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Abstract

Multiple sclerosis (MS) is considered to be a predominantly T-cell-mediated disease, and emerging evidence indicates that dendritic cells have a critical role in the initiation and progression of this debilitating condition. Dendritic cells are specialized antigen-presenting cells that can prime naive T cells and modulate adaptive immune responses. Their powerful biological functions indicate that these cells can be exploited by immunotherapeutic approaches. Therapies that inhibit the immunogenic actions of dendritic cells through the blockade of proinflammatory cytokine production and T cell co-stimulatory pathways are currently being pursued. Furthermore, novel strategies that can regulate dendritic cell development and differentiation and harness the tolerogenic capacity of these cells are also being developed. Here, we evaluate the prospects of these future therapeutic strategies, which focus on dendritic cells and dendritic cell-related targets to treat MS.

Harnessing Dendritic Cells To Treat Multiple Sclerosis

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Abstract

Although multiple sclerosis (MS) is considered to be T cell-mediated, emerging evidence points towards a critical role of dendritic cells (DCs) in the initiation and progression of this disease. DCs are professional antigen presenting cells that can prime naive T cells and control adaptive immune responses with respect to magnitude and self-tolerance. Their powerful biological functions can be exploited for the treatment of T cell-mediated autoimmune diseases. Emerging therapies aim at inhibiting immunogenic DC functions through blockade of pro-inflammatory cytokine production and T cell costimulatory pathways. Novel approaches aim at regulating DC development and differentiation and harness the tolerogenic capacity of DCs. Here, we evaluate the prospects of future strategies that focus on DCs and DC-related targets to treat MS.

Introduction

Multiple sclerosis (MS) is considered a classical antigen-driven and T cell–mediated autoimmune disease. This view is based on the cellular composition of brain and cerebrospinal fluid (CSF)-infiltrating cells, data from the most popular animal model of MS, experimental autoimmune encephalomyelitis (EAE), which can be induced by adoptive transfer of myelin-specific $CD4^+$ T cells from sick to naïve animals,[1] and by the fact that certain major histocompatibility complex (MHC) class II alleles, in particular the *HLA-DR2* haplotype carrying the *DRB1*1501* allele, represents by far the strongest genetic risk factor for MS development.[2,3] Repertoire analyses of T and B cells in CSF and brain tissue from patients with MS show clonal expansions in both populations, indicating that there is clonal reactivity to a restricted number of yet unknown disease-relevant target antigens. [4-7] Moreover, longitudinal studies over several years provide evidence for the long-term persistence of individual T-cell clones in the blood of patients with MS[8-10] indicating a strong, persisting memory response and/or ongoing antigen exposure.

Dendritic cells (DCs) are professional antigen presenting cells that can prime naïve T cells and control adaptive immune responses with respect to magnitude, memory and self-tolerance. DCs capture and process antigens, converting proteins to peptides that are presented on MHC molecules and recognized by T cells. DCs also secrete cytokines, directing naïve T cells towards different types of T helper (Th) subsets, such as Th1, Th2, Th17 cells[11] (Figure 1). In addition to their function in initiating and enhancing immunogenicity, DCs have a role in maintaining central and peripheral T cell tolerance.[12] Tolerogenic DC functions are considered to be of particular importance during the steady

state, i.e. in the absence of infection and inflammation, in order to avoid a non-appropriate response to harmless antigens that may be presented subsequently when infection strikes. The discovery of these cells and their increasingly appreciated role in regulating adaptive immune responses allowed for the development of a new generation of immunotherapies that are currently investigated in infectious, malignant, and autoimmune diseases.[13,14]

DCs: connecting innate and adaptive immunity

DCs were originally discovered and named for their stellate, tree-like shapes by Ralph M. Steinman and Zanvil Cohn in 1972.[15,16] DCs are abundant at body surfaces where they sense and migrate with environmental, self and microbial antigens to lymphoid organs in order to present processed antigen to naïve T cells and to induce antigen-specific immunity or tolerance. To increase the efficacy of antigen-uptake, DCs employ a system of endocytic receptors, often lectins, that deliver antigens to processing compartments, leading to the presentation of antigen fragments on MHC molecules. Among them are the type 1 transmembrane protein DEC-205 (CD205), the mannose receptor MRC1 (CD206) and type 2 proteins such as DC-SIGN (CD209), langerin (CD207), and CLEC4A/DCIR. By using monoclonal antibodies that bind to these receptors as surrogate ligands, one can efficiently target vaccine antigens to DCs.[14] For example, retroviral vectors gain improved immunogenicity if the envelope is engineered to target the DC-SIGN[17] and the efficacy of HIV DNA vaccines can be improved by coupling antigen to a DEC-205-specific antibody.[18]

Most DCs circulate in the body in a so-called immature state and lack many features that lead to a strong T-cell response. Immature DCs are, nonetheless, capable of capturing antigen. DC activation by different types of stimuli such as microbial ligands for pattern-recognition receptors or inflammatory cytokines leads to maturation during which DCs transport antigen-MHC complexes to the cell surface, upregulate costimulatory molecules necessary for T cell activation and survival such as CD80 and CD86, and initiate cytokine production. The latter directs T cell differentiation and assists in the activation of antibody-producing B lineage cells.

DCs can be classified into different categories based on cell surface markers, many of which are involved in pattern recognition and antigen presentation. A widely accepted classification distinguishes human DCs into two main categories: $CD11c^{+}$ myeloid DCs and $CD11c^{dim}CD123^{+}$ plasmacytoid DCs, so named because of cytologic similarities to antibody-producing plasma cells.[19,20] These subsets, which can be further subdivided based on their phenotype and functional properties, are likely to be selected to recognize distinct pathogens or forms of antigen and to initiate and regulate distinct innate and adaptive responses (Box 1).[21,22] In the steady state, most DCs in lymphoid organs arise from a blood precursor,[23,24] a process driven by the cytokine FLT3 ligand (FLT3LG).[25-27] During inflammation and infection, other cytokines, such as GM-CSF and M-CSF, additionally mobilize increased numbers of DCs that emanate from monocyte precursors.[28,29] Thus, the term DCs comprises a group of heterogeneous professional antigen-presenting immune cells. Nonetheless, all DC populations share characteristic features of the DC lineage such as the capacity to process and to present antigen, high levels of MHC class II expression, and the ability to prime naïve T cells.

DCs in autoimmune CNS inflammation

Although DCs are scant in the CNS parenchyma, their presence in vascular-rich regions of the healthy CNS such as perivascular spaces, the choroid plexus, and the meninges suggests they may have a role in immune surveillance of the CNS.[30,31] DCs accumulate in the CNS parenchyma during a wide range of inflammatory responses[31] and they are also present in inflammatory MS lesions.[32,33] Controversy remains whether DCs in the inflamed CNS differentiate from resident $CD11b^+CD11c^-$ microglia or infiltrate from a blood-borne population. Recent studies in CD11c reporter mice identified a unique population of CNS-resident $CD11b^+$ $CD11c^+$ MHC-II^- ramified cells in various regions of the developing and adult non-diseased brain parenchyma.[34] These cells upregulated MHC class II molecules and showed T cell stimulatory efficacy upon IFN-γ treatment.[35] Similar findings were made when CNS-resident microglia were treated with DC differentiation factors such as GM-CSF[36,37] which suggests that some micoglial cells have the developmental plasticity to acquire DC-like function.

During EAE development, $CD11c^+$ DCs alone are sufficient to present antigen to primed myelin-reactive T cells, thereby mediating CNS inflammation and clinical disease development.[38-40] Peripherally derived myeloid DCs appear to be superior to plasmacytoid DCs and other subsets of myeloid cells that reside or accumulate in the CNS during EAE development in inducing the activation and differentiation of CNS-infiltrating myelin-specific T cells, presumably due to enhanced expression of T cell differentiating cytokines.[41] Other studies suggested that DCs, depending on their location or differentiation state, can prevent EAE development.[42] For example, thymus-derived

DCs loaded with myelin antigen and injected intravenously into EAE susceptible hosts, were shown to inhibit disease development. This protection was lost in thymectomized recipients indicating that DCs that home to the thymus are able to confer tolerance to antigen-selected and activated encephalitogenic T cells.[43] There is also evidence that plasmacytoid DCs negatively regulate encephalotogenic $CD4^+$ T cell responses in mice.[41]

In humans, several studies report that patients with MS show altered DC phenotypes or functions. Patients with MS appear to have higher frequencies of blood myeloid DCs that express maturation markers such as CD80 and produce IL-12 and TNF-α as compared to healthy controls.[44] Similarly, DCs differentiated in vitro from blood monocytes from MS patients secrete higher levels of proinflammatory cytokines such IL-6,[45] TNF-α[45] or IL-23.[46] Altered plasmacytoid DC phenotypes, primarily associated with decreased expression of maturation markers, have also been reported in patients with MS.[47-49]

Thus, peripherally derived DCs that infiltrate the CNS appear to be crucial for EAE development and it is increasingly appreciated that overproduction of particular cytokines by certain DC subsets drives autoimmune CNS inflammation. In contrast, some DCs in lymphoid organs have the capacity to confer antigen-specific tolerance which protects from disease development. Due to their central role in T cell immunity and tolerance, DCs could be targeted for immunotherapy either by obviating their immunogenic potential or by enhancing their tolerogenic functions. Indeed, several emerging immunotherapies in various autoimmune diseases specifically target DC cytokine production or antigen presentation. Before we evaluate these new strategies, we

will discuss evidence that approved MS therapeutics utilize the DC system and innate immune mechanisms to exert their beneficial effects in MS.

Approved MS therapeutics modulate DC function

Regulatory-approved MS therapeutics such as interferon beta-1a and -1b (IFNβ), glatiramer acetate (GA), mitoxantrone, and natalizumab are reasonably effective in MS and appear to delay the time of progression to disability. These compounds, as well as the three monoclonal antibodies that are currently under active investigation for MS (rituximab, alemtuzumab, and daclizumab) and oral treatments (FTY720/fingolimod, cladribine), that have already proven to be effective in large phase III clinical trials, were originally designed to target autoreactive adaptive immune responses. However, immunological studies soon indicated that the mechanisms of action of both approved and currently tested, potential MS therapies is complex and involve, at least in part, innate immune functions such as DC-mediated polarization of effector T cells or DC-mediated expansion of regulatory immune cell compartments.

IFNβ, a pleiotropic cytokine with antiviral activity, mainly secreted by plasmacytoid DCs, became the first drug approved for the treatment of relapsing-remitting MS. Recent studies indicated that its complex mechanisms of action in MS includes an inhibitory effect on DC-mediated Th17 cell differentiation.[50] Thus, in addition to reported direct effects on T effector cells such as Th17 lymphocytes,[52] IFNβ appears to downregulate expression of Th17 cell polarizing cytokines.[53] Furthermore, the clinical response to IFNβ treatment in MS has been associated with DC function. Genome-wide expression profiling and functional studies in interferon-beta responders

vs. non-responders revealed that the latter showed higher expression levels of DC costimulatory molecules such as CD86 on myeloid DCs before treatment initiation, suggesting that patients with more pre-activated DCs are less susceptible to IFNβ therapy.[54]

GA was the first non-interferon approved for treatment of MS. GA is a synthetic polypeptide mixture containing four amino acids glutamic acid, lysine, alanine and tyrosine in a molar ratio of 1.5 : 3.6 : 4.6 : 1.0 which matches the molar ratios of the most frequent amino acids in myelin basic protein (MBP), a presumed target antigen in MS. The immunomodulatory effects of GA were believed to involve the expansion of a population of GA-reactive anti-inflammatory Th2 cells through interference with peptide/MHC binding on APCs. Hypothesizing GA also influences DC-mediated T cell polarization, Vieira et al.[55] and Kim et al.[56] showed that GA treatment led to enhancement of IL-10 and inhibition of IL-12 production in monocyte-derived DCs and blood monocytes which appeared to favor Th2 differentiation of naïve T cells.[56] The term type 1 and type 2 APCs has been introduced to characterize the ability to polarize either Th1 or Th2 cells via IL-12 or IL-10 production, respectively. In line with the aforementioned studies in humans, Weber et al. showed in experimental animal models that GA-treatment promoted the development of type 2 APCs which directed differentiation of Th2 cells and regulatory T cells.[57] Notably, adoptive transfer of GA-induced type 2 APCs reversed EAE and suppressed pathogenic T cell development.[57] Burger et al. also reported that GA inhibits pro-inflammatory monocyte function by triggering the production of the secreted IL-1 receptor antagonist (sIL-1Ra).[58] Although

not all of the abovementioned studies addressed DCs as such, they clearly extended the concept of GA's mechanisms of action to include anti-inflammatory APC functions.

The humanized monoclonal antibody natalizumab which prevents the interaction of the adhesion molecule very late activation antigen (VLA)–4 with its natural ligands, vascular cell adhesion molecule 1 (VCAM-1) and fibronectin, was designed to block trafficking of immune cells into the CNS. Indeed, the efficacy of natalizumab in MS is associated with a prolonged decrease of CSF leukocyte counts.[59] A new study indicated that natalizumab treatment, in addition to its effect adaptive immune cells, markedly reduces the number of DCs in cerebral perivascular spaces suggesting that its mechanism of action involves inhibition of DC or DC-precursor recruitment into the CNS.[60]

None of the approved MS therapeutics is as of yet a specific DC-directed therapy. However, a number of DC-based therapies have been tested in EAE and other animal models and a few of them are currently being investigated or are already approved in other human autoimmune diseases, such as rheumatoid arthritis.

Emerging DC-targeting therapies

DC-targeting therapies for autoimmune diseases aim at (i) inhibiting immunogenic DC functions or at (ii) supporting their tolerogenic potential (Figure 2). Most strategies involve the use of monoclonal antibodies (mAb) which target selective molecules expressed by DCs.

Targeting cytokines produced by DCs

DC cytokines stimulate and instruct T cells where to go and what to do. Upon maturation,

DCs produce an array of potent pro-inflammatory molecules, among them IL-1, IL-6, IL-12, and TNF-α (Figure 1). The so-called IL-1 family of cytokines, which is closely linked to innate immunity, include pro-inflammatory members such as IL-1α, IL-1β, IL-18, IL-33 as well as molecules that that suppress inflammation such as IL-1 receptor antagonist (IL-1Ra) which competes with IL-1 for receptor binding.[61] IL-1β is the most studied member of the IL-1 family because of its role in mediating autoinflammatory diseases. Mice deficient in IL-1α and IL-1β expression are resistant to EAE induction.[62] IL-1 is abundantly expressed by infiltrating myeloid cells and local microglia in inflammatory MS lesions.[63] Based on positive clinical trials, a recombinant version of IL-1Ra, anakinra, has recently been approved for treatment of patients with RA who do not responds to standard therapy. Similar to other biologics that block inflammatory key cytokines and pathways, the most important safety issues with anakinra are increased risks of serious infections and the occurrence of malignancies due to impaired immune function. So far, the safety and efficacy of anakinra or other compounds that target IL-1[61] has not been evaluated in patients with MS.

IL-6 belongs to a distinct family of proteins that are produced by innate immune cells and affect lymphocyte function. Mice deficient in IL-6 are resistant to EAE induction[64] and susceptibility is regained by exogenous administration of IL-6.[65] During EAE, IL-6 appears to shift the balance from regulatory to pathogenic effector T cells, thereby promoting disease pathology.[66] Blocking IL-6 function is, therefore, a reasonable therapeutic strategy to limit T cell-mediated autoimmunity. Tocilizumab, a humanized monoclonal antibody that competes with IL-6 for receptor binding, was recently FDA-approved for the treatment of RA. Similar to what has been noted in IL-1 targeting

studies, participants who took tocilizumab had a higher risk of serious infections compared to study participants who received placebo.[67] To our knowledge, tocilizumab or any other molecule that specifically blocks IL-6 function has not been tested in MS yet.

IL-12 and IL-23 are related heterodimeric cytokines that share a common subunit (p40) and have either p35 (IL-12) or p19 (IL-23) as a second subunit. As discussed above, IL-12 signaling directs Th1 differentiation, whereas IL-23 appears to be support the differentiation and maintenance of Th17 cells. Both IL-12/-23p40 and IL-23p19 have been detected in brain demyelinating MS lesions.[68,69] Mice that are genetically deficient in IL12p40 and IL23p19 are resistant to EAE.[70,71] Surprisingly, neutralization of the IL-12/23p40 subunit by the monoclonal antibody ustekinumab – which is effective in patients with psoriasis, psoriatic arthritis, and in patients with Crohn's disease – failed to show any efficacy in patients with relapsing-remitting MS.[72] An interpretation for this lack of efficacy is that the cytokine-mediated immunopathology observed in EAE models cannot be simply extrapolated to MS. Experience with two important T cell effector cytokines support of this hypothesis: IFN-γ is partially protective in EAE but its therapeutic use led to disease exacerbations in MS.[73] TNF-α is thought to be pathogenic in EAE whereas TNF-α neutralization led to disease exacerbations in patients with MS.[74] However, it can not be excluded that other factors such as poor CNS availability of the systemically administered antibody or the short study period (24 weeks) contributed to the negative results of the aforementioned clinical trial.[72]

The discovery of cytokines that promote DC development and differentiation such as FLT3L, GM-CSF, and M-CSF accelerated DC research.[75] GM-CSF is produced by

tissue stromal cells, and by activated T cells and NK cells and is, therefore, enriched at sites of inflammation.[76] Although GM-CSF is dispensable for steady-state DC development,[77] it supports DC differentiation from monocyte progenitors during inflammation and infection.[28,29,76] Recruitment of blood monocytes appear to sustain autoimmune tissue damage during EAE.[78] Therefore, inhibition of inflammation-induced DC differentiation from monocytes through GM-CSF blockade might have a therapeutic merit in autoimmune diseases. This approach is currently under investigation in two phase II studies in patients with RA who receive antibodies to GM-CSF (MOR103 and KB003). Therapies that specifically target DC homeostasis and inflammation-induced DC generation have, so far, not been initiated in MS.

Targeting costimulation provided by DCs

During T cell recognition of peptide/MHC complexes presented by DCs, costimulatory molecules can provide positive signals that promote T cell activation or negative signals that inhibit T cell responses. The B7:CD28 superfamily, a TNF:TNFR subfamily that lack death domains, includes a number of well characterized T cell costimulatory molecules that regulate T cell activation and tolerance. Among them are the proteins B7-1 (CD80) and B7-2 (CD86) that bind to CD28 on T cells and support T cell activation. Blockade of the B7:CD28 interaction by a fusion protein, called CTLA4-Ig, which binds to CD80 and CD86 is not only effective in limiting tissue inflammation in many preclinical models of autoimmune diseases but has also already been approved for the treatment of patients with rheumatoid arthritis (abatacept).[79,80] A recent open-label phase I clinical trial found that intravenous infusions of CTLA4-Ig were well tolerated in

patients with MS. Future studies will show whether B7:CD28 blockade as well as other and new costimulatory inhibitors, such as anti-CD40 and CD154 antagonists, will prove to be safe and effective in MS.

Targeting tolerogenic DC functions

DCs need to undergo terminal differentiation or maturation in order to coordinate protective immunity. Maturation is induced by a spectrum of environmental and endogenous stimuli, among them ligands for toll-like receptors and TNF-receptors such as CD40. In the steady state, i.e. in the absence of maturation stimuli, DCs can induce antigen unresponsiveness or T cell tolerance. In the first human study to test tolerogenic DC functions, application of immature DCs pulsed with an influenza matrix protein (MP) derived peptide led to a transient inhibition of preexisting MP-specific effector T cell functions and the appearance of MP-specific IL-10 producing cells which showed reduced IFN-γ production and lacked killer activity.[81]

Experimental models of autoimmune CNS inflammation provided evidence that antigen-specific T cell tolerance can be induced by mucosal (oral) administration of myelin antigen,[82,83] by altered peptide ligands (APLs) that interfere with the interaction of MHC/peptide complexes with encephalitogenic T cells[84,85] or by administration of high doses of either myelin peptides or soluble MHC-myelin peptide complexes which primarily aim at eliminating or inactivating myelin-specific T cells.[86-88] Based on these preclinical studies, ongoing trials are evaluating the safety, tolerability and efficacy of antigen-specific therapies in MS.[89,90] In a small 24-month randomized controlled clinical trial in patients with primary and secondary progressive MS, the intravenous injection of

soluble myelin basic protein (MBP) peptide (MBP8298, dirucotide), administered without adjuvants, was well tolerated and showed moderate effects on disease progression (as determined by changes in EDSS) in patients carrying MS-associated MHC haplotypes DR2 and/or DR4. [91] DNA vaccination is a different approach to achieve antigen-specific tolerance.[92,93] In a randomized controlled phase I/II trial, intramuscular administration of a DNA vaccine encoding full-length human MBP (BHT-3009) in patients with relapsing-remitting and secondary-progressive MS was well tolerated, led to a decrease in proliferation and IFN-γ production of myelin-reactive $CD4^+$ T cells and provided favorable trends on brain MRI metrics.[94] Larger phase II and phase III clinical trials have now been initiated to test the effect of soluble MBP peptide administration and MBP DNA vaccination in patients with relapsing-remitting and secondary progressive MS. A third strategy that is currently pursued is to re-infuse peripheral blood lymphocytes that have been chemically coupled with multiple peptides from supposedly immunodominant myelin antigens.[95] Preclinical experiments in EAE models have shown that the latter approach can prevent disease onset and ameliorate disease progression[96-99] and an open label phase I trial that tests the safety and efficacy of this strategy in patients with relapsing-remitting MS has recently been initiated.[90]

We hypothesize that the mechanisms of the aforementioned approaches involve DCs that are specialized to take up the externally administered myelin peptides or protein for antigen specific tolerance induction. In preclinical models, the administration of incompletely matured DCs loaded with myelin peptide can suppress EAE development.[100] Notably, targeting of antigens to DCs via antigen coupling to antibodies against specific uptake receptors such as the decalectin DEC-205 increases the efficacy

by which antigens are delivered to the immune system by more than 100-fold.[101-104] DC-targeting leads to either antigen-specific tolerance induction or immunity depending on the maturation state of the antigen-presenting DCs.[105-107] Hawiger et al. showed that mice pre-injected with a monoclonal antibody to DEC-205, coupled with the encephalitogenic MOG_{35-55} peptide were completely resistant to EAE development induced by subsequent MOG_{35-55} immunization.[108] Similar targeting strategies were also developed for the macrophage mannose receptor,[109] DC-SIGN,[110] DCIRs,[21,111] DNGR-1,[112] DCAR1,[113] BDCA-2[114] among others. The mechanisms that confer T cell tolerance in these animal models are diverse and involve deletion of T cells,[115] antigen-unresponsiveness[105,108] and/or the generation of regulatory T cells.[107,116]

One potential obstacle in translating these tolerization paradigms into the clinic is that patients with MS show increased frequencies of myeloid DCs expressing co-stimulatory, T cell activating, molecules, presumably due to DC maturation by chronic autoimmune inflammation,[44-46] where antigen targeted to DCs, even in the absence of adjuvants, could elicit unwanted and potentially deleterious T cell effector responses. In proof-of-concept studies for antigen-specific tolerization therapies in MS, during which patients received an altered peptide ligand (APL) based on the amino acid sequence of MBP_{83-99},[117,118] some patients experienced unexpected disease exacerbations which could be linked with unforeseen immunogenic *in vivo* effects of this APL.[118] Limiting the immunogenic potential of DCs by blocking the production or signaling of pro-inflammatory cytokines such as IL-6 might reduce the risk of triggering pathogenic T cell responses by antigen-specific therapies.[64] An alternative approach is to support tolerogenic DC functions in an antigen-unspecific way, e.g. through inhibition of DC

maturation.[119-121] Small molecule inhibitors of signaling molecules involved in DC maturation such as the Janus kinase 3 (Jak3) and the spleen tyrosine kinase (Syk)[122,123] are currently under investigation in patients with RA and psoriasis.[124,125]

Concluding remarks

DCs are central in inducing immunity and in regulating immune tolerance. In experimental models of autoimmune diseases including EAE, DCs induce and maintain pathogenic effector T cell functions. Whether DCs initiate adaptive autoimmunity in MS or whether these cells contribute to disease progression at later stages is unknown. Nonetheless, their powerful biological function makes them key targets for immunotherapeutic approaches. Emerging therapies aim at inhibiting immunogenic DC functions through blockade of pro-inflammatory cytokine production and T cell costimulatory pathways. Novel approaches aim at regulating DC development and differentiation and harness the tolerogenic capacity of DCs. Much has yet to be learned about the function of DC subsets and their T cell mobilizing potential in intact human lymphoid tissue and during chronic inflammation, as seen in MS. Nonetheless, the tools to harness DC functions in order to prevent or to limit autoimmune CNS inflammation are available and will likely become more specific and effective in the near future. These tools will allow us to address whether DC-targeting strategies either alone or in combination with established therapies will improve our ability to efficiently limit disease activity and progression in patients with MS.

Box 1: Dendritic Cell Subsets

Dendritic cells (DCs) initiate cell-extrinsic immune responses in higher eukaryotes.[126] They sense pathogen invasion and excessive cell death via pathogen associated molecular pattern (PAMP) receptors, process antigens for presentation on major histocompatibility complex (MHC) molecules to T cells and communicate information about the conditions, under which they have encountered the antigen, to innate and adaptive lymphocytes in secondary lymphoid tissues.[127,128] The DC lineage of both mouse and man is, however, composed of at least four distinct subsets. These are plasmacytoid, migratory myeloid, secondary lymphoid tissue resident myeloid and inflammatory DCs.[126] Plasmacytoid DC are the main type I interferon producing cells during immune responses.[129] Inflammatory DCs develop from monocytes at sites of inflammation.[130] Migratory DCs transfer antigen from the periphery to secondary lymphoid tissues and communicate to lymphocytes the conditions, under which they have encountered the antigen via cytokines and co-stimulatory molecules, whose up-regulation, together with enhanced antigen processing and MHC presentation, is collectively called maturation.[126] Prototypic migratory DCs are Langerhans cells of the epidermis. Resident DCs in secondary lymphoid organs are similar to migratory DCs, but might differ in MHC class I versus MHC class II antigen presentation from them.[131] It has been proposed that migratory DCs present antigens preferentially via MHC class II molecules for $CD4^+$ T cell stimulation, whereas resident DCs more efficiently cross-present antigens in secondary lymphoid tissues on MHC class I molecules to $CD8^+$ T cells after CD40/CD40L mediated maturation by activated $CD4^+$ T cells. In mice these efficiently cross-presenting DCs can be identified by their CD8 expression, whereas in humans the $BDCA3^+$ DC subset has been proposed for this

function.[132] Therefore, it is important to keep in mind that autoantigens could be presented with different efficacy by distinct DC subsets for autoreactive $CD4^+$ and $CD8^+$ T cell stimulation during MS, and that any tolerizing strategies might have to target the respective subsets to modulate $CD4^+$ or $CD8^+$ T cell mediated autoimmunity.

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Figure legends.

Figure 1: Dendritic cells stimulate and instruct T cells where to go and what to do. Following thymic selection, naïve T cells recognize their antigen in the context of MHC molecules presented by DCs. In the steady state, i.e. in the absence of inflammation, DCs can induce tolerance when they captures elf and environmental antigens. Mechanisms that confer T cell tolerance are diverse and include T cell deletion as well as well as the generation of regulatory T cells. Upon infection or other causes of DC maturation, DCs enhance their antigen processing and presenting capacities and upregulate cytokines and co-stimulatory molecules. Mature DCs can induce different types of $CD4^+$ T cells, such as Th1, Th2, and Th17. In addition to these interactions with T cells in lymphoid organs, DCs activate innate immune cells, which can further assist in the differentiation of T helper cell subsets.

Figure 2: DC-targeting therapies. DC-targeting therapies for autoimmune diseases including MS aim at (i) supporting the tolerogenic potential of DCs, e.g. through targeting of disease relevant antigens to antigen-uptake receptors such as DEC-205, by (ii) inhibiting DC development through blockade of DC-differentiating cytokines. Alternative strategies aim at (iii) limiting immunogenic DC functions such as production of inflammatory and T cell instructing cytokines or at (iv) reducing costimulatory signals required for effector T cell generation and activation. Most strategies involve the use of monoclonal antibodies (mAb) which target selective molecules expressed by DCs.

Key points

- Dendritic cells (DCs) are central in inducing immunity and in regulating immune tolerance.
- DC-targeting therapies for autoimmune diseases aim at inhibiting immunogenic DC functions or at supporting their tolerogenic potential. Most strategies involve the use of monoclonal antibodies which target selective molecules expressed by DCs.
- The DC lineage of both mouse and man is composed of at least four distinct subsets. The function of these subsets and their T cell mobilizing potential in intact human lymphoid tissue and during chronic inflammation, as seen in MS, is still incompletely understood.
- A number of DC-based therapies have been tested in EAE and other animal models and a few of them are currently being investigated or are already approved in other human autoimmune diseases, such as rheumatoid arthritis.

Review criteria

The PubMed database was searched for papers published up to May 2010 using the terms "multiple sclerosis dendritic cells", "multiple sclerosis treatment", "autoimmune dendritic cells". Articles were also identified through searches of the authors' files.

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Box 1: Dendritic cell subsets in MHC class I versus class II antigen presentation

Dendritic cells (DCs) initiate cell-extrinsic immune responses in higher eukaryotes [1]. They sense pathogen invasion and excessive cell death via pathogen associated molecular pattern (PAMP) receptors, process antigens for presentation on major histocompatibility complex (MHC) molecules to T cells and communicate information about the conditions, under which they have encountered the antigen, to innate and adaptive lymphocytes in secondary lymphoid tissues [2-3]. The dendritic cell lineage of both mouse and man is, however, composed of at least four distinct subsets. These are plasmacytoid, migratory myeloid, secondary lymphoid tissue resident myeloid and inflammatory DCs [1]. Plasmacytoid DC are the main type I interferon producing cells during immune responses [4]. Inflammatory DCs develop from monocytes at sites of inflammation [5]. Migratory DCs transfer antigen from the periphery to secondary lymphoid tissues and communicate to lymphocytes the conditions, under which they have encountered the antigen via cytokines and co-stimulatory molecules, whose up-regulation, together with enhanced antigen processing and MHC presentation, is collectively called maturation [1]. Prototypic migratory DCs are Langerhans cells of the epidermis. Resident DCs in secondary lymphoid organs are similar to migratory DCs, but might differ in MHC class I versus MHC class II antigen presentation from them [6]. It has been proposed that migratory DCs present antigens preferentially via MHC class II molecules for $CD4^+$ T cell stimulation, whereas resident DCs more efficiently cross-present antigens in secondary lymphoid tissues on MHC class I molecules to $CD8^+$ T cells after CD40/CD40L mediated maturation by activated $CD4^+$ T cells. In mice these efficiently cross-presenting DCs can be identified by their CD8 expression, whereas in humans the $BDCA3^+$ DC subset has been proposed for this function [7]. Therefore, it is important to keep in mind that autoantigens could be presented with different efficacy by distinct DC subsets for autoreactive $CD4^+$ and $CD8^+$ T cell stimulation during MS, and that any tolerizing strategies might have to target the respective subsets to modulate $CD4^+$ or $CD8^+$ T cell mediated autoimmunity.

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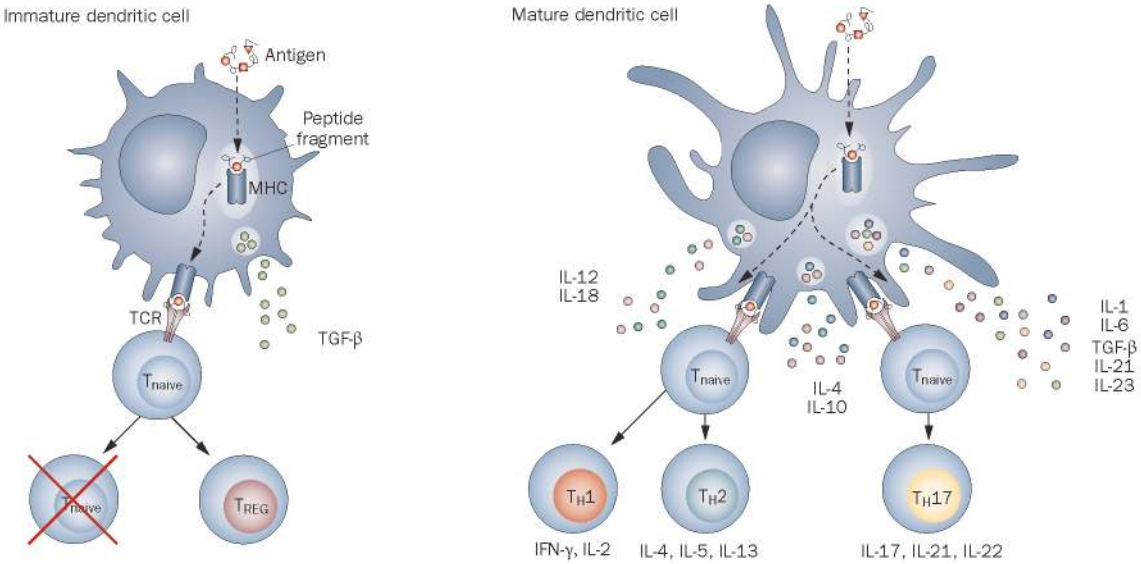
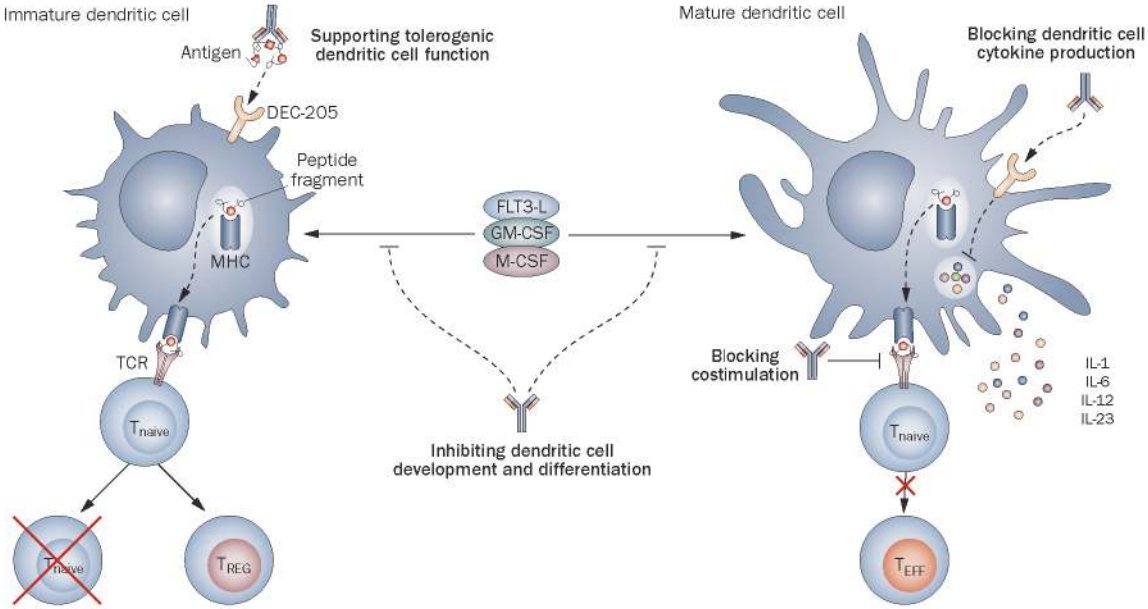

Immature dendritic cell
Antigen
Peptide fragment
MHC
TCR
TGF-β
T naive
T naive
T REG
Mature dendritic cell
IL-12
IL-18
IL-1
IL-6
TGF-β
IL-21
IL-23
IL-4
IL-10
T naive
T naive
T H1
T H2
T H17
IFN-γ, IL-2
IL-4, IL-5, IL-13
IL-17, IL-21, IL-22


Immature dendritic cell
Antigen
Supporting tolerogenic dendritic cell function
DEC-205
Peptide fragment
MHC
TCR
T naive
T naive
T REG
FLT3-L
GM-CSF
M-CSF
Inhibiting dendritic cell development and differentiation
Mature dendritic cell
Blocking dendritic cell cytokine production
Blocking costimulation
IL-1
IL-6
IL-12
IL-23
T naive
T EFF