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Title:
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Date:
2021-09-01

Citation:
Chinnery, H. R., Zhang, X. Y., Wu, C. Y. & Downie, L. E. (2021). Corneal immune cell morphometry as an indicator of local and systemic pathology: A review. Clinical and Experimental Ophthalmology, 49 (7), pp.729-740. https://doi.org/10.1111/ceo.13972.

Persistent Link:
https://hdl.handle.net/11343/298761

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Review

Corneal immune cell morphometry as an indicator of local and systemic pathology: A review

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Short running title: Dendritic cells morphometry and corneal inflammation: A review

Received 30 June 2021; accepted 3 July 2021

Funding sources / Financial disclosure: National Health and Medical Research Council Project Grant (HRC; APP1126540)

Conflict of interest: None

ABSTRACT

The corneal epithelium contains a population of resident immune cells commonly referred to as dendritic cells (DCs), or Langerhans cells. A unique advantage of the transparent cornea being situated at the surface of the eye is that these cells can be readily visualised using *in vivo* confocal microscopy. Over the past decade, interest in the involvement of corneal DCs in a range of ocular and systemic diseases has surged. For most studies, the number of corneal DCs has been the main outcome of interest. However, more recently the attention has shifted towards understanding how DC morphology may provide insights into the inflammatory status of the cornea, and in some cases, the health of the peripheral nervous system. In this review, we provide examples of recent methodologies being used to classify and measure corneal DC morphology and discuss how this relates to local and systemic inflammatory conditions in humans and rodents.

Keywords: Cornea, Imaging systems, Immunology, Inflammation, Ocular surface

1. INTRODUCTION

In vivo confocal microscopy (IVCM) is a minimally invasive imaging technique that enables the acquisition of high-resolution, *en face* images of the human cornea. The approach allows for in-depth analyses of key corneal anatomical features, including sensory nerves, endothelial cells, stromal keratocytes, and resident and infiltrating immune cells. The role and involvement of resident immune cells, in particular dendritic cells, in corneal homeostasis and pathology has generated much interest in the literature, as these cells can reflect the state of immune activation at the ocular surface. A recent systematic review has summarised the current landscape of literature pertaining to the density of resident immune cells in healthy corneas,[1] which is important for establishing normative data and for providing insights into the causes of heterogeneity across clinical studies. Another recent review has summarised the literature relating to corneal dendritic cell density in a range of ocular conditions, including herpes zoster ophthalmicus and during contact lens wear, as well as in patients with systemic diseases that have an underlying immunological aetiology, such as diabetes mellitus and rheumatoid arthritis.[2] From these reports, it is clear that the distribution and density of immune cells in different anatomical regions of the corneal epithelium serves as an important indicator of local tissue stress. In more recent years, attention has shifted towards the measurement and classification of corneal immune cells based on their morphology, as an additional potential indicator of cell activation. This review will focus on current methodologies used to qualitatively or quantitatively assess corneal immune cell morphology, as well as the approaches being used to define low-grade activation of these immune cells in human corneas. We will also report on recent methodological approaches for defining corneal immune cell morphology in rodent models of corneal inflammation.

2. TERMINOLOGY USED TO DESCRIBE IMMUNE CELLS IN THE CORNEAL EPITHELIUM

Some of the earliest descriptions of dendriform immune cells in the corneal epithelium date back to the 1800s, with reports of cells with large bodies and long processes in the basal layers of the epithelium, which resembled Langerhans cells in the skin epidermis. More robust histochemical and ultrastructural evidence for the existence of corneal Langerhans cells emerged in the early 1980s, with descriptions of $ATPase^+$ $HLA\text{-}DR^+$ cells in the human corneal epithelium, predominantly in the basal layers of the peripheral cornea.[3] A sparse population of immune cells was also identified in the central corneal epithelium; these centrally located cells appeared to lack the extensive dendrites present on the cells located in the peripheral epithelium[3]. The corneal Langerhans cells were shown to contain the characteristic intracellular organelles referred to as Birbeck granules, which at the time were thought to be an exclusive marker of these resident immunocompetent cells. Identification of Birbeck granules, using transmission electron microscopy, in widely spaced corneal immune cells is a technically challenging process. Thus, despite only a few studies reporting the presence of Birbeck granules within human[3] and mouse[4] corneal epithelium, the naming of these cells as "Langerhans cells (LC)" has persisted for decades. The classification of dendriform immune cells in the corneal epithelium as Langerhans cells was later supported further by evidence of cell-surface expression of Langerin (CD207) on intraepithelial immune cells[5]. Langerin is a c-type lectin involved in endocytosis, and is structurally associated with Birbeck granules.[6] Using *ex vivo* immunostaining of fresh human donor corneas, Mayer et al., provided compelling evidence that a subset of immune cells in the healthy corneal epithelium express Langerin, along with markers such as HLA-DR and CD1a, consistent with a 'mature' phenotype.[5] Conversely, in the central cornea, a smaller sub-population of CD45+ CD11c+ intraepithelial cells were Langerin and HLA-DR negative, suggestive of an 'immature' phenotype. There was no mention of morphological differences between the mature and immature immune cells, and thus it is possible that in studies employing IVCM to examine intraepithelial immune cells in the human cornea, the naming of all immune cells as LCs may be imprecise. Other terms used to identify corneal LCs in human IVCM studies include antigen presenting cells (APCs), dendritiform cells (DCs)[7], immune cells (IC)[8] and, most commonly, dendritic cells (DCs)[9-13]. For the sake of clarity in this review, and in line

with the majority of current reports on these corneal immune cells in studies using IVCM, the term “DCs” will be used henceforth to refer to immune cells located in the corneal epithelium.

2.1 Dendritic cell morphometry as an indicator of activation and maturation

Dendritic cells are often referred to as professional antigen presenting cells (APCs). In their immature state, DCs act as sentinels of the immune system, surveying their microenvironment for foreign antigens or ‘danger signals’, such as pathogen-associated molecular patterns (PAMPs) and damage-associated molecular pattern molecules (DAMPs)[14]. Upon endocytosing antigen, or recognising molecular signatures using pattern recognition receptors, or after exposure to signals from pro-inflammatory cytokines, DCs begin to mature, upregulating their ability to process and present antigens via MHC class II molecules on their surface. Together with expression of the co-stimulatory molecules, CD40, CD80 and CD86, DCs are equipped for successful presentation of antigenic peptides [15] to T cells to initiate adaptive immune responses. One of the hallmark features of DC activation and maturation is a morphological transformation characterised by increased cytoplasmic protrusions and dendrites, increasing the surface area and thus the likelihood of a successful interaction with a naïve T-cell.[16] In addition to enhancing cell surface area to mediate activation of adaptive immunity, DC morphology can also rapidly respond to changes in the microenvironment, which is consistent with their role in the amplification of innate immune responses.[17] Afforded by its transparency and well-defined anatomical layers, the cornea is an ideal tissue to visualise and study *en face* immune cell morphology. This approach can enable the robust measurement of subtle changes to DC morphology that are unlikely to be observable *in vivo* in other, less transparent (i.e., more pigmented) anatomical tissues.

2.2 Microglial morphology as a robust indicator of tissue stress: parallels with corneal dendritic cells?

Despite corneal DCs being a topic of interest to eye care clinicians and immunologists for several decades, until recently little attention has been paid to

how their morphology may act as an indicator of cell and tissue stress. This contrasts to the wealth of evidence that exists with respect to both subtle and robust alterations in the morphology of microglia, which are closely related to DCs, in the eye and brain. Whilst the number of studies considering corneal DC morphology in ocular conditions has risen in recent years, many more studies have used detailed morphometric approaches to characterise the state of microglial activation in the central nervous system (CNS),[18] including the retina.[19, 20] In human prefrontal cortex, four different morphological subtypes of microglia have been characterised, including 'ramified'; 'primed' (similar to ramified but with wider cell bodies); 'reactive' (wider cell bodies and less ramified) and 'amoeboid'.[21] It has long been appreciated that microglia, when triggered by local or systemic inflammation or pathology, transform from a resting, ramified shape to a reactive amoeboid phenotype.[22] Interestingly, the terminology used to describe microglia as 'resting' has become disputed, as it is now clear that these resident immune cells are highly dynamic, with their extensive processes constantly surveying surrounding neurons, endothelial cells and other glia, as well as pruning synapses in the brain parenchyma[23] and retina.[19] Similarly, in mice, resident corneal DCs actively probe their microenvironment by extending and retracting their processes between the densely packed epithelial cells.[24] In addition to the highly dynamic probing behaviours that have been reported in mouse corneal DCs, a recent *in vivo* time-lapse study in human corneas demonstrated that under steady-state conditions, corneal immune cells move rapidly in the axial direction.[25] Interestingly, only immune cells without dendrites appeared to move within the imaging window, with cells containing dendritic processes appearing more sessile. Whether this difference in behaviour of the two morphological subtypes relates to different subsets of immune cells, or perhaps indicates different stages of activation of the immune cells, is not yet known.

2.3 Approaches used to classify and measure human corneal immune cells *in vivo*

In studies examining the role or involvement of corneal DCs during ocular and systemic diseases, a range of methods are being used to classify and measure corneal immune cells, as visualised either by IVCM, or, in the case of animal studies,

by *ex vivo* image analysis of cells using immunofluorescence microscopy (Figure 1). These approaches build upon past studies where only DC density was measured, providing more insights into how corneal immune cells are affected by changes in tissue homeostasis. The capacity to assess corneal immune cell morphology in modern clinical settings is enabled by the Rostock Corneal Module, a lens adaptor that, when fitted to the Heidelberg Retinal Tomograph (HRT), enables laser-scanning confocal microscopy imaging of the cornea.[26] Despite earlier instruments, such as the Tomey Confoscan and Nidek Confoscan existing prior to the development of the HRT, the image quality was poorer, thus accurate assessment of corneal immune cell morphology using these instruments was limited.[27]

Figure 1: Corneal dendritic cells in the human (A,B) and mouse (C-E) epithelium. (A) IVCM image of "immature" DCs (white arrows) and a "mature" DC (*) in the central cornea of a healthy human eye. (B) Example of how Image J software is used to measure cell area and field area using the threshold function. (C, D). 2-D confocal microscopy image of CD45+ Iba-1^{+} DCs in the mouse central cornea, with an example of how cell area and field areas are quantified manually using ImageJ (D). Z-profile 3-D reconstruction (E) of panel C showing the vertical projections of the corneal DCs towards the superficial epithelium. Note the detailed morphology and larger field area of the DCs and the absence of "immature" DCs, in the mouse cornea compared to the human cornea.

In recent clinical studies of corneal DC morphology, DCs have been characterised on both categorical and continuous variables (Table 1). In several instances, the categories of cell morphologies are the same, but diverse terminology has been used across different studies. In some studies, DCs are classified as either "immature" or "mature" [8, 10, 28, 29], "with dendrites" or "without dendrites" [25, 30, 31], "Type 1" or "Type 2", or, in the case of rare globular cells, "Type 3".[11, 32] Less commonly, corneal DCs have been assigned a "Langerhans cell morphology (LCM)" score, whereby cells are given a score ranging from 1-3, with 1 referring to cells with no dendrites, 2 being cells with short dendrites and 3 being cells with long dendrites [33-37]. Alternatively, cells have been categorically divided into "activated" and "non-

activated" DCs.[38] An alternative approach, which is gaining in momentum, is to treat DC morphometry data as a continuous dataset, which can be achieved by performing cell morphometric analysis on a representative subset of cells using image analysis software, such as ImageJ/FIJI[7, 13, 39-45]. A benefit of this image analysis approach is that more detailed information about corneal DC morphometry can be gained, and the objective nature of the cell measurements can help reduce bias in the image analysis process (Table 1).

Table 1: Examples of clinical studies using corneal DC morphology as an indicator of cell phenotype. The type of methodological approach for quantifying corneal DC morphology (i.e. categorical or continuous), and the classification system is included.

Reference	**Disease / Conditions**	***Type* and parameters used to define morphological features**	**Major findings relating to DC morphometry**	**PMID**
Cruzat *et. al.,* 2011	Infectious keratitis	*Continuous:* Morphometric parameters: cell size and number of dendrites per cell	Higher DC size and number of dendrites in patients with acute bacterial, fungal and Acanthamoeba keratitis.	21460259
Marsovszky *et. al.,* 2013	Rheumatoid arthritis	*Categorical:* Langerhans cell morphology (LCM) score	Higher LCM scores in central cornea in patients with Rheumatoid arthritis.	23204037
Marsovszky *et. al.,* 2013	Ankylosing spondylitis	*Categorical:* Langerhans cell morphology (LCM) score	Higher LCM scores in central cornea in patients with ankylosing spondylitis.	23960273
Yamaguchi *et. al.,* 2014	Bacterial keratitis	*Continuous:* Morphometric parameters: cell area, number of dendrites per cell, and cell field area of dendritiform immune cells	Higher DC area in both affected and unaffected contralateral eyes of patients with bacterial keratitis. Number of dendrites per DC negatively correlated with level of interleukin (IL)-1ß in tear samples; DC field area negatively correlated with level of IL-17a.	25324281
Fagerholm *et. al.,* 2014	Corneal implantation	*Categorical:* Mature and immature DCs	Higher density of mature DCs in central cornea of donor corneas compared to biosynthetic implants and healthy corneas.	24374070
Kheirkhah *et. al.,* 2015	Aqueous-deficient and evaporative subtypes of dry	*Continuous:* Morphometric parameters: cell size, number of dendrites and cell field area of corneal epithelial DCs	Increased DC size, number of dendrites per cell, and DC field area in dry eye disease patients. No difference in parameters between the two DED subtypes. Within the aqueous-deficient group,	26540656

	eye disease (DED)		patients who had underlying systemic immune diseases had higher DC size and number of dendrites than patients who did not.	
Mastropasqua *et. al.,* 2016	Glaucoma and dry eye disease (DED)	*Categorical:* Mature, immature, and globular cells	Higher density of mature DCs in patients with medically controlled glaucoma, in patients with more than one anti-glaucoma drugs, and in patients with DED.	27820631
Shetty et al.	Evaporative dry eye disease	*Categorical*: DCs with or without processes	DC density (both subtypes) positively correlated with ocular surface disease index (OSDI) score and showed correlations with some of the subbasal nerve plexus parameters. Density of 'DCs with processes' negatively correlated with serum vitamin D levels.	26904676
Wu *et. al.,* 2016	Thyroid-associated ophthalmopat-hy (TAO)	*Categorical*: Langerhans cell morphology (LCM) scores	Higher LCM scores in central cornea of patients with active and inactive TAO compared to healthy controls. No difference in LCM scores between active and inactive disease groups. Central LCM scores positively correlated with clinical activity scores and ocular surface disease index scores, and inversely correlated with the Schirmer test scores.	26735162
Postole *et. al.,* 2016	Anterior uveitis	*Categorical*: Dendritic-like cells (type 1); Cell bodies lacking dendrites (type 2); Each category of cells had 3 gradings: small (a), medium (b) and large (c) cell sizes.	Patients with herpetic anterior uveitis (HAU) had higher density of type 1 cells in comparison to patients with Fuchs' uveitis syndrome. No difference in type 1 cell density between patients with HAU patients patients who had juvenile idiopathic arthritis or HLA-B27-related anterior uveitis. Difference in mean density of type 2 cells was not significant among disease subtypes.	26823398

Choi *et. al.,* 2017	Dry eye disease (DED)	*Continuous*: Morphological parameters: cell area and process length of Langerhans cells (LCs)	Increased LC area and process length in patients with DED. Both parameters positively correlated with nerve density, and negatively correlated with nerve beading.	28441413
Lagali *et. al.,* 2018	Type 2 diabetes mellitus	*Categorical*: Type 1 (Mature, mDCs), Type (Immature, imDCs), and Type 3 (globular cells). *Continuous:* Morphometric parameters for mDCs: cell size, total dendritic length, number of dendrites per cell, basoapical dendritic field area, and clustering.	Increased proportion of mDCs, decreased proportion of imDCs, and increased clustering of mDCs in patients with type 2 diabetes. Plasma concentration of tumour necrosis factor receptor super family member 9 positively associated with proportion of mDCs and negatively associated with proportion of imDCs.	30250206
Cavalcanti *et. al.* 2018	Unilateral Herpes zoster ophthalmicus	*Continuous:* Morphometric parameters: cell size, cell field and number of dendrites of corneal dendritiform cells	Increased DC size, DC field and number of dendrites in the affected eye; all parameters also increased in the contralateral eye although not statistically significant. All parameters negatively correlated with total nerve length.	28923503
Ferdousi *et. al.,* 2019	Type 1 diabetes mellitus in children and adolescents	*Categorical:* Mature and immature Langerhans cells (LCs)	Increased mature, immature, and total LC density in patients with type 1 diabetes.	31217448
Klitsch *et. al.,* 2020	Fibromyalgia syndrome	*Categorical:* Langerhans cells (LCs): DCs (cells showing dendrite-like elongations dLCs) and non-DCs (cells only consisting of a cell body)	Decreased number of dLCs that were in association with nerve fibres.	31846167

Dehghani *et. al.,* 2020	Mild cognitive impairment (MCI) and Alzheimer's disease	*Continuous:* Morphometric parameters: cell field area, field perimeter, circularity, aspect ratio, roundness of DCs	In patients with MCI: Increased DC field area and perimeter in both central cornea and the inferior whorl region of cornea, lower circularity, less roundness and higher aspect ratio of DCs at the whorl region.	33362451
Tajbakhsh *et. al.,* 2020	Systemic allergy	*Categorical:* Corneal epithelial DCs (CEDCs) morphology grades (1-3 scale)	Higher morphology grades in participants with allergies in the central cornea. In the non-allergic group, central CEDCs had lower morphology grades than mid-periphery CEDCs, but no such difference was found in the allergic group.	31743651
Colorado et al.	Healthy individuals	*Categorical:* Cells with or without dendrites	Cells without visible dendrites moved rapidly within the subbasal nerve plexus whereas cells with visible dendrites did not appear to migrate within one hour of time-lapse imaging.	32846151
Golebiowski *et. al.,* 2020	Contact lens wear	*Categorical:* Corneal epithelial DC (CEDC) morphology grades (0-3 scale)	No significant difference in CEDC morphology between contact lens wearer and non-wears, at either central or mid-peripheral cornea.	31227315
Testa *et. al.,* 2020	Multiple sclerosis (MS)	*Continuous:* DC size	DC size was similar in patients with MS versus healthy controls. Lower DC size in MS patients with a history of optic neuritis compared to MS patients without a history of optic neuritis.	33222903
Aggarwal *et. al.,* 2021	Dry eye disease (DED)	*Continuous:* Morphometric parameters: cell size, DC field and number of dendrites per cell of corneal immune dendritiform cells	Increased DC size, DC field and number of dendrites per cell in patients with DED. Higher density of dendrites in patients with more severe DED. Significant increase in DC field in patients with level 3 severity compared to level 1. Higher DC size in patients with level 3 severity in comparison to level 1 and 2.	32504855

Khan *et. al.,* 2021	Multiple sclerosis (MS)	*Categorical:* Mature and immature corneal immune cells (ICs)	Increased immature IC density but not mature IC density in patients with MS. Immature IC had greater 'near-nerve' distance in patients with MS compared to controls. Immature IC density and total IC density negatively correlated with symbol digit modalities test scores.	34003997
Levine *et. al.,* 2021	Individuals with dry eye symptoms	*Categorical:* "Activated" DCs (aDCs) and non-activated dendritic cells	Individuals with dry eye symptoms that had a systemic immune disorder were more likely to have more than 2 aDCs per image in central cornea. Number of aDCs in central cornea decreased after commencing topical anti-inflammatory therapy.	34102312
Wei *et. al.,* 2021	*Acanthamoeba* keratitis	*Continuous:* Morphometric parameters: median cell size, cell field, and dendrite length of dendritic cells	Increased median DC size, field and dendrite length. Severity of the disease positively correlated with DC size and dendrite length.	34110388

2.4 Approaches to quantify corneal dendritic cell morphometry in pre-clinical models

In pre-clinical models of corneal and ocular disease, the ability to perform detailed morphological analyses of immune cells, both *in vivo* [46, 47] and in *ex vivo* corneal preparations,[48, 49] has greatly enhanced scientific understanding of the physiology of corneal DCs. In mice, detailed immunophenotyping using antibodies and genetically modified fluorescent reporter mice, coupled with the superior resolution of confocal and fluorescent microscopy images, has enabled extensive and objective analyses to be performed.[48, 50, 51] Unlike the majority of clinical reports on corneal DC morphology, studies in mice have tended to adopt detailed image analysis protocols on either 2-D [27, 49] and 3-D datasets derived from corneal flatmounts (Table 2). Volumetric, or 3-D, image analysis of corneal DCs is ideal as it captures the three dimensional nature of these intraepithelial immune cells, as their processes are arranged in XYZ directions, extending towards the superficial layers of the epithelium (Figure 1C-E).[52] A limitation of this approach is that it is time-consuming and labour intensive, involving the manual creation of individual surfaces for each cell, which can be subject to observer bias. The range of continuous parameters frequently used to measure corneal DCs in mouse studies includes DC field area (tip-to-tip polygonal trace), cell size (total cell area occupied under the threshold), total dendrite length and circularity (Table 2).

Table 2: Examples of mouse studies reporting quantitative morphometry of corneal epithelial dendritic cells. DED, dry eye disease, dSEARCH, Dendritic surveillance extension and retraction cycling habitude.

Reference	Disease/Condition	Morphology grade/classification/terminology	Image analysis approach	Findings	PMID
(Ward *et. al.,* 2007)	Experimental thermal injury	*Continuous* DC length-tip to tip of dendrite to cell body dSEARCH index=for Langerhans cell calculated by adding the absolute values of the changes in dendrite lengths over period 6 mins	*Ex vivo* confocal time lapse imaging of corneo-limbal explants.	LCs undergo dSEARCH activity in steady-state, and exhibit more amoeba-like lateral movement during inflammation.	17250587
(De Silva *et.al.,* 2019)	Healthy young and aged (over 20 months) mice	*Continuous* Morphometric parameters: DC field/tree area	Immunostaining with *ex vivo* epifluorescent microscopy	DC morphology similar in both groups.	31242280
Seyed-Razavi *et. al.,* 2019	Local inflammation induced by cautery burn	*Continuous* Morphometric parameters: 2-D cell area, 3-D surface area, 3-D sphericity	Confocal analysis of CD11c-eYFP^{+} and Cx3cr1gfp/+ and MHC-GFP+ DCs in corneal flatmounts.	Lower DC cell area and surface area, but higher sphericity in inflamed corneas compared to controls.	30226811
(Jiao *et. al.,* 2020)	Local inflammation induced by sterile epithelial abrasion. Systemic acute inflammation induced by intraperitoneal injection of lipopolysaccharide	*Continuous* Morphometric parameters: DC field/tree area	Immunostaining with *ex vivo* epifluorescent microscopy	Healthy control-DC field area higher in periphery cornea compared to central cornea. Local inflammation-higher DC compared to controls. Systemic inflammation-lower DC field area compared to controls.	31429614

(Jiao *et. al.,* 2020)	Central nervous system tauopathy	*Continuous* Morphometric parameters: DC field area, cell area, total dendrite length, number of dendrites per cell	Immunostaining with *ex vivo* epifluorescent microscopy	Smaller DC size in mice with tauopathy, compared to healthy wild-type littermate controls.	32345316
(Wu *et. al.,* 2020)	Local inflammation induced by sterile epithelial abrasion, with or without topical decorin	*Continuous* DC Field area	Immunostaining with *ex vivo* epifluorescent microscopy	No difference in DC field area in injured corneas compared to controls. No effect of decorin treatment on DC size.	32366307
Jamali *et. al.,* 2020	Experimental model of desiccating stress-induced dry eye disease	*Continuous* Morphometric parameters: 2-D cell area, 3-D surface area, 3-D sphericity	Confocal analysis of CD11c-eYFP^{+} DCs in corneal flatmounts.	Lower DC cell area and surface area, but higher sphericity in inflamed corneas compared to controls.	32457740
Senthil *et.al.,* 2020	Short-term topical exposure to hyperosmolar saline	*Continuous* Morphometric parameters: DC field area, cell area, total dendrite length, number of dendrites per cell, DC complexity	Immunostaining with *ex vivo* epifluorescent microscopy	Higher DC field area and lower circularity in mice exposed to mild hyperosmolar saline. Lower DC field area, cell area and dendritic complexity in corneas of anaesthetised mice compared to non-anaesthetised mice.	33625479

2.5 Altered corneal dendritic cell morphology in local and systemic ocular conditions

2.5.1 Clinical studies

Many studies have provided evidence that corneal DC morphology is altered in diseases with an underlying inflammatory basis. In conditions such as dry eye disease,[43] anterior uveitis[32] and thyroid-associated ophthalmopathy[33], corneal DCs have been consistently shown to exhibit signs of 'maturation' or 'activation',[38] as characterised by longer dendritic processes, increased dendrite numbers and enlarged field areas. In individuals with acute infectious keratitis[53] and chronic herpes zoster ophthalmicus,[7] corneal DC sizes are increased, indicative of activation. Interestingly, these signs of DC activation or maturation are often also evident in the contralateral, unaffected eyes following bacterial or viral keratitis[7, 39, 44], suggesting a bilateral cross-over mechanism that may be neurogenic or systemically-driven. In dry eye disease (DED), several studies have confirmed the presence of mature corneal DCs, which is supported by quantitative cell morphometry (i.e., increased cell size and field area)[30, 43, 54]. Whilst DC morphology did not distinguish between subtypes of DED (i.e., aqueous-deficient or evaporative), people with aqueous-deficient DED and co-morbid systemic inflammatory conditions tended to have larger DC sizes.[43]

Many ocular pathologies are associated with immune-mediated conditions or auto-immune diseases that have an inflammatory basis. Given corneal DCs are a subset of immune cells, located in a peripheral tissue, it is not surprising that any condition that involves increased inflammatory mediators in the circulation would also likely induce activation of resident tissue immune cells. Despite the cornea being avascular, DCs in the central corneal epithelium are highly responsive to systemic inflammation. In individuals with type 2 diabetes mellitus, the presence of mature corneal DCs was positively associated with plasma levels of tumour necrosis factor receptor super family member-9,[11] whereas younger adults with type 1 have been found to have significantly higher densities of both mature and immature DCs in the cornea.[28] Both studies suggest that corneal DC analysis may be used as an early indicator of metabolic-induced immune activation in peripheral tissues. Changes to corneal DC density have also been proposed to be a marker of neuro-inflammation,

and an indicator of therapeutic responsiveness to systemic anti-inflammatory intervention, in small fibre neuropathy[55].

It is well known that corneal DCs located within the basal epithelium, are spatially associated with the dense network of sensory nerves that form the so-called sub-basal nerve plexus, also known as the intra-epithelial nerve plexus.[56] Studies in mice have demonstrated the structural and functional interdependence of corneal nerves and DCs,[57, 58] with the DC-derived neuropeptide ciliary neurotrophic factor shown to regulate corneal nerve homeostasis and recovery after traumatic injury.[59] The significance of neuroimmune interactions between corneal DCs and nerves is starting to emerge in the clinical literature, with evidence of corneal DC activation, as evidenced by morphological markers, in neurological diseases, including multiple sclerosis[8, 42] and mild cognitive impairment.[13] It is likely that these conditions, which primarily originate in, and affect, the CNS, are associated with low-grade systemic inflammation. This makes it challenging to determine whether the corneal DC activation is due to the neural degeneration, or the systemic inflammation, or both.

2.5.2 Pre-clinical studies

As corneal DC morphometry is increasingly being reported in published studies, an intriguing disparity between human and mouse corneal DCs is emerging. Whilst increased corneal DC size in clinical populations is overwhelmingly associated with corneal and systemic pathology, it is apparent that the rule of increased size of epithelial DCs in the context of corneal inflammation does not always hold true. Rather, corneal DCs can undergo rapid reductions in cell size within hours after exposure to systemic danger signals, such as lipopolysaccharide or endotoxin,[48] or following protocols where mice are anaesthetised, and thus blinking action and tear film homeostasis is disturbed.[49] Using a model of corneal inflammation induced by thermal cautery burn, Seyed-Razavi et.al. also reported a reduction in the size of corneal intraepithelial DCs within five days of injury.[52] In rTG4510 tauopathy mice, which overexpress hyperphosphorylated tau and thus are used to model human frontotemporal dementia, corneal DC size has been shown to be lower compared to wild-type littermates.[51] These opposing morphological responses in mouse versus human corneal DCs studies requires further exploration to understand how corneal

immune cell morphometry may be used more robustly to serve as a biomarker of disease. Whether the rapid transformation of corneal immune cell morphology also occurs in humans is unclear, however, such empirical investigations would require careful, longitudinal evaluation of immune cells pre-and post-exposure to a provocative, but relatively safe, stimulus. Clinical scenarios in which this could feasibly be undertaken include surgical procedures, whereby incision into the cornea or sclera would induce a local danger signal, and contact lens fitting, which may act as a mechanical stimulus. Such a study on the acute effect of a local stimulus (e.g., contact lens application to the eye) on resident corneal DCs has been reported in the literature,[60] but only DC density, and not morphology, was measured.

2.6 Spatial relationships between corneal sensory nerves and dendritic cells as an indicator of neuroinflammation

The physical association between corneal DCs and nerves is emerging as an additional marker of corneal neuroinflammation. Fibromyalgia is a poorly understood disorder of the CNS, characterised by chronic muscle pain and fatigue, and possibly manifests neuropathic pain. Corneal nerve fibre length and density, as measured from IVCM images, are reduced in individuals with fibromyalgia, suggesting this disorder may be a type of small fibre neuropathy.[61] Interestingly, the number of neuroimmune contacts between corneal DCs and epithelial nerves is lower in people with fibromyalgia, with fewer contacts observed particularly between “mature” corneal DCs and corneal sub-basal nerves.[31] In chronic inflammatory demyelinating polyneuropathy (CIDP), an immune-mediated peripheral neuropathy, mature dendritic cells were more frequently observed to be in contact with corneal nerves during early stage disease.[62] In more severe forms of CIDP, the number of immature, or “non-dendritic cells”, in contact with corneal nerves was lower compared to healthy controls.[62] These relatively subtle alterations in neuroimmune interactions between morphologically distinct corneal immune cells could help to stratify disease severity in conditions where peripheral neuropathy and inflammation are key pathophysiological factors.

In mouse studies of corneal neuroimmune interactions, several laboratories have demonstrated physical associations between corneal epithelial DCs and sensory

nerves, based on high-resolution, 3-D reconstructions of immunostained corneal flatmounts.[57, 63, 64] However, little is currently known about the maturation status of corneal DCs that appear to make contacts with corneal epithelial nerves; this is an area warranting further investigation.

3. CONCLUSION

Over the past few years, investigations into the biology of corneal DCs have become increasingly nuanced, as the field moves towards more detailed classifications, and unbiased objective measurements, of morphological subtypes of corneal immune cells. A more comprehensive analysis of corneal DC morphology may reveal previously unrecognised associations of these immune cells in a range of local and systemic conditions that affect corneal homeostasis. Our understanding of the identity and subsequent classification of corneal epithelial immune cells in humans may be on the precipice of change. Recent elegant studies using single cell RNA-Seq and fate mapping approaches to phenotype myeloid cells in the eye may challenge our long-held view that all $CD11c^+$ cells in the corneal epithelium represent LCs or DCs.[65] Indeed, evidence from the immunology literature lends weight to the idea that immune cells considered to be LCs may, in fact, be macrophages [66]. The heavy reliance on CD11c as an exclusive marker of corneal DCs, at least in mouse studies, may need revisiting as the paradigm shifts around the classification of resident antigen presenting cells in other organs, such as the kidney.[67] The heterogeneity in terminology used to describe corneal immune cells that are visible using IVCM is an area of concern, and efforts should be made to standardise this nomenclature to improve the clarity of reporting on these important cells in the cornea.

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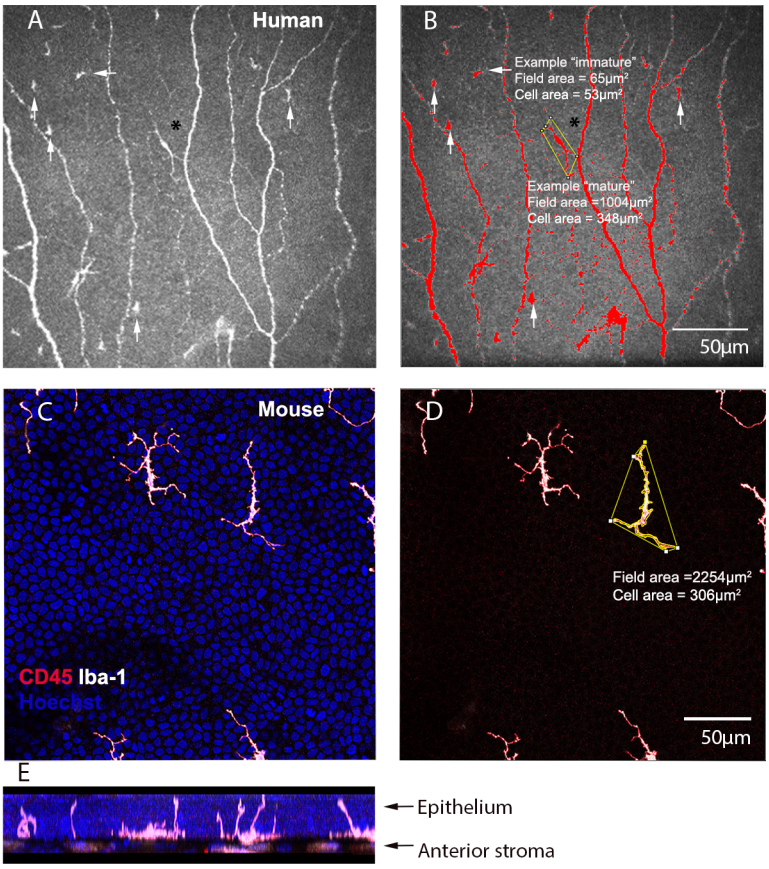

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