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Role of Dendritic Cell Subsets in Immunity and Their Contribution to Non-infectious Uveitis

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Abstract

Dendritic cells (DCs) are a heterogeneous population. Murine DCs consist of conventional DCs (cDCs) and plasmacytoid DCs (pDCs). In human, the analogous populations are myeloid DCs (mDCs) and pDCs. Though distinct in phenotypes and functions, studies have shown that these DC subsets may interact or ‘crosstalk’ during immune responses. For example, cDCs may facilitate pDC maturation, while pDCs may enhance antigen presentation of cDCs in certain pathogenic conditions or even take on a cDC phenotype themselves. The role of DCs in non-infectious uveitis has been studied primarily in the experimental autoimmune uveitis mouse model and to a more limited extent in patients. Recent evidence shows that the number, phenotype and function of DC subsets are altered in this disease. We provide an overview of selected recent developments of pDCs and cDCs/mDCs, with special attention to their interaction and the dual roles of DC subsets in non-infectious uveitis.

Keywords

Conventional dendritic cells; myeloid dendritic cells; plasmacytoid dendritic cells; non-infectious uveitis; experimental autoimmune uveitis

1. Introduction of Dendritic Cells and Non-infectious Uveitis

Dendritic cells (DCs) were discovered in the late 1970s by Ralph Steinman of Rockefeller University⁵⁸, leading to his Nobel Prize in Physiology or Medicine in 2011⁴². DCs are

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professional antigen-presenting cells (APCs) that connect the innate and adaptive immune responses with critical roles in immune tolerance and defense against pathogens⁵⁹. Although DCs all share the ability to activate naïve T cells, they are a heterogeneous population in terms of their further phenotypic and functional characteristics⁵⁷. Categorizations include: conventional (also known as classical or myeloid) DCs vs. plasmacytoid DCs; ‘steady-state’ DCs (present at all times) vs. ‘inflammatory’ DCs (develop in response to inflammation); anatomical location (e.g. lymphoid tissue ‘resident’ DCs vs. non-lymphoid tissue peripheral ‘migratory’ DCs).

The study of DC biology has elucidated these subsets in both mouse and human, identifying some degree of inter-species correspondence between subsets, but also important differences. Murine and human DCs are both comprised of two major subsets, the key distinction being between conventional (also known as classical or myeloid DCs) and plasmacytoid DCs (pDCs). There is some variation in the terms used in the literature, but for the purposes of this review we will use the terms conventional DC (cDCs) to describe the non-plasmacytoid DCs in mice and myeloid DC (mDCs) to describe the equivalent group in humans^{8; 18; 37}. In both species pDCs are the more homogeneous group, being distinguished by a non-dendritic plasma cell-like morphology in their resting state and an ability to rapidly secrete type I interferons (IFNs) in response to viral infection. The cDC/mDC grouping contains a number of different subsets with a range of functions directed towards directing T cell responses³¹. Classification of DCs and their discrimination from other mononuclear phagocytes (monocytes and macrophages) is made more challenging by plasticity especially under inflammatory conditions, which means that surface phenotype is not always a reliable guide to ontogenic relationship.

Human non-infectious uveitis is a potentially blinding condition characterized by intraocular inflammation. There is considerable evidence that most non-infectious uveitis is autoimmune (or at least auto-inflammatory) in origin³². The immune dysregulation observed in patients can be modeled and interrogated in animal models of uveitis, notably with the immunization of uveitogenic antigens supplemented with complete Freund adjuvant (CFA) in experimental autoimmune uveitis (EAU)⁶². Such models provide evidence of loss of tolerance to important intra-ocular antigens, such as S-antigen and interphotoreceptor retinoid-binding proteins (IRBP), and enable elucidation of the immune processes leading to the generation of autoreactive CD4⁺ T cells and their pathological function within the eye¹¹. Critically, EAU has also been successfully induced by intravenous injection of mature DC pulsed with uveitogenic antigens⁶¹.

Data in humans is less complete, but still compelling. There is histological evidence of T cell infiltrates at sites of inflammation in eyes with uveitis³, which is supported by flow cytometric studies of intraocular fluid samples from patients with active inflammation^{10; 44}. With regard to the role of auto-antigens in human disease, De Smet noted lymphocyte stimulation responses to peptide determinants of retinal S antigen in a range of uveitis conditions, being most frequent in uveitis associated with Behcet disease or sarcoidosis^{12; 13}. Our recent work has been directed towards determining the role of DCs in the pathogenesis of non-infectious uveitis [Ping, et al., Abstract in 2014 ARVO]. In this

review, we discuss recent advances in the understanding of DC subsets in both mice and human and explore the implications of these recent findings to non-infectious uveitis.

2. Characteristics of Murine Dendritic Cell Subsets

As introduced earlier, murine DC subsets consist of two main populations: cDCs and pDCs. In terms of surface phenotype, cDCs are CD11c⁺ and pDCs are PDCA1⁺ ⁶⁶ (Table I). Conventional DCs are found in lymphoid tissues including spleen, lymph nodes and bone marrow, but are also widely distributed amongst non-lymphoid tissues. The major cDC subsets are defined according to the presence of CD8 α and CD11b. The CD8⁺ cDC subset is well-characterized lymphoid tissue based subset with important roles in cross-presentation of exogenous antigens to CD8⁺ T cells¹⁴, and IL-12 secretion. Interestingly transcriptome profiling identified that there is an equivalent subset in non-lymphoid tissues which is CD8⁻ and is defined by the integrin CD103⁺ ⁴³; the lymphoid CD8⁺ subset and the non-lymphoid CD103⁺ subset share a number of features including responsiveness to TLR3 stimulation and expression of the chemokine receptor XCR1.

The CD11b⁺ (CD8⁻) subset is less well characterized. They are the most abundant cDC in lymphoid tissue and are also found in non-lymphoid tissues. This is a highly heterogeneous group. Further segregation has been attempted on the basis of surface markers such as CD4, but this has not been supported by transcriptome profiling²⁶. CD11b⁺ DC differ functionally from CD8⁺ DC being more effective in inducing CD4⁺ T cell responses, and capable of producing IL-6 and IL-23, whilst being poor at crosspresentation and the production of IL-12.

Plasmacytoid DCs are found in the blood and periphery. The term ‘plasmacytoid’ refers to their appearance when resting of a non-dendritic plasma cell-like morphology. Their key function is the detection of virus by toll-like receptor (TLR) 7 or TLR9, with production of high levels of type I IFNs. They are characterized by being PDCA-1⁺, but also express DEC-205 and B220.

DCs may also arise from monocytes, both *in vivo* and *in vitro*. *In vivo* such monocyte-derived ‘inflammatory’ DCs (infDCs) arise secondary to infection or inflammation. *In vitro* they may be generated from bone marrow cells (bone marrow-derived DCs; BMDCs) under the stimulation of recombinant granulocyte macrophage-colony stimulating factor (GM-CSF)^{25; 36}. A key function of infDCs is to produce large amounts of TNF- α and iNOS (so-called TNF-iNOS producing DCs or ‘Tip DCs’). They have a critical role in pathogen clearance, with an important influence in the appropriate polarization of a T cell response.

A challenge to the study of DC biology in the eye is the limitation that DC numbers *in vivo* are too low to isolate enough for performing the functional and mechanical studies. For this reason most functional studies in mouse and human have depended on the use of *in vitro* cultures of bone marrow/monocyte-derived DCs. Although we, among others, have found these model systems useful, the extent to which these *in vitro* BMDCs reflect cDCs and/or infDCs is not yet fully established. Gene expression profiles have been shown to differ significantly between cDCs (in which development is Flt3-ligand dependent) and BMDCs

(in which development is GM-CSF dependent)⁶⁷. Conversely, cDCs and BMDCs do share expression of the transcription factor Zbtb46⁵².

3. Characteristics of Human Dendritic Cell Subsets

As outlined earlier, there are shared features, but also important differences, between murine and human DC systems. Inter-species comparison based purely on surface phenotype of DC subsets is generally unhelpful, whereas more recent studies based on gene expression have been more rewarding. The key distinction of conventional DCs (hereafter referred to as myeloid DCs; mDCs) vs. plasmacytoid DCs is maintained with clear separation in both phenotype and function. Due mainly to the availability of tissue and other practical limitations, the study of DC subsets in humans has primarily been focused on peripheral blood. Indeed it was in human blood that pDCs were first identified.

As observed in the mouse, human DCs are relatively rare in the peripheral blood compared to other immune cells¹⁸. In blood there are two main populations of DCs: an mDC population, which is CD11c/BDCA-1⁺CD11c^{hi}CD123⁻ (described as mDC1) and a pDC population, which is CD11c⁻CD123⁺BDCA-2/CD303⁺^{18; 57}. There is also a second population of mDCs (mDC2), which are CD141/BDCA-3⁺CD11c^{lo}. All three subsets are negative for lineage 1 markers (Lin1⁻) and express HLA-DR (i.e. Lin1⁻HLADR⁺)^{18; 37; 70} (Table I). In humans CD11c is not restricted to DCs, with 90% of human monocytes expressing CD11c⁴⁹. Gene expression studies and the study of rare genetic mutations affecting DC function in humans supported by the detailed functional characterization across DC subsets in both species, has helped establish the equivalence of DC subsets in mouse and human. Thus CD11c/BDCA-1⁺CD11c^{hi}CD123⁻ mDC1 in the human are equivalent to CD11b⁺CD8⁻ cDCs in the mouse; CD141/BDCA-3⁺CD11c^{lo} mDC2 are equivalent to CD8⁺ cDCs, with the chemokine receptor XCR1 being expressed by this subgroup in both species; and CD11c⁻CD123⁺BDCA-2/CD303⁺ pDCs being equivalent to the murine PDCA-1⁺ pDCs⁶⁵.

In terms of function, the human subsets appear to behave similarly to their murine equivalents. pDCs secrete high levels of type I IFNs in response to viruses and other suitable stimuli; mDC1 and mDC2 are effective at presenting antigen and inducing CD4⁺ and CD8⁺ T cell responses, with mDC2 being particularly effective at cross-presentation of exogenous antigens to CD8⁺ T cells. These shared features support the idea that the study of murine DCs can support our understanding of human DC biology and related autoimmunity.

Even more than in the mouse, the concept of human ‘inflammatory’ DCs is controversial. *In vitro* it has long been established that human DCs can be derived from monocytes (MoDCs). These have been widely studied to inform human DC biology and have even been used as a tool for vaccine generation and cancer therapy⁶. Typically CD14⁺ monocytes from peripheral blood are cultured with recombinant GM-CSF and IL-4 *in vitro* for 5–7 days^{33; 55}. Further ‘maturation’ may be induced through stimulation with appropriate TLR ligands and/or pro-inflammatory cytokines²⁹. Although a number of ‘inflammatory’ DC phenotypes in humans have been identified *in vivo*, it is unclear whether these truly represent monocyte-derived DCs or whether they are a form of activated monocyte.

Monocytes may become activated during infection or inflammation with enhanced expression of CD16 and reduced level of CD14. CD16⁺ monocytes are significant producers of inflammatory cytokines (such as TNF- α) and display both DC and macrophage-like characteristics including antigen processing and presentation. When noting these parallels between the generation of MoDCs *in vitro* and the activation of monocytes *in vivo*, it is tempting to extrapolate the development of these monocytes and to assume that they become inflammatory DCs *in vivo*. In human studies, however, 'inflammatory DC' subsets, such as the 6-sulfo LacNAc (slan)⁺ DC subset, have generally been indistinguishable from activated monocytes⁹. This is clearly an area requiring further study. Table 1 summarizes several studies of the phenotypic and functional analysis in DC subsets in mice and humans.

4. Imbalanced Dendritic Cell Subset Proportions in the Immune System

Conventional DCs and pDCs are flt3-like tyrosine kinase 3 (Flt3) ligand-dependent. They are derived from a common DC precursor (CDP) that is a Lin⁻ c-Kit^{lo} CD115⁺ CX₃CR1⁺ Flt3⁺ progenitor cell, which in turn is derived from a bipotent macrophage/DC precursor (MDP; Lin⁻ c-Kit^{hi} CD115⁺ CX₃CR1⁺ Flt3⁺)^{68,121}. Studies show that Flt3 ligand is essential for the regulation of homeostatic DCs (cDCs and pDCs) development in mice spleen, where it maintains normal DC numbers by regulating cell division in the periphery⁶⁶. Another study shows that repeated injections of Flt3 ligand result in an increase in the number of functional cDCs³⁸. Although GM-CSF is commonly added to *in vitro* cultures, it does not appear to be essential for the development of any DC subsets *in vivo*, although it does have important effects on DC function, notably in cross-presentation⁵¹. Cytokines such as Flt-3 ligand not only affect the generation of DC subsets, but also influence the balance between DC subset proportions, and so may in turn impact on the pathogenesis of autoimmune diseases.

Another factor that may influence the balance of proportions between DC subsets is the conversion from one DC subset to another. Notably pDCs have been observed to convert into cDCs in response to certain stimuli. For example during viral infection, pDCs may convert to mDCs with enhanced antigen-presenting capacity and TLR upregulation⁷¹. Similarly pDCs also convert into cDCs when mice are injected with double-stranded RNA (poly I:C) and type I interferon⁶³. Furthermore, a subpopulation of pDCs, CCR9⁻ pDCs, differentiates into cDCs after they are adoptively transferred into peripheral lymphoid organs, lung, and intestine^{54; 56}. Similarly, CCR9⁻ pDCs have been shown to convert into CD11b⁺CD8 α ⁺ MHC class II^{hi} cDC-like cells under the stimulation of GM-CSF or soluble factors produced by intestinal epithelial cells. This conversion is supported by a switch from typical pDCs to CD8⁺ cDCs regulators, notably a downregulation of the E2-2 transcription factor and upregulation of ID2, PU.1 and Batf3⁵³. Therefore, the plasticity of pDCs in switching to fully functional cDCs can lead to alteration of the ratio between DC subsets *in vivo*.

5. Interaction of Dendritic Cell Subsets during Immune Responses

As explained, both murine and human DCs include two major subsets, and those subsets exhibit distinct functions. Interestingly, it appears that there is significant interrelationship

between these subsets. Kuwajima *et al.* have shown that murine cDCs fail to produce IL-12 in the absence of pDCs *in vivo*, suggesting that normal function of cDCs is dependent on the presence of pDCs. Moreover, it appeared that cell-to-cell contact (CD40–CD40L) between cDCs and pDCs was required³⁰. Similarly, in human immunodeficiency virus (HIV) infection, pDCs promote mDC function in a bystander fashion, while they produce more type I interferon in response to HIV¹⁹. This discovery has prompted further investigation into when and how cDCs/mDCs interact or crosstalk with pDCs. Furthermore Wang *et al.*³⁵ found that antigen-specific CD8⁺ T cells are increased and antitumor responses are enhanced after mice are immunized with a mixture of activated pDCs and mDCs, compared to mice immunized with either mDCs or pDCs. The increased anti-tumor responses arise because of enhanced antigen presentation in mDCs, which is facilitated by pDCs. Consistent with *in vivo* studies, co-culturing of pDCs and mDCs *in vitro* leads to pDC maturation and enhanced mDC antigen presentation (but not cytokine production) in response to bacterial infections⁴⁷. In summary, given these interactions, it is possible that the imbalance of DC subset proportions may significantly contribute to the immune responses seen in autoimmune diseases, such as uveitis.

6. Role of Dendritic Cell Subsets in Experimental Autoimmune Uveitis (EAU)

The critical role of DCs in non-infectious uveitis has been investigated in the murine model of uveitis (EAU). The immunological characteristics observed in EAU show distinct similarities to human disease states in which activation of CD4⁺ T cells is central^{17; 40}. The central role of DCs in uveitis may be argued from their key role taking up and presenting antigens to CD4⁺ T cells, thereby activating them and inducing immune responses. In autoimmune non-infectious uveitis, uveitogenic antigen-specific CD4⁺ T cells are crucial effector cells that drive inflammation and tissue damage after they are activated by DCs; however, these rare antigen-specific CD4⁺ T cells must need to encounter their uveitogenic antigens presented by APCs to successfully initiate the uveitis. As specialized APCs, DCs exhibit abnormal function in human non-infectious uveitis and EAU model, although the pathogenic mechanisms of DCs in uveitis are not fully understood (see Figure).

There is clear evidence that mDCs are present in the ocular tissues under both resting and inflammatory conditions. Choudhury *et al.* found functional mDCs in the choroid and thus may play a significant role in the inflammatory process during posterior uveitis⁷. McMenamin *et al.* have shown that antigen-presenting cells residing in the iris and ciliary body in normal rat eyes have access to ocular antigens on both sides of the blood-ocular barrier and thus are capable of activating circulating antigen-specific T cells⁴¹. Furthermore, Butler *et al.* suggested that DCs may act as local APCs in the induction of uveitis². More specifically, Gregerson and his co-workers confirmed that cDCs are distinct from microglia. They are present in quiescent retinas, and they effectively respond to injured neurons. Interestingly cDCs from quiescent retinas promote generation of Foxp3⁺ T cells and inhibit T cell activation, whereas cDCs from injured retinas inhibit Foxp3⁺ T cell generation and stimulate T cell activation. Local delivery of exogenous BMDCs increases the incidence and severity of EAU, which is induced by the adoptive transfer of activated CD4⁺ or CD8⁺ T

cells. Therefore, local conditions in the retina determine cDC function and affect the pathogenesis of EAU by both CD4⁺ and CD8⁺ T cells²². A key part of this mechanism may be a direct chemoattractant effect by IRBP and S-Ag. Howard *et al.* showed that these autoantigens attract immature DCs and T cells expressing CXCR3 and CXCR5 to initiate innate and adaptive immune responses²⁴.

In addition, the types of immune responses largely depend on the maturation status of DCs. Pretreatment with fixed immature DCs, but not fixed mature DCs, ameliorate EAU progression by inhibiting uveitogenic CD4⁺ T cell activation and differentiation⁴⁵. Suzuki *et al.* suggested that impaired cDC maturation may be the underlying mechanism for the observed anti-inflammatory effect of aminoimidazole carboxamide ribonucleotide (AICAR)⁶⁰. Impaired murine cDC maturation prevents the generation of Ag-specific Th1 and Th17 cells to attenuated EAU by the treatment of AICAR⁶⁰. Further indirect evidence of the importance of DC maturation in the pathogenesis of uveitis may be inferred from the role of pertussis toxin assisting the induction of uveitis in mice. Pertussis toxin exerts an adjuvant role on cDC to promote cDC maturation and the production of proinflammatory cytokines, thereby eliciting a Th1 response in EAU²³.

As discussed elsewhere ‘maturation’ is more complex and more plastic than a simple unidirectional linear progression, and thus there are a number of other DC states that should be considered⁵⁰. For example, IL-10-conditioning BMDCs induce an ‘immature’ phenotype which can suppress EAU development and significantly reduce IRBP-specific T-cell proliferation and IFN- γ production, but increase IL-10 production⁶⁴.

Liang *et al.* has also shown that a specific CD25⁺ splenic DC subset contributes to the development of EAU, with ablation of this DC subset resulting in decreased activation and expansion of $\gamma\delta$ T cells, with decreased activation of IL-17⁺ IRBP-specific T cells and attenuated inflammation in the EAU³⁴.

Taken together, data from the EAU model suggest that DCs have an important role in the induction or inhibition of inflammation. Now, a major challenge lies in extrapolating these data to human non-infectious uveitis.

7. Abnormalities of Dendritic Cell Subsets in Non-infectious Uveitis

Patients

An important question that remains is whether perturbations in the number, frequency, or ratio of DC subsets can lead to the development of autoimmunity. Studying DCs in non-infectious uveitis patients is a challenge owing to the low number of DCs in the peripheral blood and eye tissues. Despite this, DC subsets have been successfully quantified in patients with non-infectious uveitis secondary to Sarcoidosis, Behcet diseases, Vogt-Koyanagi-Harada (VKH) syndrome, or birdshot chorioretinopathy. Through multicolor flow cytometry analysis, we have determined that non-infectious uveitis patients have an increased frequency and absolute number of CD1c⁺ mDC1 when compared to the levels in the peripheral blood of healthy controls (Ping *et al.*, manuscript under review).

In addition to the alteration in DC numbers, DCs exhibit a more 'mature' phenotype in non-infectious uveitis patients. Studies show that the deficiencies of mDCs correlate with the clinical severity observed in sarcoidosis, but mDCs still exhibit the upregulated co-stimulatory and maturation markers (e.g., CD86, HLA-DR)³⁹. Another study revealed that increased CD86 and HLA-DR expression on mDCs in uveitis patients are strongly correlated with inflammatory activity. Interestingly, the expression of these surface molecules is not completely downregulated to the baseline levels measured in healthy controls, even during uveitis remission²⁸. We have observed that only HLA-DR, not co-stimulatory molecules such as CD80, on CD1c⁺ mDC1 is increased in the patient peripheral blood when compared with healthy controls (Ping *et al.*, manuscript under review).

Looking within the eye, Chang *et al.* used cadaveric tissue to identify DC, based on noting dendritiform stromal cells, which were HLA-DR positive and CD68 negative (a macrophage marker). This study was of interest because it showed that these cells expressed TLR4 and the associated LPS receptor complex and were localized to the uvea, but not to other ocular tissues⁴. This may be particularly relevant to those forms of uveitis that have a proposed association with gram negative bacteria such as HLA-B27 associated anterior uveitis⁵. Denniston *et al.* reported that mDCs isolated from human uveitic aqueous humor (AqH) were characterized by elevated MHC I and II, but reduced CD86 compared with matched peripheral blood mDCs¹⁶.

Unlike mDCs, there exists no consistent trend among different studies when comparing pDC frequency and function in non-infectious uveitis. Pay *et al.* reported a higher frequency of IFN α ⁺ pDCs in Behcet disease patients with higher sensitivity of these cells to CpG D ODN stimulus, which they inferred contributed to high serum IFN- α levels found in these patients promoting a Th1 type immune response⁴⁶. Plskova *et al.* studied a series of patients with severe, refractory sight-threatening uveitis pre- and post-treatment with IFN- α . They noted no significant difference between patients and control subjects in the number of pDCs, but there was a significant decrease in the capability of patients' pDCs to produce IFN- α in response to CpG and an alteration in the T cell phenotype with lower activation markers and increased intracellular T-cell IL-10 levels⁴⁸. Data from our laboratory show that pDC frequency is significantly decreased in all types of non-infectious uveitis patients regardless of disease status when comparing to that in healthy controls and that this decreased pDC frequency is highly correlated with decreased regulatory T (Treg) cells (unpublished data). This observation supports the suggestion of Plskova that pDC may help the generation of Treg cells⁴⁸. This hypothesis is also supported by observations in rheumatoid arthritis, where Kavousanaki *et al.* noted that pDC from inactive RA (but not active RA) expressed high levels of indoleamine 2,3-dioxygenase and promoted the differentiation of allogeneic naive CD4⁺CD25⁻ T cells into IL-10-secreting Treg cells that showed poor proliferation *in vitro*²⁷. While for active disease they noted reduced pDC levels (similar to our observations in uveitis), in treatment-induced remission they found elevated pDC levels (in contrast to our observations). The role of pDCs in human autoimmune disease requires further elucidation.

From practical limitations relating to the numbers of mDCs and pDCs available, the limited numbers of functional or mechanistic studies conducted in human uveitis have depended on

in vitro generation of MoDCs. Frassanito *et al.* observed that when MoDCs are generated from the peripheral blood of patients with non-infectious uveitis, these cells display an enhanced ability to stimulate cell proliferation of allogeneic T cells in mixed lymphocyte reactions (MLR) and higher levels of IL-12 production compared to healthy controls or compared to uveitis patients who had received systemic treatment²⁰. More recently, Yang *et al.* demonstrated the immunosuppressive effects of the plant extract Berberine on MoDCs from patients with active VKH⁶⁹. Similarly, Denniston *et al.* used human MoDCs as a model to test the effects of non-inflammatory and inflammatory AqH supernatant, noting that exposure of MoDCs from healthy controls to uveitic AqH from patients with active inflammation induced upregulation of MHC I and II and reduced CD86. Interestingly these changes were characteristic of the mDCs that had been directly isolated from these uveitis patients, providing evidence for the potential role of the ocular microenvironment in DC regulation, maturation and function^{15; 16}.

In light of our increased understanding of DC biology, and our own observations of alterations in DC subsets in human uveitis (notably an increase in mDCs and a reduction in pDCs), we hypothesize that these DC alterations are at least partially responsible for the biased CD4⁺ T cell immune responses observed in human non-infectious uveitis (increased Th1 and decreased Treg cells).

8. Conclusions

The investigation of DCs is complicated by the fact that they account for a relatively small proportion in the immune system. An even bigger challenge persists is the translation and application of the findings from animal models such as EAU into more complex human non-infectious uveitis. There is, however, accumulating evidence that indicates that DCs may have either a protective or pathogenic role in the uveitis, depending on their maturation status and local microenvironment.

We hypothesize that, while most studies of DCs in uveitis emphasize the impact of abnormalities in an individual DC subset, important pathogenic effects may arise from imbalances in DC subset proportions and their mutual interaction. Even as DC-based therapies for cancer and vaccines continue to progress, we need to address these more fundamental questions regarding the role of DCs in the pathogenesis of uveitis. Therefore, we argue that an equivalent depth of understanding on the function of individual DC subsets as well as their interaction in autoimmunity will be a necessary and potentially powerful tool for preventing, controlling, and effectively treating human non-infectious uveitis.

Method of Literature Search

The literature for this review was searched from PubMed, using the key words of *uveitis*, *dendritic cells*, *plasmacytoid dendritic cells*, *classical dendritic cells* and *myeloid dendritic cells*. The date range was from 1947 and 2014. Non-English manuscripts were included where a screen of the abstract identified it as being relevant.

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Abbreviations

APCs	Antigen presenting cells
AqH	Aqueous humor
BDCA	Blood Dendritic Cell Antigen
BMDCs	Bone marrow-derived dendritic cells
CFA	Complete Freund adjuvant
cDCs	Conventional dendritic cells
DCs	Dendritic cells
EAU	Experimental Autoimmune Uveitis
GM-CSF	Granulocyte macrophage-colony stimulating factor
HIV	Human immunodeficiency virus
IFN	Interferon
IRBP	Interphotoreceptor retinoid-binding proteins
MHC	Major histocompatibility complex
MLR	Mixed lymphocyte reactions
mDCs	Myeloid dendritic cells
MoDCs	Monocyte-derived dendritic cells
pDCs	Plasmacytoid dendritic cells
Treg	Regulatory T cells
Th1	T helper cell 1
TLR	Toll-like receptor

References

1. Auffray C, Emre Y, Geissmann F. Homeostasis of dendritic cell pool in lymphoid organs. *Nat Immunol.* 2008; 9:584–586. [PubMed: 18490906]
2. Butler TL, McMenamin PG. Resident and infiltrating immune cells in the uveal tract in the early and late stages of experimental autoimmune uveoretinitis. *Invest Ophthalmol Vis Sci.* 1996; 37:2195–2210. [PubMed: 8843906]
3. Chan CC, Benezra D, Rodrigues MM, et al. Immunohistochemistry and electron microscopy of choroidal infiltrates and Dalen-Fuchs nodules in sympathetic ophthalmia. *Ophthalmology.* 1985; 92:580–590. [PubMed: 3873634]
4. Chang JH, McCluskey P, Wakefield D. Expression of toll-like receptor 4 and its associated lipopolysaccharide receptor complex by resident antigen-presenting cells in the human uvea. *Invest Ophthalmol Vis Sci.* 2004; 45:1871–1878. [PubMed: 15161852]

5. Chang JH, McCluskey PJ, Wakefield D. Toll-like receptors in ocular immunity and the immunopathogenesis of inflammatory eye disease. *Br J Ophthalmol.* 2006; 90:103–108. [PubMed: 16361678]
6. Chiang CL, Kandalaf LE, Tanyi J, et al. A dendritic cell vaccine pulsed with autologous hypochlorous acid-oxidized ovarian cancer lysate primes effective broad antitumor immunity: from bench to bedside. *Clin Cancer Res.* 2013; 19:4801–4815. [PubMed: 23838316]
7. Choudhury A, Pakalnis VA, Bowers WE. Characterization and functional activity of dendritic cells from rat choroid. *Exp Eye Res.* 1994; 59:297–304. [PubMed: 7821374]
8. Colonna M, Trinchieri G, Liu YJ. Plasmacytoid dendritic cells in immunity. *Nat Immunol.* 2004; 5:1219–1226. [PubMed: 15549123]
9. Cros J, Cagnard N, Woollard K, et al. Human CD14^{dim} monocytes patrol and sense nucleic acids and viruses via TLR7 and TLR8 receptors. *Immunity.* 2010; 33:375–386. [PubMed: 20832340]
10. Curnow SJ, Scheel-Toellner D, Jenkinson W, et al. Inhibition of T cell apoptosis in the aqueous humor of patients with uveitis by IL-6/soluble IL-6 receptor trans-signaling. *J Immunol.* 2004; 173:5290–5297. [PubMed: 15470075]
11. Damsker JM, Hansen AM, Caspi RR. Th1 and Th17 cells: adversaries and collaborators. *Ann N Y Acad Sci.* 2010; 1183:211–221. [PubMed: 20146717]
12. de Smet MD, Yamamoto JH, Mochizuki M, et al. Cellular immune responses of patients with uveitis to retinal antigens and their fragments. *Am J Ophthalmol.* 1990; 110:135–142. [PubMed: 2378377]
13. de Smet MD, Bitar G, Mainigi S, Nussenblatt RB. Human S-antigen determinant recognition in uveitis. *Invest Ophthalmol Vis Sci.* 2001; 42:3233–3238. [PubMed: 11726628]
14. den Haan JM, Lehar SM, Bevan MJ. CD8(+) but not CD8(–) dendritic cells cross-prime cytotoxic T cells in vivo. *J Exp Med.* 2000; 192:1685–1696. [PubMed: 11120766]
15. Denniston AK, Kottoor SH, Khan I, et al. Endogenous cortisol and TGF-beta in human aqueous humor contribute to ocular immune privilege by regulating dendritic cell function. *J Immunol.* 2011; 186:305–311. [PubMed: 21106846]
16. Denniston AK, Tomlins P, Williams GP, et al. Aqueous humor suppression of dendritic cell function helps maintain immune regulation in the eye during human uveitis. *Invest Ophthalmol Vis Sci.* 2012; 53:888–896. [PubMed: 22247464]
17. Dick AD. Retinal antigen-specific T cells mediate experimental autoimmune uveoretinitis (EAU) in PVG rat a model for tracking antigen-specific CD4(+) T cells in the inflamed eye. *Ocul Immunol Inflamm.* 1995; 3:261–270. [PubMed: 22823273]
18. Dzionek A, Fuchs A, Schmidt P, et al. BDCA-2, BDCA-3, and BDCA-4: three markers for distinct subsets of dendritic cells in human peripheral blood. *J Immunol.* 2000; 165:6037–6046. [PubMed: 11086035]
19. Fonteneau JF, Larsson M, Beignon AS, et al. Human immunodeficiency virus type 1 activates plasmacytoid dendritic cells and concomitantly induces the bystander maturation of myeloid dendritic cells. *J Virol.* 2004; 78:5223–5232. [PubMed: 15113904]
20. Frassanito MA, Dammacco R, Fusaro T, et al. Combined cyclosporin-A/prednisone therapy of patients with active uveitis suppresses IFN-gamma production and the function of dendritic cells. *Clin Exp Immunol.* 2003; 133:233–239. [PubMed: 12869029]
21. Gilliet M, Boonstra A, Paturel C, et al. The development of murine plasmacytoid dendritic cell precursors is differentially regulated by FLT3-ligand and granulocyte/macrophage colony-stimulating factor. *J Exp Med.* 2002; 195:953–958. [PubMed: 11927638]
22. Heuss ND, Lehmann U, Norbury CC, et al. Local activation of dendritic cells alters the pathogenesis of autoimmune disease in the retina. *J Immunol.* 2012; 188:1191–1200. [PubMed: 22219322]
23. Hou W, Wu Y, Sun S, et al. Pertussis toxin enhances Th1 responses by stimulation of dendritic cells. *J Immunol.* 2003; 170:1728–1736. [PubMed: 12574336]
24. Howard OM, Dong HF, Su SB, et al. Autoantigens signal through chemokine receptors: uveitis antigens induce CXCR3- and CXCR5-expressing lymphocytes and immature dendritic cells to migrate. *Blood.* 2005; 105:4207–4214. [PubMed: 15713799]

25. Inaba K, Inaba M, Romani N, et al. Generation of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/macrophage colony-stimulating factor. *J Exp Med*. 1992; 176:1693–1702. [PubMed: 1460426]
26. Jaitin DA, Kenigsberg E, Keren-Shaul H, et al. Massively parallel single-cell RNA-seq for marker-free decomposition of tissues into cell types. *Science*. 2014; 343:776–779. [PubMed: 24531970]
27. Kavousanaki M, Makrigiannakis A, Boumpas D, Verginis P. Novel role of plasmacytoid dendritic cells in humans: induction of interleukin-10-producing Treg cells by plasmacytoid dendritic cells in patients with rheumatoid arthritis responding to therapy. *Arthritis Rheum*. 2010; 62:53–63. [PubMed: 20039433]
28. Kim TW, Kang JS, Kong JM, et al. Maturation profiles of peripheral blood dendritic cells in patients with endogenous uveitis. *Immunol Lett*. 2012; 142:14–19. [PubMed: 22108236]
29. Krutzik SR, Tan B, Li H, et al. TLR activation triggers the rapid differentiation of monocytes into macrophages and dendritic cells. *Nat Med*. 2005; 11:653–660. [PubMed: 15880118]
30. Kuwajima S, Sato T, Ishida K, et al. Interleukin 15-dependent crosstalk between conventional and plasmacytoid dendritic cells is essential for CpG-induced immune activation. *Nat Immunol*. 2006; 7:740–746. [PubMed: 16715101]
31. Lanzavecchia A, Sallusto F. Regulation of T cell immunity by dendritic cells. *Cell*. 2001; 106:263–266. [PubMed: 11509174]
32. Larson T, Nussenblatt RB, Sen HN. Emerging drugs for uveitis. *Expert Opin Emerg Drugs*. 2011; 16:309–322. [PubMed: 21210752]
33. Leon B, Lopez-Bravo M, Ardavin C. Monocyte-derived dendritic cells. *Semin Immunol*. 2005; 17:313–318. [PubMed: 15955712]
34. Liang D, Zuo A, Shao H, et al. Role of CD25+ dendritic cells in the generation of Th17 autoreactive T cells in autoimmune experimental uveitis. *J Immunol*. 2012; 188:5785–5791. [PubMed: 22539790]
35. Lou Y, Liu C, Kim GJ, et al. Plasmacytoid dendritic cells synergize with myeloid dendritic cells in the induction of antigen-specific antitumor immune responses. *J Immunol*. 2007; 178:1534–1541. [PubMed: 17237402]
36. Lutz MB, Kukutsch N, Ogilvie AL, et al. An advanced culture method for generating large quantities of highly pure dendritic cells from mouse bone marrow. *J Immunol Methods*. 1999; 223:77–92. [PubMed: 10037236]
37. MacDonald KP, Munster DJ, Clark GJ, et al. Characterization of human blood dendritic cell subsets. *Blood*. 2002; 100:4512–4520. [PubMed: 12393628]
38. Maraskovsky E, Brasel K, Teepe M, et al. Dramatic increase in the numbers of functionally mature dendritic cells in Flt3 ligand-treated mice: multiple dendritic cell subpopulations identified. *J Exp Med*. 1996; 184:1953–1962. [PubMed: 8920882]
39. Mathew S, Bauer KL, Fiscoeder A, et al. The anergic state in sarcoidosis is associated with diminished dendritic cell function. *J Immunol*. 2008; 181:746–755. [PubMed: 18566441]
40. Mattapallil MJ, Silver PB, Mattapallil JJ, et al. Uveitis-associated epitopes of retinal antigens are pathogenic in the humanized mouse model of uveitis and identify autoaggressive T cells. *J Immunol*. 2011; 187:1977–1985. [PubMed: 21765017]
41. McMenamin PG, Crewe J, Kijlstra A. Resident and infiltrating cells in the rat iris during the early stages of experimental melanin protein-induced uveitis (EMIU). *Ocul Immunol Inflamm*. 1997; 5:223–233. [PubMed: 9455739]
42. Mellman I, Nussenzweig M. Retrospective. Ralph M. Steinman (1943–2011). *Science*. 2011; 334:466. [PubMed: 22034425]
43. Miller JC, Brown BD, Shay T, et al. Deciphering the transcriptional network of the dendritic cell lineage. *Nat Immunol*. 2012; 13:888–899. [PubMed: 22797772]
44. Muhaya M, Calder VL, Towler HM, et al. Characterization of phenotype and cytokine profiles of T cell lines derived from vitreous humour in ocular inflammation in man. *Clin Exp Immunol*. 1999; 116:410–414. [PubMed: 10361227]
45. Oh K, Kim YS, Lee DS. Maturation-resistant dendritic cells ameliorate experimental autoimmune uveoretinitis. *Immune Netw*. 2011; 11:399–405. [PubMed: 22346781]

46. Pay S, Pekel A, Simsek I, et al. Pronounced interferon-alpha production from plasmacytoid dendritic cells in patients with Behcet's disease following CpG D ODN stimulation. *Clin Exp Rheumatol.* 2009; 27:S37–S42. [PubMed: 19796531]
47. Piccioli D, Sammicheli C, Tavarini S, et al. Human plasmacytoid dendritic cells are unresponsive to bacterial stimulation and require a novel type of cooperation with myeloid dendritic cells for maturation. *Blood.* 2009; 113:4232–4239. [PubMed: 19176317]
48. Plskova J, Greiner K, Muckersie E, et al. Interferon-alpha: a key factor in autoimmune disease? *Invest Ophthalmol Vis Sci.* 2006; 47:3946–3950. [PubMed: 16936109]
49. Randolph GJ, Inaba K, Robbiani DF, et al. Differentiation of phagocytic monocytes into lymph node dendritic cells in vivo. *Immunity.* 1999; 11:753–761. [PubMed: 10626897]
50. Reis e Sousa C. Dendritic cells in a mature age. *Nat Rev Immunol.* 2006; 6:476–483. [PubMed: 16691244]
51. Sathe P, Pooley J, Vremec D, et al. The acquisition of antigen cross-presentation function by newly formed dendritic cells. *J Immunol.* 2011; 186:5184–5192. [PubMed: 21422244]
52. Satpathy AT, Kc W, Albring JC, et al. Zbtb46 expression distinguishes classical dendritic cells and their committed progenitors from other immune lineages. *J Exp Med.* 2012; 209:1135–1152. [PubMed: 22615127]
53. Schlitzer A, Loschko J, Mair K, et al. Identification of CCR9- murine plasmacytoid DC precursors with plasticity to differentiate into conventional DCs. *Blood.* 2011; 117:6562–6570. [PubMed: 21508410]
54. Schlitzer A, Heiseke AF, Einwachter H, et al. Tissue-specific differentiation of a circulating CCR9- pDC-like common dendritic cell precursor. *Blood.* 2012; 119:6063–6071. [PubMed: 22547585]
55. Schreibelt G, Klinkenberg LJ, Cruz LJ, et al. The C-type lectin receptor CLEC9A mediates antigen uptake and (cross-)presentation by human blood BDCA3+ myeloid dendritic cells. *Blood.* 2012; 119:2284–2292. [PubMed: 22234694]
56. Segura E, Wong J, Villadangos JA. Cutting edge: B220+CCR9- dendritic cells are not plasmacytoid dendritic cells but are precursors of conventional dendritic cells. *J Immunol.* 2009; 183:1514–1517. [PubMed: 19570827]
57. Shortman K, Liu YJ. Mouse and human dendritic cell subtypes. *Nat Rev Immunol.* 2002; 2:151–161. [PubMed: 11913066]
58. Steinman RM, Cohn ZA. Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. *J Exp Med.* 1973; 137:1142–1162. [PubMed: 4573839]
59. Steinman RM. The dendritic cell system and its role in immunogenicity. *Annu Rev Immunol.* 1991; 9:271–296. [PubMed: 1910679]
60. Suzuki J, Yoshimura T, Simeonova M, et al. Aminoimidazole carboxamide ribonucleotide ameliorates experimental autoimmune uveitis. *Invest Ophthalmol Vis Sci.* 2012; 53:4158–4169. [PubMed: 22562518]
61. Tang J, Zhu W, Silver PB, et al. Autoimmune uveitis elicited with antigen-pulsed dendritic cells has a distinct clinical signature and is driven by unique effector mechanisms: initial encounter with autoantigen defines disease phenotype. *J Immunol.* 2007; 178:5578–5587. [PubMed: 17442940]
62. Tang J, Zhou R, Luger D, et al. Calcitriol suppresses antiretinal autoimmunity through inhibitory effects on the Th17 effector response. *J Immunol.* 2009; 182:4624–4632. [PubMed: 19342637]
63. Toma-Hirano M, Namiki S, Miyatake S, et al. Type I interferon regulates pDC maturation and Ly49Q expression. *Eur J Immunol.* 2007; 37:2707–2714. [PubMed: 17823983]
64. Usui Y, Takeuchi M, Hattori T, et al. Suppression of experimental autoimmune uveoretinitis by regulatory dendritic cells in mice. *Arch Ophthalmol.* 2009; 127:514–519. [PubMed: 19365033]
65. Villadangos JA, Shortman K. Found in translation: the human equivalent of mouse CD8+ dendritic cells. *J Exp Med.* 2010; 207:1131–1134. [PubMed: 20513744]
66. Waskow C, Liu K, Darrasse-Jeze G, et al. The receptor tyrosine kinase Flt3 is required for dendritic cell development in peripheral lymphoid tissues. *Nat Immunol.* 2008; 9:676–683. [PubMed: 18469816]

67. Xu Y, Zhan Y, Lew AM, et al. Differential development of murine dendritic cells by GM-CSF versus Flt3 ligand has implications for inflammation and trafficking. *J Immunol.* 2007; 179:7577–7584. [PubMed: 18025203]
68. Yang GX, Lian ZX, Kikuchi K, et al. Plasmacytoid dendritic cells of different origins have distinct characteristics and function: studies of lymphoid progenitors versus myeloid progenitors. *J Immunol.* 2005; 175:7281–7287. [PubMed: 16301633]
69. Yang Y, Qi J, Wang Q, et al. Berberine suppresses Th17 and dendritic cell responses. *Invest Ophthalmol Vis Sci.* 2013; 54:2516–2522. [PubMed: 23482469]
70. Ziegler-Heitbrock L, Ancuta P, Crowe S, et al. Nomenclature of monocytes and dendritic cells in blood. *Blood.* 2010; 116:e74–e80. [PubMed: 20628149]
71. Zuniga EI, McGavern DB, Pruneda-Paz JL, et al. Bone marrow plasmacytoid dendritic cells can differentiate into myeloid dendritic cells upon virus infection. *Nat Immunol.* 2004; 5:1227–1234. [PubMed: 15531885]

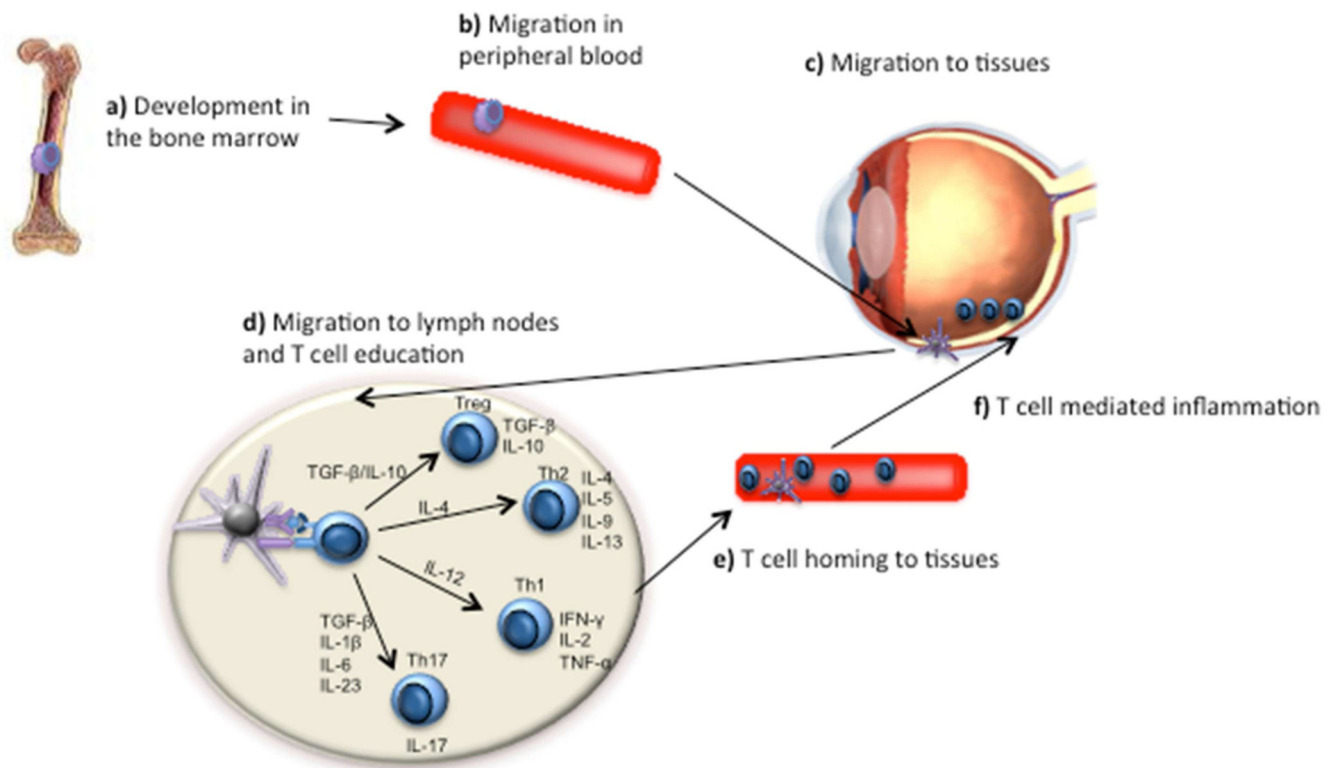


Figure 1. Role of DCs in Non-infectious Uveitis

a) Precursors of DCs from bone marrow migrate into peripheral blood and become immature DCs in response to microenvironment challenge. **b)** Immature DCs circulate in the peripheral blood and eye under the attraction of chemokine or cytokines. **c)** Immature DCs sense and take up the antigens in the eye. **d)** Immature DCs become mature after antigen uptake, which express high MHC and co-stimulatory molecules. Mature DCs migrate into draining lymph nodes where they educate naïve T cells to be Th1, Th2, T regulatory cells and Th17 cells depending on signals. **e)** Educated T cells migrate into eyes to secrete cytokines, which cause non-infectious uveitis.

Table 1

Comparison of Human and Murine DC Subsets

	Subsets	Phenotypes	Function	References
Human	pDCs	BDCA2 (CD303) BDCA4 (CD304) CD123 TLR7, 9	Type I interferon production in response to viral or self nucleic acids (via TLR7, 9)	5; 6; 7; 48
	mDC1	BDCA1 (CD1c) CD11c TLR1–8	Stimulate CD4 ⁺ T cells; Wide-range of TLR and lectins; Secrete IL-12, IL-8, IL-10, IL-23 and TNF α	5; 6
	mDC2	BDCA3 (CD141) XCR1	Antigen cross presentation to CD8 ⁺ T cells	5; 6
Mouse	pDCs	PDCA1	Type I interferon production in response to viral or self nucleic acids (via TLR7, 9)	15
	CD8 ⁺ /CD103 ⁺ cDCs	CD11c CD8 α (lymphoid) or CD103 (tissue) XCR1	Antigen cross presentation to CD8 ⁺ T cells	15
	CD8 ⁻ cDCs	CD11c CD11b	Stimulate CD4 ⁺ T cells; Wide-range of TLR and lectins; Secrete IL-12, IL-8, IL-10, IL-23 and TNF α	

* CD8⁺ cDCs in the lymphoid tissues and CD103⁺ in non-lymphoid tissues share XCR1, and the ability to cross-present antigen to CD8⁺ T cells. They are therefore grouped as the same subset here.