Dendritic cells: inciting and inhibiting autoimmunity
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Dendritic cells are considered the most influential antigen presenting cells in the body because of their unique role in initiating immunity against threatening antigens. Recent studies addressing the consequences of self-antigen presentation by dendritic cells revealed the unexpected ability of these antigen presenting cells to inhibit T cell-mediated autoimmune diseases. The specific mechanisms by which dendritic cells suppress immune responses have been explored during the past year. These efforts indicate that extrathymic dendritic cells control autoimmunity by inducing peripheral T cell tolerance, a function intimately linked to their state of maturation.

Central tolerance is a critical step for the establishment of peripheral tolerance as well as immunity [2]. Genetic or environmental factors that alter the immunostimulatory capacity of DCs could impair peripheral tolerance induction leading to the onset of autoimmune disease.

The paradoxical ability of DCs to incite and inhibit autoimmune disease, as well as their features in autoimmune tissues, are reviewed here. Recent studies examining the specific mechanisms by which DCs induce peripheral tolerance are also discussed.

Introduction

Dendritic cells (DCs) are bone marrow-derived antigen presenting cells (APCs), unrivalled in their capacity for activating naïve and effector T cells. The life history of DCs unfolds in two main developmental stages, termed immature and mature. Immature DCs form lattice-like networks in virtually every tissue where they peruse the extracellular milieu, avidly endocytosing diverse antigens. Signaling by select pathogens, pro-inflammatory mediators, or CD40L, triggers immature DCs to embark on an irreversible differentiation process that results in mature DCs displaying remarkable immunostimulatory might. Before reaching peak maturity, DCs pass through an intermediate stage that is obligatory for Langerhans cells (LCs), red pulp splenic DCs and bone marrow-derived DCs. At this stage, DCs exhibit a transitional or semi-mature phenotype in terms of TCR ligand and accessory molecule expression; however, their functional contributions have not been well characterized. Maturation radically boosts the immunogenicity of DCs by inducing the stable expression of peptide–MHC complexes, upregulation of costimulatory and adhesion molecules, secretion of chemokines and stimulatory cytokines, and swift migration to T cell zones of regional lymph nodes (LNs). As mature DCs are poised for the optimal stimulation of naïve T lymphocytes [1], antigen presentation by mature DCs is a critical checkpoint in the generation of primary immune responses.

Central tolerance is an imperfect process, thereby allowing some autoreactive T lymphocytes to escape riddance in the thymus. DCs undoubtedly process self-proteins that are either expressed endogenously or acquired during endocytosis. DC presentation of self-antigens during infection or tissue injury could lead to the misguided generation of autoaggressive T lymphocytes. Despite the presence of autoreactive lymphocytes in the circulation and the presentation of self-epitopes by ‘nature’s adjuvant’, most individuals escape the pathological consequences of autoimmunity. Thus, extrathymic mechanisms for subduing the autoreactive lymphocyte repertoire must exist. These elusive mechanisms are collectively referred to as peripheral tolerance. Emerging evidence indicates that DCs are responsible for the establishment of peripheral tolerance as well as immunity [2]. Genetic or environmental factors that alter the immunostimulatory capacity of DCs could impair peripheral tolerance induction leading to the onset of autoimmune disease.

The paradoxical ability of DCs to incite and inhibit autoimmune disease, as well as their features in autoimmune tissues, are reviewed here. Recent studies examining the specific mechanisms by which DCs induce peripheral tolerance are also discussed.
results from defects in this function of DCs. In examining the mechanisms of peripheral tolerance induction by DCs, we might begin to understand how autoimmune disease unfolds.

**Controlling autoimmunity by peripheral tolerance induction**

**Elimination of autoreactive T lymphocytes**

How do the same APCs that mount primary immune responses and precipitate autoimmunity also inhibit autoimmune disease? One possibility is that tolerance is mediated by immature or semi-mature DCs expressing low levels of T cell-receptor ligands and costimulatory molecules, whereas immunity is generated by mature DCs expressing high levels of these molecules. This would require the presentation of tissue antigens by immature DCs in secondary lymphoid tissue, a scenario that seems to conflict with dogma at first glance; however, this idea may not be at odds with the classical notion of ‘DC migration upon maturation’ because some DCs migrate on a continuous basis [16]. Homing of migratory DCs to secondary lymphoid tissue, in the absence of maturation stimuli, could allow immature or semi-mature DCs to present tissue antigens to cognate T cells in a substimulatory context. But do DCs actually traffic peripheral antigens to regional LNs in the steady state? For some time it has been known that transport of antigenic cargo is critical for generating immunity against pathogens that invade non-lymphoid tissues.

Administration of trackable antigens, such as microbes, contact sensitisers or latex microparticles, revealed that transport of antigen from non-lymphoid to lymphoid tissue is facilitated by DCs. Because such procedures inevitably release pro-inflammatory mediators, they cannot be used to assess antigen delivery in the steady state. This dilemma was recently resolved by two studies in which gut- and skin-restricted antigens were identified within DCs of nearby LNs [17,18]. Indeed, a population of migratory DCs samples antigens in peripheral tissues and transports them to draining LNs under homeostatic conditions.

The role of immature DCs in T cell engagement has largely been ignored for two main reasons: first, immature DCs are inefficient at antigen processing; and second, their positioning in peripheral tissues makes an encounter with naïve T cells unlikely. That DCs transport peripheral antigens to LNs in the steady state, however, implies that this process is carried out by immature DCs, and that antigen presentation by these cells has immunological consequences. In an important investigation, Hawiger et al. [19] addressed these issues and found that presentation of antigen by DCs in the absence of inflammation or infection leads to bona fide tolerance. Soluble antigen was targeted to the MHC class II pathway of DCs in situ by non-inflammatory measures, using an antibody specific for a specialized DC endocytosis receptor. Under these conditions, antigen presentation by DCs prompted a short proliferative burst of cognate CD4+ T cells followed by their deletion. The lack of a response to subsequent peptide immunization indicated that the recipients had been rendered tolerant. In agreement with a previous report that links peripheral tolerance to bone marrow-derived APCs, the Hawiger study established that DCs induce peripheral tolerance by eliminating autoreactive T cells [20]. In contrast, when antigen targeting was accompanied by a strong DC maturation stimulus, such as anti-CD40, the outcome was converted to immunity. These findings indicate that self-antigen presentation by immature DCs is pivotal in the elimination of autoreactive T lymphocytes and that the fate of any immune response is shaped by the ‘maturity’ of the DC presenting antigen.

Mature or semi-mature DCs may also contribute to the maintenance of peripheral tolerance by deleting specific subsets of autoreactive T cells. In an *in vitro* system described by Albert et al. [21], memory CD8+ T cells are tolerized by DCs matured in TNF-α and prostaglandin E2 (PGE2), but not by macrophages or immature DCs. Maturation triggered by CD40 signaling, however, changed the outcome from T cell elimination to cytotoxic T lymphocyte (CTL) generation. Thus, the combination of TNF-α and PGE2 conditioned the DCs differently than anti-CD40 treatment, possibly by providing only partial maturation stimuli or by activating entirely distinct signal transduction pathways that render the DC tolerogenic. Whatever the case may be, some degree of DC maturation may be necessary for peripheral tolerance induction. It has been hypothesized that autoimmune syndromes result from defects in peripheral tolerance induction. If mature DCs participate in peripheral tolerance, then genetic or environmental factors that impair DC maturation could hinder the suppression of autoreactive T lymphocytes resulting in unchecked activation of these cells. Interestingly, blood DCs obtained from patients with autoimmune diabetes, systemic lupus erythematosus (SLE), Grave’s disease and multiple sclerosis (MS), as well as individuals at risk of diabetes, exhibit a relatively immature phenotype [22–26]. When compared with healthy controls, DCs derived from patient blood were impaired in T cell stimulation and generally expressed lower levels of costimulatory molecules. Alternatively, semi-mature DCs may be required for the induction of regulatory cells, suggesting that functionally impaired DCs underlie immunoregulatory defects in autoimmune patients.

Finally, peripheral tolerance might be mediated by a specialized subset of DCs in secondary lymphoid tissue that constitutively expresses and presents peripheral antigens for the purpose of perpetual elimination of autoreactive T cells. Pugliese et al. [27] recently identified a small subset of spleen DCs expressing pancreatic islet-specific antigens. Intriguingly, apoptotic lymphocytes were found in close proximity to DCs expressing self-antigen, suggesting that this DC population may induce T cell tolerance by direct induction of programmed cell death.

**Altering the effector functions of T cells**

DCs also induce peripheral tolerance by generating regulatory T cells that impede the functions of effector T cells...
through suppressive cytokines or a contact-dependent mechanism [28]. IL-10-producing CD4+ and CD8+ regulatory T cells can be induced by immature DCs [29,30**,31**]. It has been proposed that peripheral tolerance is maintained by immature DCs presenting antigens that are captured during normal cellular turnover, although no convincing link has been made between the presentation of apoptotic material and the induction of regulatory T cells by DCs [32]. A role for this form of tolerance induction was recently investigated in a model of autoimmune diabetes. Here, the induction of regulatory T cells by immature DCs correlated with disease prevention [33**]. Importantly, protection from diabetes appeared to be dependent on presentation of antigen derived from apoptotic β cells. DCs at an intermediate stage of maturation are also equipped to inhibit experimental autoimmune encephalomyelitis (EAE) through CD4+ T regulatory cell induction [11*].

Control of immunity by DCs is no doubt affected by costimulation and hence by the external factors that affect costimulatory molecule expression. Systemic administration of IL-4 inhibits both spontaneous and virus-induced diabetes. IL-4 prevents diabetes in non-obese diabetic mice by generating a Th2 response in a typically pathogenic Th1 environment [34]. The specific mechanism by which IL-4 inhibits lymphocytic choriomeningitis virus (LCMV)-induced diabetes was recently explored. King et al. [35**] found that IL-4, selectively expressed in β cells, acts directly on local DCs by differentially altering expression of B7 molecules. Exposure of DCs to IL-4 caused CD80 directly on local DCs by differentially altering expression of B7 molecules. Importantly, protection from diabetes appeared to be dependent on presentation of antigen derived from apoptotic β cells. DCs at an intermediate stage of maturation are also equipped to inhibit experimental autoimmune encephalomyelitis (EAE) through CD4+ T regulatory cell induction [11*].

Dendritic cell physiology in autoimmune tissues

Our understanding of the role of DCs in autoimmune disease stems, in part, from direct examination of these APCs in tissues of autoimmune subjects. DCs have been detected in lesions associated with numerous autoimmune diseases, including diabetes, rheumatoid arthritis (RA), psoriasis, EAE, thyroiditis, Sjögren’s syndrome and SLE, and they are among the first cells to infiltrate target organs [37–44]. Unique functions of DCs in autoimmune tissues may coordinate the recruitment and/or activation of other immune players [8,45]. For example, abnormal chemokine secretion by DCs in tissues of nephritic mice preferentially acts on B1 cells, which may trigger autoantibody production in lupus [46*].

The origin of DCs in autoimmune lesions may illuminate their specific role(s) in autoimmune pathology. Although DCs in autoimmune lesions are primarily mature, it is unclear whether they arrive as fully mature cells or as DC precursors that undergo differentiation upon contact with specific tissue factors [8,38,39,47]. Santiago-Schwarz et al. [48] explored the composition of DCs in autoimmune synovial fluid and found that multiple stages of DC development were represented. In addition to mature DCs, they detected proliferating CD34–CD33+ myeloid progenitors that gave rise to non-proliferating myeloid dendritic progenitors. Interestingly, RA synovial fluid promoted differentiation of myeloid dendritic progenitors into functionally mature DCs in vitro; however, this was not the case when myeloid dendritic progenitors were cultured with synovial fluid from osteoarthritic patients or with normal human serum, or when CD34+ progenitors were cultured with RA synovial fluid. Thus, autoimmune synovial fluid contains factors that attract myeloid dendritic progenitors and promote their differentiation into mature DCs.

Likewise, Blanco et al. [49*] demonstrated that serum from SLE patients enhanced monocyte differentiation into immunostimulatory DCs and that this potential correlated directly with disease activity. IFN-α, which is elevated in the blood of patients with SLE, RA, Sjögren’s syndrome and scleroderma, was the culprit driving DC development from monocytes. Paradoxically, the overall numbers of myeloid DCs, their monocyte precursors, and IFN-α-producing plasmacytoid DCs in patient blood were reduced. A decrease in blood DCs could be accounted for by augmented migration to tissues, as seen in murine lupus [46*,50]. Abnormalities in myeloid DC development and maturation have also been observed in murine models of diabetes and lupus [51–56]. Unfortunately, the reports are not in agreement with each other, preventing a general consensus from being reached. Elucidating these putative defects in myelopoiesis will be critical for understanding DC differentiation in autoimmune disease.

Conclusions

Our understanding of DCs and their roles in autoimmune disease has broadened in several ways this past year. We have learned that DCs not only promote immunity but also mediate peripheral T cell tolerance by direct elimination, T regulatory cell induction or counter-regulation. Tolerance can be induced by adoptive transfer of immature or semi-mature DCs, or by DCs presenting self-antigens under steady state conditions; however, the functional attributes that distinguish a tolerogenic from an ‘autoimmunogenic’ DC have yet to be defined. The efficacy of DCs in inducing peripheral tolerance may be useful for the treatment of ongoing human autoimmune
conditions and preventing autoimmune disease onset in disease-prone subjects.

Studies of subjects with acute autoimmune disease indicate that DCs in autoimmune lesions exhibit an altered mature phenotype, whereas DCs obtained directly from blood or differentiated from blood precursors exhibit developmental defects as well as an altered immature phenotype; however, whether these alterations are causes or effects of autoimmune disease remains unclear. Taken together, these studies demonstrate that DCs are extremely versatile APCs capable of actively subduing autoimmune under homeostatic or immunosuppressive conditions and mounting protective immunity during infection or inflammation. Autoimmune disease may arise when peripheral tolerance mechanisms are disrupted or when the immunogenicity of DCs is aimed at self-antigens.

Update
A specific role has been identified for the enigmatic CD4+ DC population in controlling autoimmune disease. In contrast to their CD8+ counterparts, which trigger autoimmunity via robust IL-12 production, splenic CD4+ DCs reverse CNS homogenate-induced EAE [57*]. CD4+ DCs that have internalized aggregated Ig-MOG via FcγRI suppress autoimmunity by secreting IL-10.

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References and recommended reading
Papers of particular interest, published within the annual period of review, are highlighted as ••.


This study underscores the important point that not all DC maturation stimuli should be regarded as equal. The authors demonstrate that serial transfers of bone marrow-derived DCs pretreated with MOG-peptide and TNF-α completely protect recipients from EAE by inducing CD4+ T regulatory cells, whereas anti-CD40 and LPS treatment generated classical mature DCs with no tolerogenic potential. Thus, partially mature or intermediate DCs are conditioned, albeit in unknown ways, for peripheral tolerance induction.


18. Hemmi H, Yoshino M, Yamazaki H, Naito M, Iyoda T, Omatsu Y, Shimoyama S, Letterio J, Nakabayashi T, Tagaya H et al. Skin antigens in the steady state are trafficked to regional lymph nodes by transforming growth factor-beta1-dependent cells. Int Immunol 2001, 13:895-904. Using hyperpigmented mice to monitor trafficking of skin-restricted antigens, the authors show that melanin granules are transported to draining LNs in the absence of inflammation. Melanin granules are undetectable in LNs of transforming growth factor (TGF)-β-deficient mice (which lack epidermal DCs or Langerhans cells), indicating that insoluble skin antigens are continuously ferried to LNs by migratory DCs that have captured melanocyte fragments rather than by lymph- or macrophage-mediated transport pathways.


This work establishes a functional role for DC-mediated antigen transport which occurs under homeostatic conditions. A mononuclear antibody specific for DEC205, an endocytic receptor highly expressed on DCs, was used to efficiently target specific peptides to DCs in vivo. The authors show that presentation of soluble antigen by DCs in healthy, uninfected subjects leads to tolerance by deletion of antigen-specific CD4+ T cells.


This study characterizes the requirements for in vitro cross tolerisation of human memory CD8+ T cells. The authors demonstrate that an increase in B7 expression induced by CD40 engagement is necessary for deletion of antigen-specific CD8+ T cells by DCs.


27. Pugliese A, Brown D, Gazza D, Murchison D, Zeller M, Redondo M, **Diez J, Eisenbarth GS, Patel DD, Ricordi C**: Self-antigen-presenting cells expressing diabetes-associated autoantigens exist in both thymus and peripheral lymphoid organs. J Clin Invest 2001, 107:555-564. This investigation demonstrates that DCs in human spleen and thymus express tissue-specific autoantigens such as GAD, proinsulin and IA-2. This evidence supports the provocative hypothesis that a specialized subset of extrathympic DCs induces tolerance by presenting endogenously expressed autoantigens followed by direct killing of autoreactive T lymphocytes.


This study characterizes a novel population of DX5 regulatory DCs that inhibit LCMV-induced diabetes in response to CD40L blockade. This cell population exhibits functional and phenotypic features of NK cells and macrophages; however, their origin and mechanism of action remain unclear.


46. Ishikawa S, Sato T, Abe M, Naga S, Onai N, Yoneyama H, Zhang Y, Suzuki T, Hashimoto S, Shirai T et al.: Aberrant high expression of B lymphocyte chemokine (BLC/CXCL13) by C11b+CD11c+ dendritic cells in murine lupus and preferential chemotaxis of B1 cells towards BLC. J Exp Med 2001, 193:1393-1402. This work demonstrates that thymic DCs in mice with lupus produce abnormally high levels of B lymphocyte chemokine and that CXCR5+ B1 cells are selectively recruited by this chemokine. Evidence provided in this report supports the hypothesis that abnormal chemokine production by DCs coordinates the formation of autoimmune infiltrates by attracting specific lymphocyte populations.


Using adoptive transfers of specific DC populations, the authors discover a function for CD4+ DCs in establishing peripheral tolerance. IL-10 production by CD4+ DCs counters the effects of IL-12 made by CD8α+ DCs, leading to reversal of ongoing EAE.