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Increased CD1c$^+$ mDC1 with mature phenotype regulated by TNFα–p38 MAPK in autoimmune ocular inflammatory disease

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Abstract In this study we investigated the role of blood CD1c$^+$ myeloid dendritic cells 1 (mDC1), a key mDC subtype, in patients with autoimmune uveitis. We observed a significant increase of blood CD1c$^+$ mDC1 in uveitis patients. The increased CD1c$^+$ mDC1 exhibited high HLADR expression and less antigen uptake. CD1c$^+$ mDC1 were divided into two subpopulations. CD1c^{hi} mDC1 subpopulation showed less antigen uptake and higher HLADR expression compared to CD1c^{lo} mDC1 subpopulation. Importantly, the CD1c^{hi} mDC1 subpopulation was increased in uveitis patients. *In vitro*, mature monocyte-derived dendritic cells (MoDCs), characterized by lower levels of antigen uptake, induced more CD4$^+$CD62L$^-$ T helper cell proliferation. The mature phenotype and function of CD1c$^+$ mDC1 were regulated by TNFα *via* a p38 MAPK-dependent pathway. These data show that alterations in the systemic immune response are involved in the pathogenesis of autoimmune uveitis and invite the therapeutic possibility of attenuating uveitis by manipulating blood CD1c$^+$ mDC1.
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Abbreviations: mDC1, myeloid dendritic cells 1; HCs, healthy controls; MoDCs, monocyte-derived dendritic cells; PBMCs, peripheral blood mononuclear cells; Th, T helper; DCs, dendritic cells; mDCs, myeloid dendritic cells; pDCs, plasmacytoid dendritic cells; Lin1, lineage 1; Alb, albumin; iNKT, invariant natural killer T.

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1. Introduction

Human autoimmune ocular inflammatory diseases, particularly non-infectious uveitis (abbreviated as uveitis), are currently responsible for 10% of the visual handicap in the United States [1]. The most commonly recognized mechanism of uveitis is $CD4^+$ T helper (Th) cell mediated immune dysregulation [2–4]. It has been suggested that the immune dysregulation is characterized by the imbalance between regulatory and pathogenic effector T cells, increased inflammatory and decreased anti-inflammatory cytokines, hyperresponsiveness to self-antigens, and dysfunctional dendritic cells (DCs), which are all suspected to contribute to the pathogenesis of autoimmune disease [5].

Dendritic cells (DCs) play a central role in immune regulation since they are involved in both innate and adaptive immune responses. An indispensable part of initiating the immune response in the eye is the permeability of the blood-ocular barrier to inflammatory cells. Infiltrating cells include monocytes/macrophages, lymphocytes, neutrophils, and myeloid DCs (mDCs). Moreover, an emerging body of literature supports a pivotal role for mDCs in uveitis in humans and mice [6–8]. However, the actual source of mDCs in eye tissue, along with the factors that trigger DC maturation, take up antigens and help T cell responses, remains unidentified.

DCs are derived from either progenitors in the bone marrow or monocytes in the peripheral blood. Blood DCs contain three subsets: $CD1c^+$ (BDCA1) myeloid dendritic cell 1 (mDC1), $CD141^+$ (BDCA3) myeloid dendritic cell 2 (mDC2), and $CD303^+$ (BDCA2) plasmacytoid DCs (pDCs) [9], which are from Lineage 1^- (Lin1, including CD3, CD14, CD16, CD19, CD20 and CD56) $HLADR^+$ cells. Myeloid DCs actively infiltrate into the eye and can be seen suspending in the aqueous humor of uveitis patients [10].

In this study, we observed an increase in blood $CD1c^+$ mDC1 with enhanced HLADR expression and lower levels of antigen uptake indicating a more mature phenotype. Moreover, this was associated with an increase in a subpopulation of $CD1c^{hi}$ mDC1, which had very low levels of antigen uptake. In an *in vitro* model we observed that MoDCs with a similar low-antigen-uptake phenotype promoted more $CD4^+CD62L^-$ T cell proliferation, than those taking up high levels of antigen. Our work also supports the hypothesis that in uveitis the mature phenotype of $CD1c^+$ mDC1 was regulated by TNFα *via* a p38 MAPK-dependent pathway.

2. Material and methods

2.1. Study population

Peripheral blood was obtained from patients with non-infectious uveitis (n = 74) in the Clinical Center of the National Eye Institute (NEI), and from healthy controls (HCs, n = 96) in the Blood Bank of the National Institutes of Health (NIH). All protocols were approved by the NIH Neuroscience Institutional Review Board (IRB). Informed consents were obtained from all subjects before the study commenced. Ocular disease activity, *i.e.* active and inactive uveitis, was recorded according to the Standardization of Uveitis Nomenclature (SUN) [11]. According to SUN criteria, less than one cell, or alternatively, no flare in the anterior chamber, constitutes a graded score of 0. SUN criteria go on to address "trace" cells in the vitreous, correlating this clinical impression with a score of 0.5. A grade of 0 is regarded as inactive, whereas, a grade $\geq$0.5 is considered slightly active, and a grade $\geq$1 or haze in vitreous is considered active. Here, we put slightly active and active uveitis into one group as "active".

To investigate the phenotype and function of mDC1 in the peripheral blood, the effects of systemic immunosuppressive therapy on mDC1 were considered. The presence or absence of systemic immunosuppressive therapy was thus documented; this included systemic corticosteroids, secondary immunomodulatory therapy and biologic response modifiers (see Table 1).

2.2. Identification of mDCs and pDCs in human peripheral blood

Blood DCs were identified by 4-color staining performed on whole fresh peripheral blood using the following monoclonal antibodies: PE-CD1c (BDCA1, Miltenyi Biotec, Auburn, CA) or PE-CD141 (BDCA3, Miltenyi Biotec), PERCP-HLADR (Miltenyi Biotec), APC-CD303 (BDCA2, Miltenyi Biotec), and FITC-labeled mAbs against lineage markers CD3, CD14, CD16, CD19, CD20 and CD56 (BD Biosciences, San Jose, CA). Cells that were not labeled with these lineage markers were designated as $Lin1^-$. Myeloid DC1 were $CD1c^+$, mDC2 were $CD141^+$, and pDCs were $CD303^+$. These DCs were gated on $Lin1^-HLADR^+$ total DC population. Cells were analyzed on FACSCalibur (BD Biosciences). DC number was calculated by multiplying the percentage of DCs by the total number of white blood cells (WBC) in 1 ml blood (DC number = % of DCs × number of WBC/ml).

Table 1 Characteristics of uveitis patients and HCs.

Parameter	Uveitis (n = 74)	HCs (n = 96)
Age (y), median (range)	44 (17–74)	46 (19–76)
Gender [n (%)]		
Male	44 (59)	61 (64)
Female	30 (41)	35 (36)
Race [n (%)]		
Caucasian	28 (38)	80 (83)
African American	28 (38)	15 (16)
Others	18 (24)	1 (1)
Anatomic type of uveitis [n (%)]		N/A
Anterior	10 (14)	
Intermediate	7 (9)	
Posterior	17 (23)	
Panuveitis	40 (54)	
Disease association [n (%)]		N/A
Sarcoidosis	17 (23)	
Idiopathic	32 (43)	
Birdshot	8 (11)	
VKH	5 (7)	
Behcet's disease	8 (11)	
Serpiginous choroidopathy	4 (5)	
Systemic immunosuppressive therapy [n (%)]	57 (77)	N/A
Active uveitis [n (%)]	18 (24)	N/A