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Experimental models to investigate the function of dendritic cell subsets: challenges and implications

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Summary

The dendritic cell (DC) lineage is remarkably heterogeneous. It has been postulated that specialized DC subsets have evolved in order to select and support the multitude of possible T cell differentiation pathways. However, defining the function of individual DC subsets has proven remarkably difficult, and DC subset control of key T cell fates such as tolerance, T helper cell commitment and regulatory T cell induction is still not well understood. While the difficulty in assigning unique functions to particular DC subsets may be due to sharing of functions, it may also reflect a lack of appropriate physiological *in-vivo* models for studying DC function. In this paper we review the limitations associated with many of the current DC models and highlight some of the underlying difficulties involved in studying the function of murine DC subsets.

Keywords: animal models/studies – mice/rats, antibody engineering, dendritic cells (myeloid, plasmacytoid, monocyte-derived), T cells, transgenics/knock-outs

Introduction

Dendritic cells (DCs) are professional antigen-presenting cells critically required for the initiation of T cell responses. Some DC subsets sample antigens in peripheral tissues and transport them to the lymph node (LN), where DCs come into contact with recirculating naive T cells. Other DC subsets are strategically positioned within secondary lymphoid organs to capture blood-borne antigens and present them to T cells (reviewed in [1]). In addition to 'classical' antigen presentation pathways involving exogenous antigen uptake and presentation to $CD4^+$ T cells in association with histocompatibility complex class II (MHC II), DCs are capable of presenting autophagosome-derived, endogenous antigens to $CD4^+$ T cells and cross-presenting exogenous antigens to $CD8^+$ T cells in association with MHC class I (reviewed in [1]).

Interactions between naive T cells and DCs are believed to control both primary T cell activation and subsequent T cell fate, and thus the outcome of the adaptive immune response. How DCs perform such a complex feat remains unclear. The currently accepted view is that immune outcomes are determined primarily by factors external to both DCs and T cells, such as the microbe-derived signals that radically alter the activation state of DCs [1]. An alternative view is that the DC lineage is comprised of distinct DC subpopulations committed to predetermined functions [2,3]. These functions, including generation of T cell tolerance or immunity, are then amplified by exposure to microbial signals. In this model, the outcome of an immune response depends upon how T cells integrate signals derived from the mix of preprogrammed DCs to which they are exposed during priming.

The DC lineage in the mouse has been subdivided into populations on the basis of surface phenotypes that correlate with differences in ontogeny, microanatomical location and requirements for specific cytokines and transcription factors. In the currently accepted schema, expression of high levels of CD11c and MHC II defines conventional DCs (cDCs), which are generated from precursors residing in secondary lymphoid organs such as LN and spleen [1]. cDCs are then subdivided into $CD8^+$ ($Xcr1^+Clec9a^+$) and $CD11b^+$ ($Sirpa^+$) subsets that correlate with the human $CD141^+$ ($Xcr1^+Clec9a^+$) and $CD1c^+$ ($Sirpa^+$) DC subsets (reviewed in [4,5]). In addition to cDCs, LNs contain migratory DCs (mDCs) that have entered the LN via afferent lymphatic vessels. In murine LNs draining the skin, mDCs are defined as $CD11c^{int}MHC\ II^{high}$, and comprise four distinct subsets: radioresistant migratory epidermal Langerhans cells (mLCs) and three subsets of radiosensitive

Table 1. Key citations highlighting overlapping subset-specific functions in the dendritic cell (DC) literature to date.

	Resident cDCs		Migratory DCs			
	$CD8^+$	$CD11b^+$	$CD11b^-$ $CD103^+$	$CD11b^+$ $CD103^-$	$CD11b^-$ $CD103^-$	mLCs
MHC I presentation	++	++	++	++	++	++
Cross-presentation	++	+ [18–22]	++	+ [23–25]		+ [25,26]
Apoptotic cell uptake	++	+ [91]	++			
MHC II presentation	++	++	++	++	++	++
IL-12 production	++	+ [12,13]				
Th1 induction	++	+ [14–16]	++	+ [92,93]		
Th2 induction	+ [14]	++	+ [92]	++	++	
Th17 induction	+ [94]	+ [95,96]	++	+ [97]	+ [97]	+ [49]
iT_{reg} induction	++	+ [98,99]	++	+ [95]		
Tolerance committed						+ [8]
Humoral immunity	++	++	++	++	++	++

For a comprehensive set of DC reviews, see *Immunology Reviews*, 2010, volume 234(1). Commonly attributed subset functions are denoted with ++. MHC: major histocompatibility complex; IL: interleukin; Th: T helper; iT_{reg}: induced regulatory T cells.

migratory dermal DCs (mDDCs) that differ in expression of CD11b and CD207/Langerin [6] and/or CD103 (reviewed in [1,7]). Migration of antigen-bearing DCs into the LN is essential for generating both peripheral adaptive immune responses and tolerance to antigens present within non-lymphoid tissues such as the skin [6,8]. Migratory DC subset equivalents in humans have not been established fully, but recent reports have identified multiple distinct DC populations in human skin and LNs [9–11].

Attributing specific functions to individual DC subsets has proven far more difficult than the analysis of phenotype. DC subsets capable of driving CD4 and CD8 responses, regulating T helper type 1 (Th1)/Th2/Th17 bias, generating inducible regulatory T cells (T_{regs}) and/or inducing tolerance are highly model-dependent (see Table 1). For example, $CD11b^+$ cDCs are usually held to be responsible for Th2 priming but can produce interleukin (IL)-12 [12,13] and prime both Th1 [14–16] and non-polarized Th responses [17]. Even cross-presentation capacity, which has been attributed solely to $CD8^+$ cDCs and $CD103^+$ mDDCs in many models, has also been observed in mLCs, $CD11b^+$ mDDCs and/or $CD11b^+$ cDCs [18–26]. In this review we will discuss how underlying limitations of murine experimental models may have led to these apparently contradictory findings.

Ex-vivo assays

DC subset function is often inferred from *ex-vivo* assays that measure the response of antigen-specific T cells co-cultured with DC subsets purified from the draining LNs and/or spleens of immunized or infected mice. Additionally, lymphatic cannulation of larger mammals such as in rats, pigs, sheep and cattle has been used to recover migrating dendritic cells for *ex-vivo* phenotypical and functional studies (reviewed in [27]).

T cell proliferation and effector function in these *ex-vivo* assays generally reflect the extent of antigen presentation at the time of DC harvest, and thus provide an indirect measure of the efficiency of *in-vivo* antigen uptake and processing by a given DC subset. However, *ex-vivo* assays can also be affected by changes in DC immunogenic properties resulting from the physical manipulation involved in DC isolation [28,29]. In addition, co-culture overrides microanatomical factors that may constrain the probability of *in-vivo* contact between DCs and T cells within the T cell zones of lymphoid organs. For example, the majority of splenic $CD11b^+$ cDCs are located outside the T cell zone in the steady state and would contact T cells only after Toll-like receptor (TLR)-dependent signals drive their relocation into the T cell zone, yet they may still present antigen to activate T cells *in vitro* [30]. In skin-draining LN, the peak arrival of mLCs after immunization is on day 4, compared with days 1–2 for mDDCs [6], so that assays performed on day 2 would not detect the capacity of mLCs migrating from the immunization site to present antigen [31].

Another major limitation of *ex-vivo* assays is that *in-vitro* T cell responses do not always mimic their *in-vivo* counterparts [3,32,33]. Effective concentrations of cytokines such as IL-2 are higher *in vitro* yet T cell division times are longer, and are accompanied by much higher rates of spontaneous cell death [33]. T cell cytokine production tends to be polarized more strongly *in vitro* than *in vivo* (reviewed in [34]). Long-term regulation of T cell effector and memory differentiation *in vitro* is also highly dependent on addition or withdrawal of exogenous cytokines.

Most importantly, the conditions that induce T cell deletion *in vivo* are not replicated effectively *in vitro*. *In-vivo* tolerogenic responses to soluble peptide begin with a proliferative burst that is followed rapidly by deletion in the absence of effector cytokine production [33,35]. *In vitro*, however, the same T cells make high levels of interferon

(IFN)-γ and survive long-term if provided with exogenous IL-2 [3].

DC adoptive transfer

To overcome the limitations of *in-vitro* assays, antigen-pulsed DC subsets have been transferred into naive animals in order to assess their ability to generate *in-vivo* T cell responses [36,37]. However, the ensuing immune response may not reflect the true functional capacity of unmanipulated DCs. Multiple reports have shown dramatically inefficient DC trafficking after intraperitoneal [38], intradermal [39] or subcutaneous [40] administration, with only 0–4% of injected DCs reaching the LN. Human studies have provided very similar results [41]. Paradoxically, antigen-pulsed murine splenic $CD8^+$ cDCs, injected either subcutaneously [42] or intratracheally [43], failed to enter the draining LN but still induced a specific T cell response in the node. In general, the T cell response to pulsed DC injection is crucially dependent upon endogenous LN DCs, which may present antigen or antigen–MHC complexes transferred from the injected DCs [44–46]. The end result is that the DC responsible for T cell activation may not have the same functions as the immunizing DC. Therefore, caution is required when using the results of DC adoptive transfer experiments to infer DC subset function or to predict the capacity for priming effective responses against pathogens or tumours.

Antibody-mediated targeting

Rather than introducing exogenous antigen-pulsed DCs, antigen can be selectively targeted to DC subsets *in situ* when delivered in a complex with antibodies against DC subset-specific surface markers. The main benefit of such an approach is that antigen can be targeted to DC subsets in unmanipulated mice in which DCs retain their normal trafficking to LN. However, the applicability of this approach for determining the function of individual DC subsets, rather than for testing the efficacy of potentially therapeutic antibody–antigen complexes, remains unclear.

The attribution of an observed function to the targeted subset, independent of the nature of the targeting molecule, can be extremely difficult. In the case of splenic cDCs, most surface molecules are also expressed on mDCs and other immune cell populations. For example, anti-CD205 (DEC205) will target antigen to $CD205^{high}$ $CD8^+$ cDCs, but may also target mLCs [6], mDDCs [6], activated $CD11b^+$ cDCs [47], macrophages [48] and B cells, all of which express CD205 at lower levels [48]. This lack of specificity can be overcome by antibody-targeting a transgene-encoded receptor whose expression is limited to a single DC subset. In this way, Igyarto *et al.* recently delivered antigen to murine LCs expressing a transgene-encoded human CD207 by means of an anti-human CD207 antibody [49].

A second constraint is that the measured function of a DC subset may be dependent upon the particular molecule targeted. For instance, when targeted via Dectin-1, $CD11b^+$ cDCs were more efficient at generating $CD4^+$ T cell responses than $CD8^+$ cDCs targeted via DEC205 [50], whereas they were less efficient when targeted via Dcir2 [51]. While the capacity to prime $CD8^+$ T cell responses is usually attributed to $CD8^+$ cDCs, they can also be primed by Dectin-2 (Clec4n)-targeted $CD11b^+$ cDCs [52], but not by Dcir2 (Clec4a4)- [53] or Dectin-1 (Clec7a)-targeted $CD11b^+$ cDCs [50]. Lastly, targeting different specificities on the same DC subset can result in different immune outcomes. For example, $CD8^+$ cDCs induced a strong antibody response without adjuvant when targeted via the 10B4 anti-Clec9a (DNGR1) antibody but not via CD205 [54] or the 7H11 Clec9a antibody [55]. Similarly, $CD8^+$ cDCs induced strong $CD8^+$ T cell responses when targeted via CD207, CD205 or Clec9a [51,54], whereas a weaker response was observed when targeting Clec12a [54].

These distinctions may reflect differences in the expression or signalling properties of the targeted molecule [56] and/or the properties of the targeting antibody itself, including its lifespan *in vivo* [54]. Thus, targeting experiments, while crucial in determining the therapeutic potential of particular antigen–antibody complexes, may not add substantially to our understanding of the function of DC subsets *in vivo*.

DC ablation models

DC ablation models have been used to test whether a DC subset is required for a particular T cell response. DC ablation models generally rely upon expression of diphtheria toxin or its receptor to delete DCs either constitutively or inducibly (reviewed in [57]). In addition to killing DCs, ablation may have significant secondary effects due to changes in the immune microenvironment, interference with feedback loops involving other cell types, and so on. Constitutive removal of the entire DC compartment not only prevented immune responses to immunization, but also resulted in gross secondary syndromes ranging from myeloproliferative disorders to spontaneous fatal multi-organ autoimmunity [58,59].

Inducible ablation of individual DC subsets, which would be predicted to have fewer unforseen secondary effects, has been achieved by administration of diphtheria toxin into mice expressing the high-affinity diphtheria toxin receptor (DTR) under appropriate promoters, or by means of treatment with horse cytochrome c. When CD11c-DTR mice were treated with diphtheria toxin, T cell responses to bacterial, viral and parasitic infections were reduced dramatically [57]. However, a range of CD11c-negative/low macrophage and monocyte subsets were also depleted [60], while the majority of the mDC subsets were unaffected

[57]. CD11c-DTR mice also developed a chemokine-dependent neutrophilia after dendritic cell ablation [61]. An alternative CD11c-Cre DTR model has been developed recently. In this model, Cre recombinase-mediated excision of a floxed-stop codon allows for constitutive DTR expression in CD11c-Cre-positive cells [62].

Langerin-DTR models have been used to assess the role of LCs in the immune response, but the results from these experiments have been heavily model-dependent. For example, LC deletion resulted in increased [63] or decreased [64] contact hypersensitivity responses depending on the mouse line. In the murine-Langerin-DTR models, developed originally to target only LCs, it was realized subsequently that both CD207/Langerin$^+$ DDCs and LCs were ablated by diphtheria toxin treatment. Because the two DC subsets reconstituted with different kinetics, interpretation of the effect on T cell responses was complex [63–65]. Finally, depletion of CD205$^+$ DCs in CD205-DTR mice dramatically reduced CD4$^+$ and CD8$^+$ T cell responses to bacterial and viral infections [48]. However, given that the steady-state frequency and distribution of T_{regs}, Th1 and Th17 cells was grossly altered by diphtheria toxin treatment, it was difficult to attribute the effect solely to CD205$^+$ DCs, without considering the effect of the altered immune environment [48].

CD11c-cre and Langerin-cre mice have also been used to generate targeted knock-outs of multiple immune signalling molecules, including recombination signal binding protein for immunoglobulin kappa J (RBPJ) [66], signal transducer and activator of transcription 3 (STAT3) [67], tumour necrosis factor, alpha-induced protein 3 (TNFAIP3) (A20) [68] and myeloid differentiation primary response gene 88 (Myd88) [69]. These applications suffer from the same subset specificity issues as the DTR models, due to model-dependent artefacts and the complex expression patterns of Langerin and the CD11c transgene [70,71].

Administration of horse cytochrome c is an alternate strategy used to ablate cross-presenting DCs via specific induction of the apoptosis pathway in cells possessing cross-presentation machinery [72]. Experiments using this treatment have suggested that cross-presentation is limited to a subset of splenic CD8$^+$ cDCs, although the model was complicated by the partial depletion of CD11b$^+$(CD4$^+$) cDCs, which are usually considered to be incapable of cross-presentation [73].

In addition to inducible ablation, transcription factor knock-out mice have been used to define *in-vivo* DC subset function, as they show complete or partial deficiencies in well-defined DC subsets (reviewed in [1,74]). For example, the comparison of interferon regulatory factor 4 (IRF4$^{-/-}$) mice (lacking CD11b$^+$ DCs) with Id2$^{-/-}$ or IRF8$^{-/-}$ mice (both lacking CD8$^+$ DCs) has supported the paradigm that CD11b$^+$ DCs promote Th2 cytokine production, while CD8$^+$ cDCs promote Th1 cytokine production [75,76]. Similarly, basic leucine zipper transcription factor, ATF-like 3 (BATF3$^{-/-}$) mice have been used to demonstrate that cross-presentation is confined to the CD8$^+$ cDC and CD103$^+$ mDC subsets, which are selectively deficient in these mice [77]. Interestingly, while both CD205-DTR [48] and BATF3-deficient mice [77] lack CD8$^+$ cDCs, only in the CD205-DTR model were splenic CD4$^+$ T cell responses affected. An additional complexity in transcription-factor knock-out mice is that the targeted transcription factors are expressed, albeit at lower levels, in the remaining DC subsets [74,78]. For example, the CD11b$^+$(CD8$^-$) DCs in IRF8$^{-/-}$ mice showed altered *in-vitro* response to microbial ligands, even though their *in-vivo* numbers and surface phenotype appeared normal [79].

Thus, the data from ablation models cannot be interpreted without also taking into account the actual rather than predicted ablation patterns, the kinetics of deletion and regeneration, the effect on the remaining DC compartment and the role the depleted cell populations may play in immune homeostasis in the steady state.

DC subset-restricted expression of MHC molecules

Models in which MHC alleles required for specific antigen presentation are expressed only by a defined DC subset would overcome most, if not all, of the problems associated with DC immunization, antibody targeting and ablation strategies. By retaining the entire complement of DC subsets with their normal transcriptional and biochemical programme, these models have the potential to define DC biology in a physiological context. So far, this aim has been achieved only for radioresistant DC subsets, namely LCs.

A number of published models have studied responses to LCs in MHC-disparate bone marrow (BM) chimeras in which LCs remain of host origin, whereas the majority of DDCs and cDCs are replaced [6,8,80–82]. The functional capacity of LCs can then be assessed using well-characterized TCR transgenic T cells whose specificity is restricted by an MHC allele encoded within the radioresistant host genome. MHC I-restricted models have made use of the fact that the K^{bm1} mutant allele does not allow presentation of the ovalbumin (OVA) epitope to CD8$^+$ OT-I TCR-transgenic T cells. In these models, OT-I stimulation capacity is restricted to LCs and radioresistant stromal cells of the H-2^k host reconstituted with H-2K^{bm1} BM [82]. The preservation of deletion of OT-I cells in response to skin-derived antigen has been interpreted as indicating that LCs can induce CD8$^+$ T cell deletion *in vivo*, but the possibility that the effect was mediated via MHC I-expressing LN stromal cells cannot be excluded [82].

In contrast, MHC II-dependent skin responses are effectively restricted only to LCs in MHC II-disparate chimeras, as LN stromal cells do not express MHC II [8]. Two groups have published results from such models. Allen *et al.* used wild-type hosts reconstituted with MHC II-knock-out (H2-Ab1$^{-/-}$) BM and concluded that LCs were unable to

support $CD4^+$ T cell proliferation [80]. However, reconstitution with MHC II-knock-out BM would generate an immune system in which tonic MHC II-dependent TCR signalling was deficient due to a lack of MHC II expression by the vast majority of DCs [83–86]. Such tonic TCR signalling is known to be critical for the maintenance of TCR sensitivity and responsiveness to activation, motility and memory generation within the $CD4^+$ T cell compartment [87–90]. Thus the lack of $CD4^+$ T cell response may have been due to the failure of most DCs to express MHC II, rather than an inability of LCs to support T cell proliferation under physiological conditions.

In contrast, our studies have shown that LCs drive a strong proliferative burst in a BM chimera engineered to avoid the unphysiological consequences of MHC II gene knock-out [6,8]. Our model makes use of selective *in-vivo* expression of individual MHC II alleles on a C57BL/6 (IA^b IE^{neg}) background, which reconstitute IE^{db} expression and thereby allow presentation of moth cytochrome c (MCC) to the 5C.C7 TCR. Using host mice transgenic for the MHC II IE alpha chain, we have restricted expression of IE to radioresistant LCs, while maintaining normal T cell homeostasis via expression of IA^b on all host and donor-derived DCs. We have demonstrated that LCs, as the sole antigen-presenting subset in this model, induce deletion of $CD4^+$ T cells even when highly activated by exposure to multiple TLR and inflammasome-mediated signals. Thus our results indicate that LCs are precommitted to the induction of immunological tolerance. LCs can also inhibit the immune response driven by radiosensitive, immunogenic DC subsets. The use of this model has thus allowed the first direct investigation of the *in-vivo* function of LCs, in contrast to the essentially indirect ablation studies in which the function of multiple DC subsets is assessed in the presence or absence of LCs [8].

While chimeric models are useful for assessing the function of LCs, restricting functional presentation capacity to defined DC subsets in tissues such as gut and lung remains a challenge. The development of further transgenic and knock-in models that will allow functional analysis of individual DC subsets in mice possessing the full complement of MHC-expressing DCs remains a high priority.

Conclusions/future perspectives

The goal of DC subset biology, in the context of T cell responses, is to understand how DCs control the many classes of immune responses that are generated *in vivo*. Defining the individual functions of DC subsets should allow us to develop a more complete understanding of the mechanisms controlling T cell-mediated immunity and tolerance, maximizing the therapeutic potential of targeting DC subsets for future translation into the clinic. The recent demonstration that mouse and human DC subsets are related much more closely than previously believed underlines the importance of studying DC biology in the mouse using physiological models.

Table 2. Summary of the limitations of the common dendritic cell (DC) models.

Models	Limitations and challenges
Ex vivo	Change in DC immunogenicity due to *in-vitro* manipulation
	Static snapshot of the immune response
	Differences in T cell behaviour *in-vitro versus in vivo*
	Cannot test deletional tolerance *versus* immunological memory
	Oversimplification of the *in vivo* microenvironment
Adoptive transfer	Differences in subset trafficking
	Low efficiency trafficking to DLN
	Antigen transfer to endogenous DCs
Antibody targeting	Complex expression patterns
	Molecule-specific not subset-specific responses
	Same subset but different responses
	Targeting functional molecules
DC ablation	Defects in immune and T cell homeostasis
	Incomplete or complex depletion patterns
	Effects on remaining DC populations
MHC restriction	Lack of TCR threshold signalling in MHC knock-out models
	Knock-in/transgenic MHC expression required
	Complex chimeric strategies
	Can compare only radioresistant *versus* radiosensitive DCs

MHC: major histocompatibility complex; DC: dendritic cells; DLN: draining lymph nodes; TCR: T cell receptor.

The limitations in the models currently available to study DC subset control of T cell responses (summarized in Table 2) highlight the importance of careful interpretation of the results from these models. The improvement and combination of current models should allow for a clearer picture of DC biology.

Disclosure

The authors have no competing interests.

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