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Short reviews

Are dendritic cells central to regulatory T cell function?

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ABSTRACT

The role of dendritic cells (DCs) as sentinels and inducers of immunity has been amply documented in the past 35 years. More recently, experimental evidence has suggested that DCs may also be critical to maintain tolerance to self-antigens. These opposing functions are complementary and would ensure the integrity of the organism in an environment full of pathogens. In this review, we summarize the observations supporting the hypothesis that DCs induce and maintain tolerance by a mechanism involving regulatory T cells.

1. DCs display opposite functions

The role of dendritic cells (DCs) as sentinels in the periphery and inducers of immunity in the lymphoid organs has been amply documented since their discovery by Steinman and Cohn in 1973 [1]. Among antigen-presenting-cells, cells of the dendritic family display unique features which confer to them the capacity to trigger, in secondary lymphoid organs, an immune reaction to microbial antigens encountered earlier in peripheral tissues. In the presence of a "danger signal" (such as infection or inflammation), DCs shift from an antigen-capturing mode to a T cell sensitizing mode, a phenomenon called "maturation" which is usually associated with their migration to lymphoid organs. *In vitro* and *in vivo* observations therefore suggested that DCs would be the "nature adjuvant": immature DCs would capture antigen in the periphery and, in the presence of maturating agents (infection, inflammation), would carry it to immune cells which are confined in organized lymphoid structures. In this case, new migrant DCs are at a mature stage and express the costimulatory signals required for the optimal activation of naïve T cells. Of note, this specialization of function over time and location will favour immune reactions to non-self antigens able to trigger DC maturation.

Surprisingly, in 1996, Finkelman et al. reported observations suggesting that DC function could vary according to the nature of the stimulation [2]. They showed that injection of mice with a rat mAb (33D1) specific for an unknown DC antigen induced specific T cell and B cell tolerance for the rat IgG. This unexpected result seemed to indicate that, in the absence of supplementary stimuli, DCs would be able to present antigen in a tolerogenic fashion. In agreement with this hypothesis, Viney et al. demonstrated that treatment with Flt3L (hematopoietic growth factor) increased the number of DC *in vivo* and induced tolerance in mice fed soluble antigens [3]. Of note, tolerance could be induced in FLT3L-treated mice using very low doses of antigen that were ineffective in control animals. Other studies similarly suggested that tolerance to autoantigens could be induced by bone-marrow-derived antigen-presenting-cells [4,5].

Consistent with tolerogenic properties of DCs, two studies subsequently showed that, in steady state conditions, i.e. in the absence of inflammation, DCs migrated constitutively from peripheral tissues. In the rat, Huang et al. identified a subpopulation ($OX41^-$) of DCs which carry apoptotic intestinal epithelial cells to mesenteric lymph node through afferent lymph. $OX41^-$ DCs are weak APCs despite expressing high levels of B7 molecules, whereas another subset $OX41^+$ is immunostimulatory but does not seem to reach the T cell area in the absence of inflammation [6]. In the lung, antigen transport from the airway mucosa to the thoracic lymph nodes was studied *in vivo* by intratracheal instillation of fluorescein isothiocyanate-conjugated macromolecules [7]. A strong FITC signal was detected in migratory airway-derived lymph node DCs, suggesting that DCs rapidly transport antigen from the airways to the thoracic lymph nodes. These observations suggested that DCs may capture and present tissue-specific self-antigens even in steady state conditions, and that recruitment to lymphoid organs and maturation and be independently regulated.

Direct evidence for a tolerogenic role of immature DCs was provided by Hawiger et al. who used a mAb specific for DEC-205 (an endocytic receptor) coupled with a peptide from hen egg lyzozyme (HEL) to deliver antigen to DCs *in vivo* [8]. Mice were transferred with HEL-specific transgenic $CD4^+$ T cells and treated, 24 h later,

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with the fusion protein (αDEC/HEL). 2 d after transfer, T cells isolated from draining lymph node proliferated strongly, but 7 d later, the T cells either disappeared or became anergic to antigenic restimulation. These findings contrasted with the prolonged T cell activation observed in mice co-injected with DC-targeted antigen and anti-CD40 agonistic antibody, suggesting that, in the steady state, the primary function of DC was to maintain peripheral tolerance. Probst et al. used an elegant system to demonstrate, without any cell transfer, that resting DCs induced peripheral tolerance: they injected double-transgenic DIETER mice with tamoxifen to induce the presentation of three transgenic CTL epitopes by DCs [9]. This presentation resulted in peripheral tolerance if the DCs were resting and in protective immunity if DCs were activated *in vivo*. More recently, the authors demonstrated that PD-1 and CTLA-4 acted synergistically for peripheral tolerance induced by resting DCs [10].

2. Nature of the tolerogenic DC

These results convincingly demonstrate that immature DCs have the capacity to induce tolerance in the periphery. However, whether a specialized subset exists which is tolerogenic is still a matter of speculation and would suggest that the tolerogenic property would be intrinsic to DC subpopulation(s). Alternatively, the function of DCs would be dictated by the cytokinic environment.

The family of DCs appears heterogenous and includes interstitial DCs, Langerhans cells, inflammatory DCs, veiled cells and plasmacytoid-derived DCS. Interstitial DCS are found in most organs, including lymphoid organs where they comprise distinct subsets. In particular, splenic DCs can be separated according to the expression of the CD8α marker. The $CD8\alpha^+$ DCs are positive for the C-type lectin 205 ($CD8\alpha^+$ $DEC205^+$) while $CD8\alpha^-$ DCs lack CD8α but express the antigen recognized by the 33D1 mAb ($CD8\alpha^-$ $33D1^+$). Early studies, in 1996, suggested that splenic $CD8\alpha^+$ DC were able to suppress *in vitro* allogeneic T cell responses via an apoptosis-dependent mechanism, whereas $CD8\alpha^-$ DCs induced a vigorous proliferative response in $CD4^+$ T cells in the same conditions [11]. The tolerogenic capacity of $CD8\alpha^+$ DCs was also observed *in vivo* but appears restricted to tumor/self-peptide P815AB [12]. Consistent with a role in tolerance induction, $CD8\alpha^+$, but not $CD8\alpha^-$, DCs have been shown to take up apoptotic splenocytes (osmotically shocked) *in vivo* [13]. Likewise, Lu et al. have derived from normal mouse liver, a $DEC\text{-}205^+$ non-parenchymal population with DC morphology and tolerogenic function [14]. *In vivo* administration of liver $DEC205^+$ $B220^+$ $CD19^-$ cells significantly prolonged survival of vascularized cardiac allografts in an allo-antigen specific manner. By contrast, Dudziak et al. have shown that delivery of antigens by the mean of anti-DEC205 ($CD8\alpha^+$ DC) or anti-33D1 ($CD8\alpha^-$ DC) mAb leads to T cell tolerance suggesting that both DC subsets are able to induce an immune and tolerogenic response depending on their maturation stage [15].

Another subset of DC has been shown to induce tolerance: DCs which derive from plasmacytoid cells (IFN-α/β-producing cells). This population belongs to pre-DCs as these cells need an inflammatory stimulus to acquire typical DC features. De Heer et al. showed that a single intratracheal injection of OVA before the repeated challenges leads to tolerance [16]. Depletion of plasmacytoid DCs using anti-120G8 Ab in this tolerogenic model abrogated the unresponsiveness, whereas intravenous injections of bone marrow-derived OVA-pulsed plasmacytoid DC before the induction of asthma strongly inhibited airway inflammation, suggesting that these DCs induced tolerance in response to inhalation of harmless protein antigens. Similarly, Ochando et al. demonstrated that plasmacytoid DCs were critical for the tolerance of vascularized allograft [17]. These cells acquired alloantigen in the allograft and moved through the blood to draining lymph nodes, where they induced the development of Treg. Finally, other cells of the dendritic family have been shown to display tolerogenic properties, such as Langerhans cells [18], $CD103^+$ DCs at mucosal sites [19] and IL-10-producing $CD11c^{low}$ $CD45RB^{high}$ DCs [20].

The capacity to induce tolerance may be intrinsic to DCs and/or be influenced by the environment. It is generally admitted that IL-10 and TGF-β are involved in the induction and maintenance of peripheral tolerance (see later discussion). Svensson et al. have shown that spleen-derived stromal cells cluster with progenitor cells in culture and induce the development of $CD11c^{lo}$ $CD45RB^+$ DCs which have a partially IL-10-dependent, but TGF-β-independent, regulatory function [21]. Of note, stromal cells from mice infected with Leishmania donovani more effectively supported the differentiation of these regulatory DCs, which may cause the observed chronic infection.

3. DCs as inducers of Treg

The mechanisms by which DCs would induce tolerance are still elusive and could be either direct (induction of anergy or deletion of responding T cells) or indirect (development of regulatory T cells). There is little evidence, *in vivo*, that T lymphocytes would become anergic to further stimulation when they recognize their cognate antigen under conditions of suboptimal costimulation. Recent studies would rather support a role of regulatory T cells.

Regulatory T cells are divided into two groups on the basis of their lineage. Natural Tregs arise in the thymus through homeostatic processes and express the IL-2 receptor-α chain (CD25) while induced Treg develop extrathymically under appropriate stimuli. The expression of the transcription factor Foxp3 is the hallmark of regulatory T cells; even though $Foxp3^-$ regulatory T cells have been described. The crucial role of Tregs in tolerance to self is highlighted by the observation that Foxp3-null mice die from an autoimmune syndrome [22]. The mechanisms by which regulatory T cells control immune response are still unclear. Recent reports have suggested a role for IL-35 [23], cytokine deprivation-mediated apoptosis [24], perforin-granzyme pathway [25], cAMP [26] in the function of natural Treg.

Yamazaki et al. have shown that DCs could induce the proliferation of natural regulatory T cells *in vitro* and *in vivo* [27]. This finding was unexpected as regulatory T cells were described as anergic, i.e. unresponsive to TCR stimulation in the presence of splenic APCs. Mature, and to a lesser extent immature, bone marrow-derived OVA pulsed DCs induced the proliferation of OVA specific $CD4^+$ $CD25^+$ T cells. Transwell experiments indicated that cell contact with bone marrow-derived DCs was crucial for initiating Treg proliferation. *In vivo*, both steady state and mature antigen-pulsed DCs induced proliferation of adoptively transferred OVA specific $CD4^+CD25^+$ T cells. The same authors have shown that bone marrow derived DCs, which are considered as inflammatory DCs, appear more efficient than splenic DCs, suggesting that discrete subpopulations of DC may be able to trigger Treg proliferation. Another study confirmed that transferred TCR transgenic Treg proliferated *in vivo* in response to antigen in IFA [28].

Although it was generally admitted that DCs at the immature stage would induce the differentiation and/or function of natural Treg thereby preventing self-reactions, we and others have shown that natural Treg controlled immunity to non-self antigens [29]. In particular, the development of class I and class II-restricted IFN-γ producing cells was strongly enhanced in the absence of Treg in mice immunized by injection of mature DCs pulsed with foreign antigens (KLH, OVA peptides). By contrast, depletion of Treg did