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Density and morphology of corneal epithelial dendritic cells are different in allergy.

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ABSTRACT

Purpose: The role of corneal epithelial dendritic cells (CEDC), a subtype of antigen presenting cells, in ocular allergy remains largely unknown. This cross-sectional study evaluated the density and morphology of CEDC in participants diagnosed with systemic allergy, to increase our understanding of the role of CEDC in ocular inflammation associated with systemic allergy.

Materials and methods: A convenience sample of 50 participants was categorised into allergic and non-allergic groups (31 allergic and 19 non-allergic) based on the results of skin prick test (SPT). Ocular allergy symptoms, clinical ocular surface signs and serum IgE were assessed. *In vivo* confocal microscopy was performed on the right eye only. The number of CEDC in a 1mm² region at both the central and mid-peripheral cornea was manually counted. CEDC morphology was graded on a 1 to 3 scale.

Results: Ocular surface symptoms, signs (other than eyelid oedema), and serum IgE were significantly higher in the allergic (SPT+) group. CEDC density at the mid-peripheral cornea was significantly lower in the allergic group ($p=0.003$). CEDC morphology grades were significantly higher in allergic participants in the central cornea ($p=0.02$), with the highest grade morphology observed only in allergic participants. No associations were evident between CEDC density or morphology and ocular signs, symptoms or serum IgE.

Conclusions: The study showed reduced CEDC density and cells with longer dendrites in allergic participants. The more mature CEDC morphology in the allergic group is suggestive of an inflammatory or immune response.

Key words: Corneal epithelial dendritic cell, Allergy, *In vivo* confocal microscopy

Introduction

Allergy is a growing problem throughout the world, mainly due to environmental and lifestyle factors.¹ It is reported that in subjects with allergy, ocular symptoms are more common than nasal symptoms alone.² Dendritic cells are an important subtype of leukocytes responsible for the initiation of the allergic response.³ In recent years, the introduction of clinical confocal microscopy has provided an opportunity to observe dendritic cells *in vivo*, in different areas of normal and diseased corneas with high resolution and contrast. Changes in density and/or morphology of dendritic cells have been observed with altered systemic and ocular immune states, including atopic allergy.³⁻⁵

The role of dendritic cells in ocular allergy is not well established. An increased number of corneal epithelial dendritic cells in the active phase of vernal keratoconjunctivitis has been reported, but morphology has not yet been fully studied.^{6,7} As ocular symptoms are widely reported in atopic individuals, we sought to assess CEDC in allergic participants.

Materials and methods

In this study, we documented density and morphology of CEDC in a convenience sample of 50 allergic and non-allergic participants (mean age 30.9 ± 12.7 years, 56% female).⁸ Study participants were aged 18 years or older and included those with and without symptoms of ocular allergy or prior diagnosis of allergic rhino-conjunctivitis or hay fever. Thirty-one participants were classed as allergic based on the positive response to skin prick testing (SPT), and 19 were non-allergic. There was no significant difference between the two groups in the distribution of age ($p=0.25$, Mann-Whitney U test) or sex ($p=0.77$, Fisher's Exact test). A standard SPT for common Sydney-region indoor and outdoor allergens was conducted, by using extracts of ten chosen allergens, including four grass pollens, two tree pollens, two dust mites, and cat and dog dander (Stallergenes Corporation, Antony, France). Exclusion criteria included: use of topical or systemic antihistamines, mast cell stabilizers or nasal corticosteroid sprays in the week prior to the study, immunotherapy for aeroallergens, uncontrolled asthma, past anaphylactic episode, pregnancy or breastfeeding, regular contact lens wear, refractive surgery and ocular diseases. None of the participants had a history of ocular surgery or used topical/systemic steroids in the week prior to the study. The study was approved by the University of New

South Wales Human Research Ethics Committee and adhered to the tenets of the Declaration of Helsinki. Informed consent was obtained from all participants.

The study was conducted in Sydney during spring, summer and autumn, due to the high levels of environmental pollen present. Frequency⁹ and severity¹⁰ of ocular allergy symptoms in the week prior to the study were assessed using allergy-specific questionnaires. Bulbar conjunctival hyperaemia¹¹ and chemosis¹², lid roughness¹¹, and eyelid oedema¹² were graded using published grading scales, and serum IgE concentration was measured using a commercially available ELISA kit after venipuncture.

CEDC were imaged *in vivo* using confocal microscopy (Heidelberg Retina Tomograph II with Rostock Corneal Module), in the right eye only. Confocal microscopy was done immediately before SPT. CEDC were identified as hyperreflective linear objects with or without dendritic processes at the sub-basal nerve plexus level of the corneal epithelium. The number of CEDC was manually counted by a masked investigator, in two 1mm² areas, at the corneal centre and in the temporal mid-periphery 2.5 mm from the centre. CEDC morphology was graded on a 1 to 3 scale, based on a previously published scoring system, where grade 1 indicates cell bodies without dendrites, grade 2 indicates cells with short dendrites and grade 3 indicates cells with long dendrites⁴; images devoid of CEDC were not considered in the morphology analysis (Figure 1). If more than one grade was present in an image, the predominant morphology was considered for grading.

Mann-Whitney U test, Wilcoxon signed rank test, and Fisher's Exact test were used to compare clinical signs and symptoms, CEDC density and morphology between groups and between corneal locations. Spearman correlation and Chi-Square (Cramer's V) tests were used to assess the associations between variables, and between corneal locations; partial correlation was used to control for the effect of SPT status. Statistical significance was set at $p \leq 0.05$.

Results

Frequency and severity of ocular allergy symptoms, bulbar conjunctival hyperaemia and chemosis, lid roughness and serum IgE concentration were significantly higher in allergic (SPT+) participants than in the non-allergic (SPT-) group, but there was no difference in lid oedema (Table1).

CEDC were observed in 80% of allergic participants and 90% of non-allergic participants in the central cornea. In the mid-peripheral area, CEDC were observed in 78% of allergic and 100% of non-allergic participants. CEDC density in the central cornea was not significantly different between allergic and non-allergic participants, while in the mid-peripheral corneal area it was significantly lower in the allergic group than the non-allergic group ($p=0.003$) (Table 1, Figure 2). There was no significant difference between central and mid-peripheral CEDC density in allergic ($p=0.11$) and non-allergic group ($p=0.46$). The median ratio of central:mid-peripheral CEDC in the allergic group was 1.37 and this was not significantly different to the ratio of 1.15 in the non-allergic group ($p=0.56$). There was a moderate association between central and mid-peripheral CEDC density which was significant in both allergic ($\rho=0.65$, $p=0.001$) and non-allergic participants ($\rho=0.69$, $p=0.001$). CEDC density at either corneal location was not associated with frequency or severity of ocular allergy symptoms, clinical signs or serum IgE concentration; with no effect of SPT status on these relationships.

CEDC morphology grade was observed to be significantly higher in allergic than non-allergic participants at the central cornea ($p=0.02$), but not in the mid-periphery (Table 1, Figure 3). Grade 3 cells (longest dendrites) were observed only in the allergic group, at both corneal locations. In the non-allergic group, CEDC were observed to have a lower morphology grade in the central cornea than in the mid-periphery ($p=0.01$), but there was no such difference in the allergic group ($p=0.83$). CEDC morphology was not associated with CEDC density nor with any of the clinical indicators and this was not affected by SPT status.

Discussion

The results of this study showed systemic sensitization has an unexpected effect on CEDC: reduced density in the central and mid-peripheral cornea and longer cell dendrites. To our knowledge, this is the first study to examine CEDC in mild forms of ocular allergy and to compare CEDC density and morphology in the central and mid-peripheral cornea of allergic and non-allergic participants. Previous

studies showed an increased density of CEDC at central, far peripheral and limbal cornea in severe forms of active chronic ocular allergy.^{6,7} It was therefore hypothesized that CEDC density would likewise be affected in common allergic conditions. The central and mid-peripheral CEDC density findings from this study sit within the range of values described for normal corneas in a recent meta-analysis of central CEDC density.¹³ The distribution of CEDC do not show a marked density gradient, but rather a slight reduction in density from the central to mid-peripheral cornea, in agreement with our earlier study in normal corneas.¹⁴ Reduced CEDC in allergic participants is an unexpected finding and in contrast with the reports in severe allergy.^{6,7} It is possible that the different findings in our study reflect the milder allergic manifestation in this participant group, as is demonstrated by the ocular signs and symptoms results above (Table 1), or that allergy may not have been in the active phase in these participants. Further investigation of participants with common forms of allergy during the active phase of ocular allergy may help elucidate these findings. Alternatively, although participants were screened for use of immunosuppressants in the week before to the study, participants may have had prior exposure to systemic immunosuppressive treatment which has been shown to result in lower CEDC density.¹⁵

A key finding is the presence of CEDC with a higher-grade morphology in the central and mid-peripheral cornea of the allergic group. This is in agreement with previous studies which qualitatively described corneal dendritic cell morphology in patients with severe vernal keratoconjunctivitis.^{6,7} Higher-grade cells may represent a more mature phenotype and indicate the activation of inflammation and immunity in the ocular surface of allergic participants.^{16,17} In the non-allergic participants, CEDC with long dendrites were not observed at either corneal location. An earlier study of CEDC in the normal human cornea found the corneal centre to be devoid of cells with long dendrites and observed the more mature morphology in the peripheral cornea only.¹⁸ These results are consistent with *ex vivo* studies in animals.¹⁹

CEDC density and morphology were not associated with ocular allergy symptoms or signs, nor with serum IgE in this study. These associations have not previously been investigated in mild or severe ocular allergy. These relationships require further exploration. Based on these, we speculate that

CEDC changes may be a more sensitive marker of ocular allergy-induced inflammation than clinically evident signs of symptoms.

These findings suggest that systemic allergic sensitization may affect activation of dendritic cells in the cornea, but that the recruitment of dendritic cells into the corneal centre may not occur, in contrast to severe forms of ocular allergy. The results highlight the need for further research to reach a definitive conclusion for the role of CEDC in allergic conditions.

Declaration of Interest:

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Figure 1: *In vivo* confocal microscopic images for the CEDC morphology grading scale, showing examples of (A) cell bodies without dendrites, grade 1; (B) cell with short dendrites, grade 2; (C) cells with long dendrites, grade 3.

Figure 2: Corneal epithelial dendritic cell (CEDC) density in the central and mid-peripheral cornea in allergic (n=31) and non-allergic (n=19) participants. The number of CEDC in the mid-peripheral cornea was significantly lower in the allergic (SPT+) group.

Figure 3: Corneal epithelial dendritic cell (CEDC) morphology in the central and mid-peripheral cornea in allergic (n=31) and non-allergic (n=19) participants. Numbers within bars indicate the number of participants. More cells with long dendrites were found in the allergic (SPT+) group at both corneal regions.

Table 1: Summary of findings for ocular allergy symptom frequency and severity (total of individual symptom scores calculated from the EAPIQ and AUAQ, ocular surface clinical signs, serum IgE concentration, CEDC density and morphology in allergic (SPT+) and non-allergic (SPT-) participants. Values are reported in median (range). Ocular signs were graded in integers except for bulbar conjunctival hyperaemia and lid roughness which were graded in 0.1 steps. Statistically significant values ($p < 0.05$) are indicated in bold/italics. EAPIQ, Eye Allergy Patient Impact Questionnaire; AUAQ, Aston University Allergy Questionnaire; CEDC, corneal epithelial dendritic cells; IgE, immunoglobulin E.

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		Allergic SPT+ (n=31)	Non-allergic SPT- (n=19)	p-value
Ocular allergy symptoms	Frequency score (EAPIQ, 0-25)	4.5 (0-25)	1.5 (0-8.5)	<0.001
	Severity score (AUAQ, 0-21)	7 (0-17)	1 (0-15)	<0.001
Ocular surface signs	Bulbar conjunctival hyperaemia (0-4)	2.2 (1.2-3.8)	1.8 (1.2-2.3)	0.01
	Lid roughness (0-4)	2.4 (1.0-3.0)	1.5 (0-2.9)	<0.001
	Conjunctival chemosis (0-3)	1 (0-2)	0 (0-2)	0.02
	Eye lid oedema (0-3)	0 (0-1)	0 (0-0)	0.26
Serum IgE (ng/ml)		30.5 (0-133.2)	0 (0-87.4)	0.01
CEDC density	Centre	5.0 (0-91.1)	9.0 (0-146.3)	0.11
	Mid-periphery	3.0 (0-93)	12.0 (1.0-54.0)	0.003
	Centre : mid-periphery	1.37	1.15	0.56
CEDC morphology	Centre	2 (1-3)	1 (1-2)	0.02
	Mid-periphery	2 (1-3)	2 (1-2)	0.44

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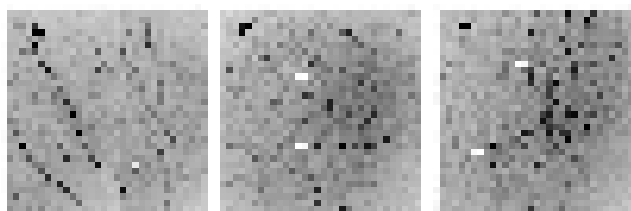


FIG 1

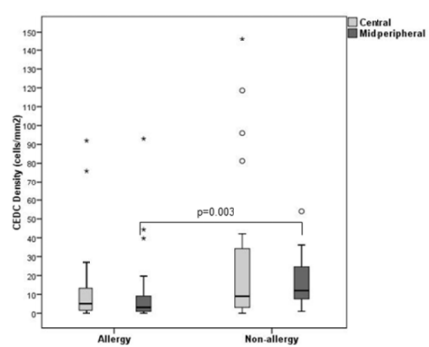


FIG 2

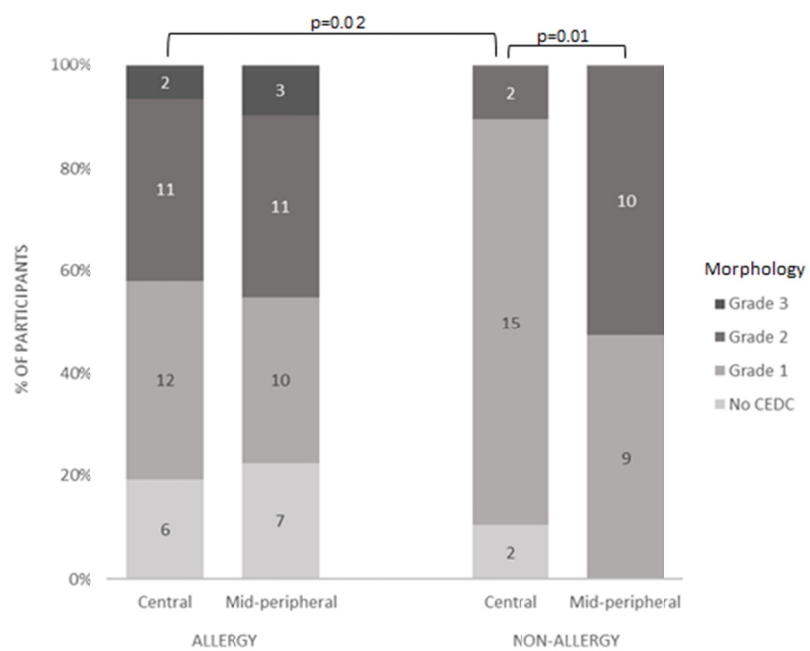


FIG 3