a lymphatic system. The BBB only allows molecules below a certain size to pass freely from the circulation across the vascular endothelium into the CNS. The tight junctions between endothelial cells prevent the diffusion of proteins and other charged molecules into the intercellular space. The BBB was considered to be a barrier not only for solutes, but also for cells. However, it is now clear that the BBB does not completely isolate the CNS from the peripheral immune system, and leukocytes manage to penetrate an intact BBB and reside in the CNS. In the past, the lack of lymphatic drainage and MHC antigen expression have been considered as contributing factors to the immune-privileged status of the CNS. For instance, the prolonged survival rate of tissue transplants in the CNS suggests the inability of the host to mount a rejection response, which involves participation of immune cells. However, accumulating evidence suggests that the normal CNS does contain cells which express MHC antigens, that lymphatic drainage occurs via the Virchow-Robin spaces, and that tissues allotransplanted into the CNS are subjected to immunological rejection, albeit at a slower rate than in other anatomical compartments.

Additional evidence to support the occurrence of active immunological responses in the CNS is based on data showing that CNS fluids and intracerebrally injected antigens drain into cervical lymph nodes along perivascular pathways in the meninges and brain parenchyma. The perivascular spaces surrounding cerebral and meningeal blood vessels contain macrophages (the perivascular cells) which are thought to play an important role as first-line CNS scavengers and APCs. Perivascular cells exhibit both constitutive and inflammation-upregulated expression of MHC class II and costimulatory molecules. However, their capacity to travel to regional lymph nodes along perivascular spaces and to prime T cells has yet to be determined. Perivascular cells are slowly, but continuously, replaced by blood-derived cells of the monocyte/macrophage lineage. Since monocyte precursors can also differentiate into DCs, it is possible that some of the cells trafficking through the perivascular space may have acquired DC-like properties under the influence of inflammatory stimuli in the periphery.

Microglial cells

Although it has long been established that glia exhibit important supportive functions within the CNS, increased attention has recently been paid to the potential role of these cells in inflammation and immune responses. Astroglial cells are the predominant cell type in the CNS, while microglia and oligodendrocytes only account for a minor proportion of the cells (approximately 10 and 5%, respectively). In early studies, Del Rio Hortega described microglia as being ubiquitous with minimal regional variations in the CNS. This assumption was later proved incomplete, and the distribution of microglia is in fact heterogeneous. A large number of microglial cells are located in close vicinity to neurons in the gray matter and between fiber tracts in the white matter of the CNS. There are more than 5 times as many microglial cells in the gray matter than...
in the white matter. Interestingly, microglia are regularly distributed within a structure, with each cell occupying its own territory. Attempts to manipulate the density of the microglia in the retina failed to disrupt the regularity of the array of cell distribution.

The origin of ramified microglia has been a long-standing controversial issue. Bone marrow-derived precursors which are of mesodermal origin, and neuroectodermal matrix cells, which also serve as precursors, for astrocytes and oligodendrocytes, both have been considered as potential sources of developing microglia. However, microglia do share most surface molecules with bone marrow-derived macrophages, making a distinction between these two cell types in pathological settings difficult. With the development of more specific immunocytochemical techniques, microglia can unequivocally be distinguished from other resident, non-neuronal cells in the CNS. Resting microglia constitutively express complement receptor (CR3, also termed Mac-1 or CD11b/CD18), which is similarly found on peripheral blood monocytes and can be further upregulated upon activation.

In general, resting microglia show a low-level expression of the cell surface molecules CD14, CD80 (B7-1) and HLA-DR. This property has been speculated to represent an adaptation of the microglia to the specialized microenvironment of the CNS. However, the ability of microglia to respond rapidly to a variety of stimulatory molecules suggests that their apparent quiescent state does not obscure their vigilance to changes in the surrounding extracellular milieu. Neuronal brain lesions induce prompt inflammatory responses associated with the activation of microglia at the edges of the lesions and are accompanied by infiltration of hematogenous macrophages within hours after injury. The activated microglia proliferate locally and reach a maximal cell expansion at day 2–3. These cells are able to rapidly phagocytose and clear debris, and produce the proinflammatory cytokine IL-1, which further stimulates microgliosis and neovascularization. The most commonly described changes in activated microglia are altered cell morphology and up-regulation of constitutively expressed cell surface CR3 (Mac-1, CD11b/CD18). A significantly enhanced expression of MHC class II molecules is observed within the first 7 days post-injury. The expression of a variety of chemotactic receptors, which mediate migration of microglial cells, is also induced by inflammatory and immunological signals. For instance, the Gram-negative bacterial endotoxin LPS upregulates the expression of formyl peptide receptor 2 (FPR2), a homolog of human formyl peptide receptor-like 1 (FPRL1) on murine microglia. This is a functional receptor for a great variety of chemotactic agonists, including the bacterial formylated peptide FMLF, the HIV-1 envelope protein-derived chemotactic peptides, as well as the 42-amino-acid form of amyloid β (Aβ1-42) peptide, a key pathogenic agent of Alzheimer’s disease. LPS concomitantly downregulates murine microglial expression of CXCR4, a receptor for the chemokine SDF-1α, which is involved in hematopoiesis and development.

Therefore, LPS, by selectively upregulating the expression and function of the formyl peptide receptor, may promote a more specialized microglial response to exogenous and endogenously produced chemotaxants.

In response to inflammatory stimulants, including LPS, microglia produce TNF-α, which in turn further activates microglia to secrete pro-inflammatory cytokines and neurotoxic mediators, including nitric oxide (NO). A pivotal indicator of microglia as sensors of insult in the CNS is reflected by the early nuclear induction of two active DNA-binding subunits of NF-κB, p50 and p65, in disease models. For instance, in mouse experimental allergic encephalomyelitis (EAE), 4 days after transfer of encephalitogenic T cells, p50 and p65 are detected in activated microglia but not in astrocytes. Thus, microglia play a central role in mediating pro-inflammatory and immune responses in the CNS.

**Dendritic cells**

Initially, due to the low levels of detectable MHC class II molecules, it was believed that DCs might be absent from the CNS. However, using the monoclonal antibody OX62, which specifically reacts with an integrin only expressed in rat DCs, a small number of DCs have been identified in the meninges and stroma of the choroid plexus of rat brain. Microglia, obtained from newborn rodent or adult human and expanded in vitro in the presence of astrocytes (mixed glial culture), exhibit a clear-cut macrophage phenotype and resemble ameboid, phagocytic microglia in the developing or injured CNS. However, when granulocyte macrophage colony-stimulating factor (GM-CSF) is added to the culture, microglial cells dramatically expand in number and express the typical DC surface markers Dec-205 and CD11c. In contrast, incubation with macrophage colony-stimulating factor (M-CSF) skews the microglial population toward a more defined macrophage phenotype, expressing high levels of CD11b and low levels of CD11c. Interestingly, Santambrogio et al. suggested that brain-resident microglia, in contrast to
Dendritic Cells in Experimental Allergic Encephalomyelitis and Multiple Sclerosis

Multiple sclerosis (MS) is a chronic, demyelinating disease affecting the white matter of the CNS. The etiology of MS is unknown, but several lines of evidence support the hypothesis that the pathogenesis is mediated by autoreactive T lymphocytes. Regardless of its cause, it is clear that the progression of this demyelinating disease is associated with an inflammatory reaction that involves activated lymphocytes, macrophages, and endogenous glial cells (astrocytes and microglia) in the brain. At early stages of inflammation, the disease involves the adhesion and transmigration of leukocytes across the BBB into the CNS, which is correlated with an increased expression of adhesion molecules.

In human MS patients, DCs generated from adherent blood mononuclear cells did not differ substantially with respect to their yield and morphology from DCs obtained from healthy subjects, except that the DCs derived from MS patients expressed higher levels of CD1a. In addition, patients with MS had higher levels of DCs secreting IFN-γ, TNF-α, and IL-6 than healthy subjects, suggesting that DCs from MS patients are in a more activated state. However, the involvement of DCs in human MS has not been elucidated.

EAE is a disease model with many features similar to human MS. EAE can be reliably elicited in different rodent species by immunization with either CNS tissue or with purified components of CNS myelin, such as myelin basic protein (MBP) and proteolipid protein (PLP). Sensitized T lymphocyte lines, propagated in vitro and then injected intravenously, generate “adoptive-transfer EAE,” giving conclusive proof of the pivotal pathogenic role of T cells.

The presence of DCs has been reported in lesions of acute EAE in Lewis rats induced by subcutaneous (s.c.) injection with MBP in Freund’s complete adjuvant (FCA). The presence of OX62+ DCs was detected in many EAE lesions in the CNS. In general, the function of DCs in EAE is associated with the initiation of immune responses, although they also participate in the effector phase of the immune responses. For instance, NO produced by DCs not only induces apoptosis of auto-reactive T cells, but also of DCs themselves, thereby disrupting continual antigen presentation during periods of remission of EAE.

In a study by Dittel et al., irradiated B10. PL mice were injected s.c. with bone marrow DCs pulsed with MBP Ac1-11 peptide. One day later, the mice were injected i.v. with MBP-TCR CD4+ T cells isolated from the spleen of MBP-TCR transgenic mice. The antigen-pulsed DCs efficiently presented MBP Ac1-11 peptide to antigen-specific T cells and induced EAE in recipient mice.

When administered in vivo, DCs can present specific antigens to T cells in either immunogenic or tolerogenic fashion, depending on the route of administration, numbers of DCs administered, and the maturation state of DCs. As observed by Fischer and Reichmann, functional maturation of brain DCs oc-
curs following the onset of encephalitis and these DCs seem to be differentiated from resident microglia. In brain cells from SJL mice during EAE, DCs were identified and a correlation between the number of brain DCs and the severity of the disease was evident. The concept of local expansion of DCs in CNS under inflammation is supported by the findings that DCs can be differentiated in vitro from resting CD11c+ microglial cells. It is speculated that the emergence, maturation, and prolonged activity of DCs in EAE brain contribute to chronic Th1 responses.

Xiao et al. have demonstrated that DCs, upon being pulsed in vitro with encephalitogenic MBP peptide 68–86 (MBP 68–86) and injected s.c. back into healthy Lewis rats, transfer immune tolerance to EAE induced by immunization with MBP 68–86 and FCA. Considering the observation that MBP 68–86 peptide-pulsed DCs can induce tolerance to EAE, it is possible that vaccination of MS patients with autologous blood DCs pulsed in vitro with autoantigen may be beneficial for disease control. However, a major obstacle to utilizing this scheme as a general therapeutic approach lies in the lack of characterized autoantigens responsible for the human disease.

**Dendritic Cells in Alzheimer’s Disease**

Alzheimer’s disease (AD) is a progressive neurological disorder that is characterized by memory loss, disorientation and behavioral changes. There is good evidence indicating that aberrant production and accumulation of the β-amyloid (Aβ) peptides, the 42-amino-acid form of Aβ (Aβ42) in particular, are primary events in the pathogenesis of AD. As a proteolytic cleavage product of the β-precursor protein (APP), both soluble and aggregated Aβ42 have been reported to be directly toxic to neurons. It has also been established that inflammatory response in the brain, mediated by Aβ42-activated astrocytes and microglia, plays an important role in the disease progression of AD. Aβ-activated astroglial cells elaborate pro-inflammatory cytokines, such as IL-1β, IL-6 and TNF-α, as well as other mediators, which promote neuronal death. On the other hand, studies suggest that as phagocytic cells in the brain, activated microglia may also play a role in the up-take and clearance of Aβ peptides, thus limiting their accumulation.

Since AD appears to be a degenerative disease associated with chronic inflammation and does not involve active participation of the adaptive immune system, the role of DCs in the pathological events of AD may not be as important as in MS. Nevertheless, observations, limited up to now, have revealed some unique features of DC interaction with Aβ peptide. Schmidt et al. monitored the survival, cytokine release, and surface marker expression by human DCs after exposure to Aβ peptides. Some peptide aggregates were found in intracellular vacuoles of DCs, suggesting an active uptake of Aβ peptides by the cells, presumably through phagocytosis. This process did not increase the production of TNF-α and did not change the surface expression of CD18, CD11a or CD11b by DCs. However, a decrease in the surface expression of MHC class II molecules was observed. DCs pulsed with Aβ aggregates in vitro were unable to stimulate T cells in an autologous co-culture system. This observation suggests that amyloid peptide may escape immune recognition by inhibiting MHC class II surface expression on DCs and suppressing their capacity to act as APCs.

Interestingly, recent studies indicate that activation of an effective adaptive immune response in AD may have therapeutic potential. This approach has been tested in transgenic mice overexpressing human APP, which develop AD-like syndromes with Aβ42 deposition in the brain and dementia. Vaccination of mice with aggregated human Aβ42 reduced the formation of Aβ plaques, and in older animals, vaccination resulted in a reduction in the ratio and the number of existing plaques. Aβ42-vaccinated mice exhibited significant cognitive improvement, suggesting a highly effective immunotherapy in AD-like animals. While the mechanism of plaque clearance by Aβ42 vaccination remains to be determined, Bard et al. reported that injection of an antibody specific to Aβ42 in transgenic mice initiated the clearance of antibody-bound Aβ aggregates in the brain by microglial cells.

It is therefore clear that in animal models of AD, vaccination with Aβ42 elicits a potent antibody response, which most likely requires antigen recognition and presentation by DCs in the periphery. However, whether vaccination is also suitable for human patients should be handled with caution because, as discussed earlier, treatment of human DCs with Aβ42 could actually reduce MHC class II expression on the cells and suppress their APC capacity.

**Dendritic Cells in Prion Diseases**

Prion diseases (transmissible spongiform encephalopathies, TSE) are a group of fatal progressive neurological disorders including Creutzfeldt-Jakob disease, Gerstmann-Sträussler-Scheinker syndrome and fatal
familial insomnia in humans, as well as bovine spongiform encephalopathy (BSE) and scrapie in other species. The etiological agent of these diseases is proposed to be an aberrant isomer of a cell surface glycoprotein, the prion protein (PrPc)\textsuperscript{63}. To maintain TSE infection, host cells must express the normal cellular isomer of the prion protein, as mice deficient in PrPc (PrP\text{+/–} mice) do not develop disease after peripheral injection of the infectious PrPc isoform\textsuperscript{13, 57}. Following experimental peripheral infection with scrapie, the pathologic isoform of PrPc (PrPSc) rapidly accumulates in lymphoid tissues\textsuperscript{8, 46, 56}, long before its appearance in the CNS. This peripheral “replication” leads to a progressive invasion of the CNS, in which PrPSc is deposited in the extracellular space of the diseased CNS infiltrated by activated astrocytes and mononuclear phagocytes (microglia)\textsuperscript{65}. The mechanisms of PrPSc toxicity for neurons are not clear, but seem to involve activation of microglia and oxidative stress. In cell culture experiments, Brown and Kretzschmar\textsuperscript{11} demonstrated that a pathogenic fragment of human PrPc, PrP\text{106–126}, is toxic to cells from the cerebellum and that this toxicity requires the presence of microglia. In response to PrP\text{106–126}, microglia increase their production of oxygen radicals and pro-inflammatory cytokines. Le et al.\textsuperscript{54} showed that PrP\text{106–126} is chemotactic for human monocytes through the use of a G protein-coupled receptor FPRL1, which has been reported to interact with a diverse array of exogenous and endogenous ligands. Upon stimulation by PrP\text{106–126}– FPRL1 underwent a rapid internalization and, furthermore, PrP\text{106–126} enhanced monocyte production of pro-inflammatory cytokines, which was inhibited by pertussis toxin\textsuperscript{53}. Recently, the expression of a FPRL1 homolog, FPR2, on murine microglial cells has been demonstrated\textsuperscript{19}. Thus, FPRL1 in human or FPR2 in mice may provide a molecular basis for the interaction of PrP\text{106–126} with microglial cells.

TSE displays a long incubation period and, initially, the prion titer increases in peripheral lymphoid organs\textsuperscript{8}, where follicular dendritic cells (FDCs) appear to support prion replication. Recent studies have shown that FDCs of the germinal centers of the spleen, lymph nodes and Peyer’s patches are key players in TSE pathogenesis. The normal functions of FDCs are to trap and retain native antigens in the form of immune complexes for presentation to B cells\textsuperscript{81}. Complement also plays an important role in the localization and retention of scrapie to FDCs during the first few days after infection\textsuperscript{55}. The classical complement activation pathway was most likely to be involved in C3 activation during scrapie infection, as the incubation period was markedly prolonged in mice deficient in both classical and alternative pathways (H2-Bj/C2–/– mice)\textsuperscript{82} or in mice with classical pathway deficiency (C1qa–/– mice)\textsuperscript{9, 47, 55}.

High levels of PrPSc are detected on FDCs in TSE-infected mice\textsuperscript{85}. Abnormal PrPSc accumulation is also seen within the germinal centers in Creutzfeldt-Jakob disease\textsuperscript{41} and sheep scrapie\textsuperscript{85}. It has been reported that SCID mice are much less susceptible than immunocompetent mice to peripheral challenge with TSE agents, although they are fully susceptible when infectious agents are introduced directly into their brains\textsuperscript{43}. This resistance to peripheral challenge is attributed to an inability of SCID lymphoid tissues to support PrPSc replication, which requires participation of FDCs. B cells may also be important in terms of their requirement for the maturation of FDCs. However, involvement of FDCs alone is not sufficient to explain how the infectious forms of the prion protein are transported from the gastrointestinal tract to the lymphoid organs and CNS. FDCs reside within B cell areas of lymphoid organs where they bind and retain antigens on the cell surface for months or years\textsuperscript{85}. These cells have a very slow turnover rate and are believed not to recirculate. Moreover, cellular PrPc has not been found to be associated with the FDCs in enteric lymphoid tissue\textsuperscript{37}. Therefore, some missing links within the chain of transportation of infectious PrPSc require further clarification. Interestingly, CD11c\textsuperscript{+} DCs, a cell type unrelated to FDCs, are able to propagate infectious prions from the periphery to the CNS in the absence of any additional lymphoid element\textsuperscript{4}. In addition, Burtheim et al.\textsuperscript{14} demonstrated that high levels of PrPc are present on myeloid DCs that surround the splenic white pulp. These myeloid DCs are ontologically and functionally distinct from FDCs. Consistent with these observations, expression of PrPc was strongly induced during the generation of mature myeloid DCs in vitro. In these cells, PrPc colocalized with MHC class II molecules. Given the close anatomic and functional connection of myeloid DCs with lymphoid follicles, it is suggested that myeloid DCs may also play a role in the propagation of PrPSc in humans.

**Concluding Remarks**

Although the complex interactions between the CNS and the immune system are not fully understood, accumulating evidence suggests that immune cells play important roles in the pathogenesis and progression of neurodegenerative diseases. The low frequency of DC precursors and mature DCs in the normal CNS initially
suggested that DCs might not be relevant to neurodegenerative diseases. However, under inflammatory conditions, the number of DCs increases significantly so that such cells are readily detectable and can be isolated from the CNS. Furthermore, the resident macrophages in the brain, the microglia, have the capacity to differentiate into effective APCs. While the role of DCs in antigen presentation and cross-talk with other immune cells in the CNS requires further investigation, DCs in the periphery have been shown to be pivotal in some forms of neurodegenerative diseases, in which immune responses to self antigen are the causative factors. In prion diseases, both FDCs and myeloid DCs are involved in the replication and transmission of infectious prion isoforms. In AD, although immune responses have not been implicated in the development and progression of the disease, successful vaccination in mouse models of AD suggests a novel therapeutic approach based on activation of the immune system, which surely requires antigen recognition and processing by APCs. Thus, a better understanding of DCs, or APCs in general, in neurodegenerative diseases will promote the elucidation of the pathogenic mechanisms and the development of therapeutic approaches.

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