tvst

Cornea & External Disease

Ocular Surface Immune Cell Profiles in Contact Lens–Induced Discomfort

Archana Padmanabhan Nair[1], Swaminathan Sethu[1], Harsha Nagaraj[2], Vijay Kumar[2], Sriharsha Nagaraj[2], Zohra Fadli[3], Charles Scales[3], Mike Chemaly[3], Xiao-Yu Song[3], Arkasubhra Ghosh[1], and Bailin Liang[3]

[1] GROW Research Laboratory, Narayana Nethralaya Foundation, Bangalore, India
[2] Department of Cornea and Refractive Surgery, Narayana Nethralaya, Bangalore, India
[3] Johnson & Johnson Vision, Jacksonville, FL, USA

Correspondence: Arkasubhra Ghosh, GROW Research Laboratory, Narayana Nethralaya Foundation, 3rd Floor, Narayana Nethralaya, #258/A Hosur Road, Bommasandra, Bangalore 560099, India. e-mail: arkasubhra@narayananethralaya.com Bailin Liang, Johnson & Johnson Vision, 7500 Centurion Parkway North, Jacksonville, FL 32256, USA. e-mail: bliang1@its.jnj.com

Received: March 23, 2022
Accepted: June 14, 2022
Published: July 20, 2022

Keywords: contact lens-induced discomfort; immunophenotyping; impression cytology; neutrophils; natural killer cells; T cells; dendritic cells

Citation: Nair AP, Sethu S, Nagaraj H, Kumar V, Nagaraj S, Fadli Z, Scales C, Chemaly M, Song XY, Ghosh A, Liang B. Ocular surface immune cell profiles in contact lens–induced discomfort. Transl Vis Sci Technol. 2022;11(7):16, https://doi.org/10.1167/tvst.11.7.16

Purpose: Contact lens–induced discomfort (CLD) remains a primary factor in discontinuation or prevention of contact lens wear. Thus, we investigated the role of ocular surface immune cells in subjects with CLD.

Methods: Habitual contact lens (CL) wearers with CLD ($n = 19$; 38 eyes) and without CLD ($n = 21$; 42 eyes) as determined by the Contact Lens Dry Eye Questionnaire-8 was included in a trial. Enrolled subjects used either of the two types of CL (designated as CL-A or CL-D). Ocular surface cells from the bulbar conjunctiva were obtained by impression cytology. The collected cells were phenotyped using fluorochrome-conjugated antibodies specific for leukocytes ($CD45^+$), neutrophils ($CD66b^{+,High,Low}$), macrophages ($CD163^+$), T cells ($CD3^+CD4^+$, $CD3^+CD8^+$), natural killer (NK) cells ($CD56^{+,\,High,\,Low}$), natural killer T (NKT) cells ($CD3^+CD56^+$), and gamma delta T ($\gamma\delta$T) cells ($CD3^+\gamma\delta TCR^+$) by flow cytometry. Further, corneal dendritic cell density (cDCD) was also determined using in vivo confocal microscopy.

Results: Significantly higher proportions of $CD45^+$ cells were observed in subjects with CLD compared to those without CLD. The percentages of $CD66b^{Total,Low}$, $CD163^+$, pan T cells, $CD4^+$T cells, $CD8^+$T cells, $CD56^{Total,High,Low}$ (NK) cells, and NKT cells, as well as the CD4/CD8 ratio, were significantly higher in CLD subjects. The proportion of T cells (CD4, CD8, CD4/CD8 ratio, NKT cells) and macrophages exhibited a direct association with discomfort score. The percentages of $CD45^+$, $CD66b^{Total,Low}$, $CD163^+$, $CD3^+$, $CD56^{Total,High,Low}$, and NKT cells and cDCD were significantly higher in CLD subjects wearing CL-D. The percentages of $CD66b^{High}$, $CD4^+$T cells, $CD8^+$T cells, NKT cells, and CD4/CD8 ratio were significantly higher in CLD subjects wearing CL-A.

Conclusions: Increased proportions of ocular surface immune cells are observed in CLD, and the lens type could impact the immune cells associated with CLD.

Translational Relevance: The association between the proportion of altered ocular surface immune cell subsets and contact lens–induced discomfort underpins the importance of considering immune-related aspects during contact lens development and in the clinical management of ocular surface pain.

Introduction

Over 140 million people use contact lenses (CLs) worldwide,[1] and CLs have improved the quality of vision and life among their users. However, 12% to 51% of CL users have discontinued their use,[2–5] primarily due to contact lens–induced discomfort (CLD).[2,5] Decades of CL research and development have resulted in significant improvements in materials and optical and mechanical designs, as well as multipurpose solutions to enhance comfort and safety for CL wearers. Despite the progress, CLD remains the foremost reason why consumers abandon use of

their CLs,[2,5] highlighting the need to further elucidate the mechanisms underpinning CLD. Regardless of the subjectivity and psychological aspects associated with reporting CLD, the spectrum of causal factors that do contribute to CLD includes the physical and chemical characteristics of CL material, the CL ocular surface/tear film interface, and CL-induced molecular alteration on the ocular surface that reduces threshold of nociception.[6–8]

The influence of polymer chemistry and various other material attributes, including water content, hydration, ionicity, oxygen transmissibility, modulus, and mechanical factors such as coefficient of friction, wettability, and surface modification, have been investigated intensively for their contributions to CLD.[7] None of these attributes, with the one exception of coefficient of friction, has appeared to be associated directly with CLD. Because CLD affects only a subset of CL users, it would be beneficial to determine the ocular surface profiles of molecular and cellular components with the ability to influence nociception at the ocular surface in these subjects. Inflammatory factors are known to mediate pain by reducing the threshold to nociceptive stimuli via sensitization of polymodal nociceptors, thermoreceptors, and mechanonociceptors and by altering the expression or conformation of ion channels.[9,10] Levels of inflammatory mediators at the ocular surface are altered following CL use, including those reporting CLD.[11] Hence, tear film dynamics and lipid layer disruption due to CL use contribute to changes in ocular surface inflammatory factors and discomfort.[6,8,9] The magnitude of the changes in these factors following CL use could vary among subjects depending on the presence of ocular surface comorbidities, subject-specific responses to CLs, and possibly the type of CL used, thus predisposing some to CLD.

The ocular surface structural cells could also contribute to the levels of inflammatory factors in response to various stimuli and immune response.[12,13] Changes in the proportions of immune cell subsets and their activity on the ocular surface would result in compromise of corneal epithelial barrier function and disruption in ocular surface homeostasis, resulting in discomfort and vision disturbance.[14–17] A variety of immune cells are known to be present in the corneal and ocular surface mucosa to render protection and maintain homeostasis. Neutrophils, natural killer (NK) cells, macrophages, dendritic cells (DCs), and T-cell subsets are some of the innate and adaptive immune cells present on the ocular surface that have been implicated in ocular surface conditions such as dry eye disease (DED) and keratoconus.[16,18–21] However, the proportion of these cells and that of other immune cell subsets on the ocular surface of subjects with CLD are yet to be investigated. Further, the knowledge regarding the orchestrated interaction between immune cells and structural cells of tissues in various tissues and organs[22] suggests the need to study the status of immune profiles on the ocular surface in the context of CLD. Flow cytometry–based phenotyping of cells obtained from the impression cytology procedure is one of the most commonly used methods for assessing the status of inflammatory and immune status of the ocular surface in human subjects.[23–26] Hence, in the current study, we investigated the proportions of total leukocytes, neutrophils, macrophages, DCs, NK cells, pan-T cells, CD4 T cells, CD 8 T cells, and gamma delta T ($\gamma\delta$T) cells on the ocular surface of subjects with and without CLD and on two different types of CLs in a non-invasive manner using conjunctival impression cytology samples.

Methods

Study Cohort and Design

This study was a non-masked, non-randomized, non-interventional, stratified, four-arm parallel group, multi-visit clinical trial (CTRI/2017/12/010950). The study was approved by the respective institutional ethics committees of Narayana Nethralaya, Bangalore (EC ref. no. C/2017/12/03) and Sankara Nethralaya, Chennai. Subjects were enrolled into one of four arms as shown in Table 1 based on their habitual CL type, the difference between comfortable wearing time (CWT) and average wearing time (AWT), and their Contact Lens Dry Eye Questionnaire-8 (CLDEQ-8) scores.

Inclusion and Exclusion Criteria

Inclusion Criteria

1. The subject must read, understand, and sign the statement of informed consent and receive a fully executed copy of the form.
2. The subject must appear able and willing to adhere to the instructions set forth in this clinical protocol.
3. The subject must appear able and willing to adhere to a no-CL-wear period of at least a month.
4. The subject must be between 18 and 45 (inclusive) years of age at the time of screening.
5. The subject must currently be wearing one of the two habitual contact lenses in both eyes for at least 3 months. The habitual lens types included in the study were CL-A (1-DAY ACUVUE MOIST; Johnson & Johnson

Table 1. Study Cohort Characteristics

	1	2	3	4
Habitual contact lens	1-DAY ACUVUE MOIST (CL-A)	1-DAY ACUVUE MOIST (CL-A)	DAILIES AquaComfort Plus (CL-D)	DAILIES AquaComfort Plus (CL-D)
Discomfort status	Asymptomatic	Symptomatic	Asymptomatic	Symptomatic
Difference between CWT and AWT (h)	<1	≥3	<1	≥3
CLDEQ-8 score at baseline	≤7	≥15	≤7	≥15
Sample size (subject; eyes), *n*	10; 20	9; 18	11; 22	10; 20
Male; female, *n*	7; 3	1; 9	3; 9	4; 6
Age (y), mean ± SD	25.0 ± 5.1	24.8 ± 5.4	22.8 ± 2.4	26.1 ± 4.6

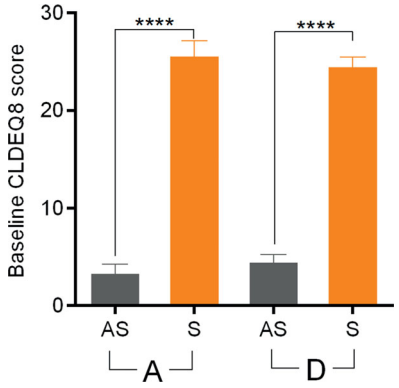

Figure 1. CLDEQ-8 scores for CLD subjects wearing the different lens types. The graph indicates the CLDEQ-8 scores for subjects without CLD (As) and with CLD (S). A, CL-A users; D, CL-D users. CL-A As (20 eyes, $n = 10$), CL-A S (18 eyes, $n = 9$), CL-D As (22 eyes, $n = 11$), and CL-D S (20 eyes, $n = 10$). The *bar graph* represents mean ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (Mann–Whitney test).

Vision, Jacksonville, FL) and CL-D (DAILIES AquaComfort Plus; Alcon, Geneva, Switzerland). Habitual CL wear was defined as having a minimum wearing time of 3 hours per day, minimum of 3 days per week for at least 3 months.

6. The subject must have visual acuity of at least 20/40 or better in each eye with their habitual lenses.
7. Subjects must meet one of the following criteria in order to be classified as either asymptomatic or symptomatic group: for the asymptomatic group, CLDEQ-8 score of ≤7 and difference between CWT and AWT of <1 h/day; for the symptomatic group, CLDEQ-8 score of ≥15 and difference between CWT and AWT of >3 h/day. The baseline CLDEQ-8 scores of the four study groups at the time of recruitment are shown in Figure 1.
8. The subject must have healthy eyes with no evidence of abnormality or disease (i.e., no active ocular pathological conditions or infections of any type).
9. The subject must have a pair of spectacles that provide corrected binocular visual acuity of 20/40 or better. If they have no spectacles, subjects must have unaided binocular visual acuity of 20/40 or better.

Exclusion Criteria

1. The subject is currently pregnant or lactating.
2. The subject has any diseases (e.g., Sjögren's syndrome), allergies, infectious disease (e.g., hepatitis, tuberculosis), contagious immunosuppressive diseases (e.g., HIV), autoimmune disease (e.g., rheumatoid arthritis), diabetes, or other diseases, by self-report that are known to interfere with contact lens wear and/or participation in the study.
3. The subject has unacceptable lens fit with their habitual lenses in either eye based on a study investigator's judgement.
4. The subject has any extended wear modality.
5. The subject has used medications, including topical eye drops, for the management of ocular surface discomfort or any other condition in the last 3 months.

Study Design

Following enrollment into the study, the subjects were categorized into one of the two categories (CL-A or CL-D). Following two weeks of CL wear, the wearer's discomfort was recorded based on a visual analog scale (VAS) that is frequently used to measure temporal characteristics of contact lens discomfort and is useful for assessing qualities such as duration, onset, and chronicity. Thereafter, impression cytology samples were collected from each study eye as described below to phenotype the immune cells on the ocular surface.

Impression Cytology

Ocular surface cells were collected non-invasively by impression cytology using an impression cytology device (EYEPRIM; Opia Technologies, Paris, France) at the sixth visit for each subject as per the manufacturer's instructions. The sampling area of impression cytology was central bulbar conjunctiva inferior and proximal to the cornea. After the impression cytology sampling, the membrane from the device with the cellular material was ejected and collected in a 1.5-mL microcentrifuge tube. Two-thirds of the membrane of the impression cytology device was cut and immediately immersed in pre-chilled phosphate-buffered saline (PBS, pH 7.4) with fixative (0.05% paraformaldehyde) in 1.5-mL microcentrifuge tubes and stored at 4°C until further processing.

Immunophenotyping by Flow Cytometry

The cells bound to the membrane of the impression cytology device were detached prior to staining with the relevant cell-type–specific fluorochrome-conjugated antibodies. Briefly, the cells from the membrane were detached by gentle agitation (500 rpm) at 4°C using a ThermoMixer (Eppendorf, Hamburg, Germany) in PBS with fixative for 30 minutes; the membrane was removed and centrifuged (1600 rpm for 5 minutes). The pellet was resuspended in 50 µL of ice-cold staining buffer (5% fetal bovine serum [FBS] in 1× PBS, pH 7.4), and the cell surface molecule staining and analysis protocol were performed as follows. The fluorochrome-conjugated antibodies used to phenotype immune cells included leukocytes (CD45; allophycocyanin [APC]-H7, clone 2D1; BD Pharmingen, San Diego, CA); neutrophils (CD66b; AF647, clone G10F5; BD Pharmingen); macrophages (CD163; fluorescein isothiocyanate [FITC], clone GHI/61; BD Pharmingen); T cells (CD3; phycoerythrin [PE], clone HIT3a; BD Pharmingen); $\gamma\delta$T cells ($\gamma\delta$ T-cell receptor [$\gamma\delta$TCR]; peridinin chlorophyll protein [PerCP]-Cy5.5, clone B1; BD Pharmingen); and NK cells (CD56; PeCy7, clone B159; BD Pharmingen). CD4 and CD8 T cells were phenotyped using BD Multitest (BD Pharmingen), a four-color direct immunofluorescence panel (FITC, PE, PerCP, and APC) for CD3, CD8, CD45, and CD4. Staining of cell surface markers was done using fluorochrome-conjugated antibody cocktail diluted in staining buffer (50 µL) and incubated for 45 minutes at room temperature. The cells were washed twice and then resuspended in 400 µL 1× PBS (pH 7.4) for data acquisition. Data acquisition was performed on a BD FACS-Canto II flow cytometer using BD FACSDiva software (BD Bioscience), and post-acquisition analysis was done using FCS Express 6 Flow Research Edition. The analysis included a compensation protocol to adjust for the spectral overlap between the multiple fluorochromes used for the different cell surface molecules. The compensation matrix was calculated from single stained controls. Universal negative and fluorescence minus one control were used to determine gating regions. A gating strategy was employed for immune cell subset identification after acquisition, where the cells were first defined based on side scatter (SSC) and CD45-positive staining followed by identification of cell subsets based on positive staining, as shown in Supplementary Figures S1 and S2.

Corneal Dendritic Cell Density Assessment by In Vivo Confocal Microscopy

Corneal dendritic cell density (cDCD) in the study subjects was measured by in vivo confocal microscopy (IVCM) imaging using a Rostock Corneal Module/Heidelberg Retina Tomograph II (RCM/HRTII; Heidelberg Engineering, Heidelberg, Germany) as described previously.[27] All subjects underwent a 2-month no-CL period to wash off the

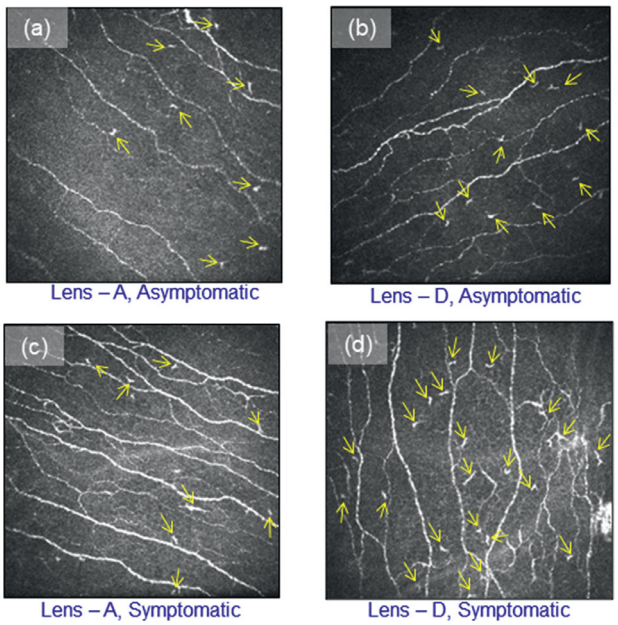

Figure 2. Dendritic cells at the level of subbasal nerve plexus in the cornea. The representative image for each of the study categories shows dendritic cells (indicated by *yellow arrows*) at the subbasal nerve plexus region. Panels shown are representative IVCM images with frame size 400 × 400 µm. (a) Representative image from CL-A user without CLD (asymptomatic). (b) Representative image from CL-D user without CLD (asymptomatic). (c) Representative image from CL-A user with CLD (symptomatic). (d) Representative image from CL-D user with CLD (symptomatic).

effects of CL wear. Both eyes were included for IVCM-based investigations in the subjects. Prior to the procedure, 0.5% proparacaine drops were used to anesthetize the cornea. The cDCD (cells/mm^2) and dendritiform structures were quantified using Cell Count software (Heidelberg Engineering).[27,28] Figure 2 shows representative IVCM images from the study cohort with corneal dendritic cells.

Statistical Analysis

The distribution status of the dataset was determined by the Shapiro–Wilk normality test. Statistical analyses including the Mann–Whitney test and Spearman's rank correlation were performed with either Prism 6.0 (GraphPad Software, San Diego, CA) or MedCalc 12.5 (MedCalc Software, Ostend, Belgium). $P < 0.05$ was considered to be statistically significant.

Results

Ocular Surface Immune Cell Subset Profile in CLD

A significant increase in discomfort rating was recorded in symptomatic subjects compared to asymptomatic subjects using either type of CL (Fig. 3). No significant difference was observed between symptomatic subjects using the different types of CL (Fig. 3). Leukocytes and all of the immune cell subsets intended to be phenotyped were identified in the impression cytology samples. A significantly higher percentage of CD45$^+$ (pan leukocyte marker) cells was observed in the cell population obtained by impression cytology in subjects with CLD compared to those without CLD (Fig. 4a). The percentages of CD45$^+$CD66b^{Total} (neutrophil) and CD45$^+$CD66b^{Low} (inactive neutrophil) cells were observed to be significantly higher in subjects with CLD, but no difference was noted in the percentage of CD45$^+$CD66b^{High} (activated neutrophil) cells between subjects with and without CLD (Fig. 4b). The proportion of NK cell subtypes (CD45$^+$CD56^{Total}, CD45$^+$CD56^{Low}, CD45$^+$CD56^{High}) and CD45$^+$CD163$^+$ (macrophages) was significantly greater in CLD subjects as shown in Figures 4c and 4d. The proportion of CD45$^+$CD3$^+$ (pan T cell marker) cells, CD45$^+$CD3$^+$CD4$^+$ (T helper) cells, CD45$^+$CD3$^+$CD8$^+$ (cytotoxic T) cells, and CD45$^+$CD3$^+$CD56$^+$ (NKT) cells, as well as the CD4$^+$/CD8$^+$ ratio, were significantly higher in CLD subjects (Figs. 4e–4h, 4j). No difference was observed in the proportion of CD45$^+$CD3$^+\gamma\delta$TCR$^+$ ($\gamma\delta$T) cells between subjects with and without CLD (Fig. 4i). cDCD showed no difference between subject groups with and without CLD (Fig. 4k). The proportion of macrophages, pan T cells, CD4 T cells, CD8 T cells, and NKT cells and the CD4/CD8 ratio exhibited a direct association with the discomfort score (Table 2). These findings indicate the presence of altered ocular surface immune cell subsets in subjects suffering from CLD.

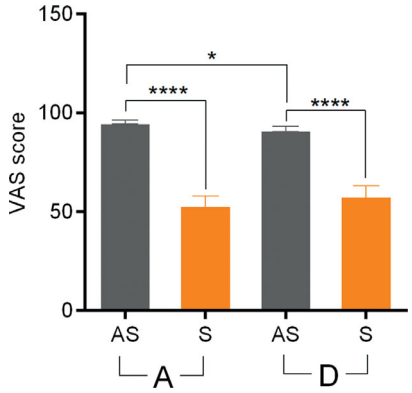

Figure 3. VAS score in CLD subjects wearing the different lens types after 2 weeks of CL wear. The graph indicates the VAS scores in subjects without CLD (As) and with CLD (S). A, CL-A users; D, CL-D users. CL-A As (20 eyes, $n = 10$), CL-A S (18 eyes, $n = 9$), CL-D As (22 eyes, $n = 11$), and CL-D S (20 eyes, $n = 10$). The *bar graph* represents mean ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (Mann–Whitney test).

Effect of Lens Type on the Profile of Ocular Surface Immune Cell Subsets in CLD

Lens type–specific differences in the ocular surface immune cell subsets in subjects with and without CLD are shown in Figures 5 to 8. A significantly higher proportion of CD45$^+$ cells was observed in CLD subjects wearing CL-D compared with those wearing CL-A (Fig. 5a). Similar observations were made regarding the proportions of neutrophils, inactive form of neutrophils, and macrophages (Figs. 5b, 5c, 5e). In contrast, the percentage of activated neutrophils was significantly higher in CLD subjects wearing CL-A compared with those wearing CL-D (Fig. 5d). In addition, the proportion of inactive/quiescent neutrophils was significantly lower in non-CLD subjects wearing CL-D compared with those wearing CL-A (Fig. 5c).

NK cells and their subtypes (based on CD56 expression, CD56^{Low} and CD56^{High}) were also significantly higher in CLD subjects wearing CL-D compared with those wearing CL-A (Figs. 6a–6c). Similar to what

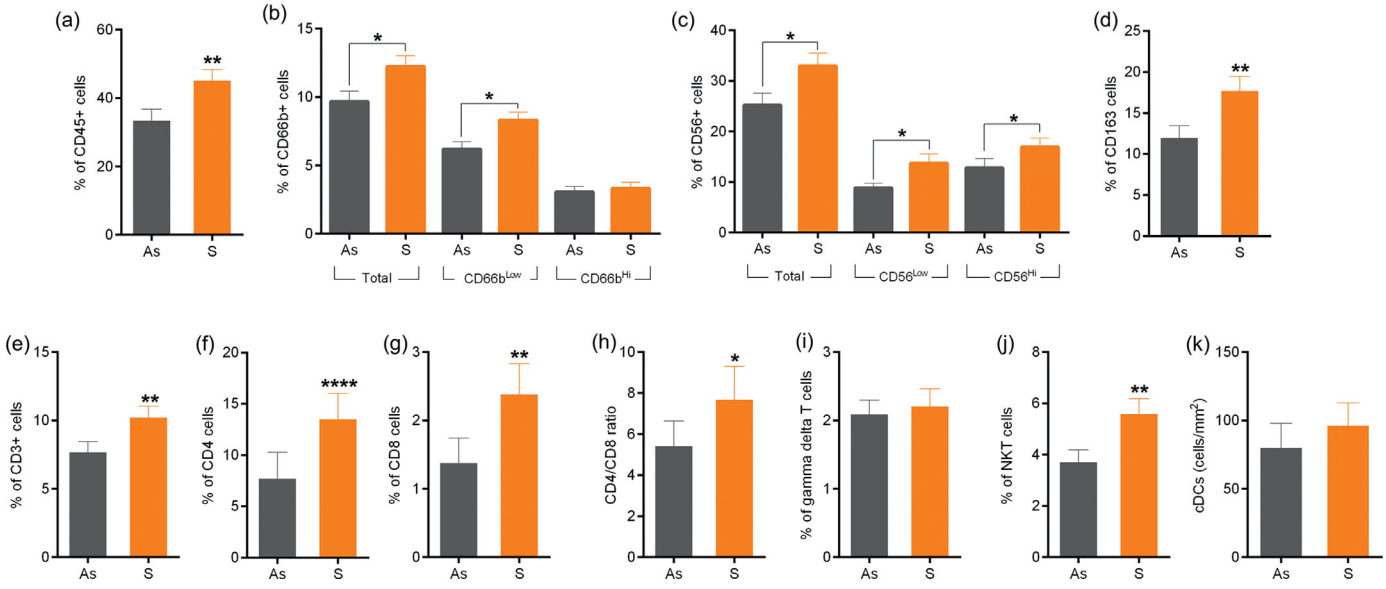

Figure 4. Profiles of ocular surface immune cell subsets in CLD. (a) The graph indicates the percentage of $CD45^+$ cells (pan leukocyte marker) in the impression cytology samples from subjects without CLD (As, asymptomatic) and with CLD (S, symptomatic) as determined by flow cytometry. (b) The graph indicates the percentage of $CD45^+CD66b^{Total}$ (neutrophils), $CD45^+CD66b^{Low}$ (inactive neutrophils), and $CD45^+CD66b^{High}$ (activated neutrophils). (c) The graph indicates the percentages of NK cell subtypes ($CD45^+CD56^{Total}$, $CD45^+CD56^{Low}$, $CD45^+CD56^{High}$). Other graphs indicate (d) $CD45^+CD163^+$ cells (macrophages), (e) $CD45^+CD3^+$ cells (pan T cells), (f) $CD45^+CD3^+CD4^+$ cells (T helper cells), (g) $CD45^+CD3^+CD8^+$ cells (cytotoxic T cells), (h) $CD4^+/CD8^+$ ratio, (i) $CD45^+CD3^+\gamma\delta TCR^+$ cells ($\gamma\delta$T cells), and (j) $CD45^+CD3^+CD56^+$ cells (NKT cells). (k) The graph indicates the average cDCD (cells/mm^2) as determined by IVCM in subjects without CLD (As) and with CLD (S). (a–j) As (42 eyes, $n = 21$); S (38 eyes, $n = 19$). (k) As (14 eyes, $n = 7$); S (12 eyes, $n = 6$). The *bar graph* represents mean ± SEM. *$P < 0.05$, **$P < 0.01$, ****$P < 0.0001$ (Mann–Whitney test).

Table 2. Association Between Discomfort Scores and Ocular Surface Immune Cell Subset Proportions in the Study Subjects

	VAS Score	
	R	*P*
Pan leukocytes		
$CD45^+$ cells	−0.161	0.159
Neutrophils		
$CD66b^{Total}$ cells	−0.201	0.077
$CD66b^{Low}$ cells	−0.119	0.301
$CD66b^{High}$ cells	−0.110	0.340
Macrophages		
$CD163^+$ cells	−0.225	0.048
NK cells		
$CD56^{Total}$ cells	−0.116	0.313
$CD56^{High}$ cells	−0.148	0.195
$CD56^{Low}$ cells	−0.048	0.679
T-cell subsets		
Pan $CD3^+$ T cells	−0.25	0.028
$CD4^+$ T cells	−0.541	<0.0001
$CD8^+$ T cells	−0.418	0.0002
CD4/CD8 ratio	−0.262	0.024
$\gamma\delta$T cells	0.066	0.568
NKT cells	−0.257	0.023

R, Spearman's rank correlation coefficient.

was observed with inactive/quiescent neutrophils, the $CD56^{Total}$ and $CD56^{Low}$ NK cells were significantly lower in non-CLD subjects wearing CL-D compared with those wearing CL-A (Figs. 6a, 6b). NKT cell numbers was significantly higher in the CLD subjects irrespective of the type of lens used (Fig. 6d). The number of $\gamma\delta$T cells, on the other hand, was not observed to be altered based on CLD status or the type of lens worn (Fig. 6e).

Overall, the number of T cells was higher in the CLD subjects; the increase was significant in CL-D users and nearly significant in CL-A users (Fig. 7a). The number of CD4 and CD8 T cells was significantly greater in CL-A users with CLD compared with those wearing CL-D with CLD (Figs. 7b, 7c). The CD4/CD8 ratio was higher in subjects with CLD in both CL-A and CL-D users (Fig. 7d). It is interesting to note that the number of CD4 and CD8 T cells was significantly lower in non-CLD subjects wearing CL-A compared with those wearing CL-D (Figs. 7b, 7c). Significantly higher cDCD was observed in CL-D users with CLD compared with CL-A users with CLD (Fig. 8). cDCD was not observed to differ between CL-A or CL-D users without CLD (Fig. 8). The observations indicate that CLD and lens type are associated with an altered proportion of immune cell subsets on the ocular surface.

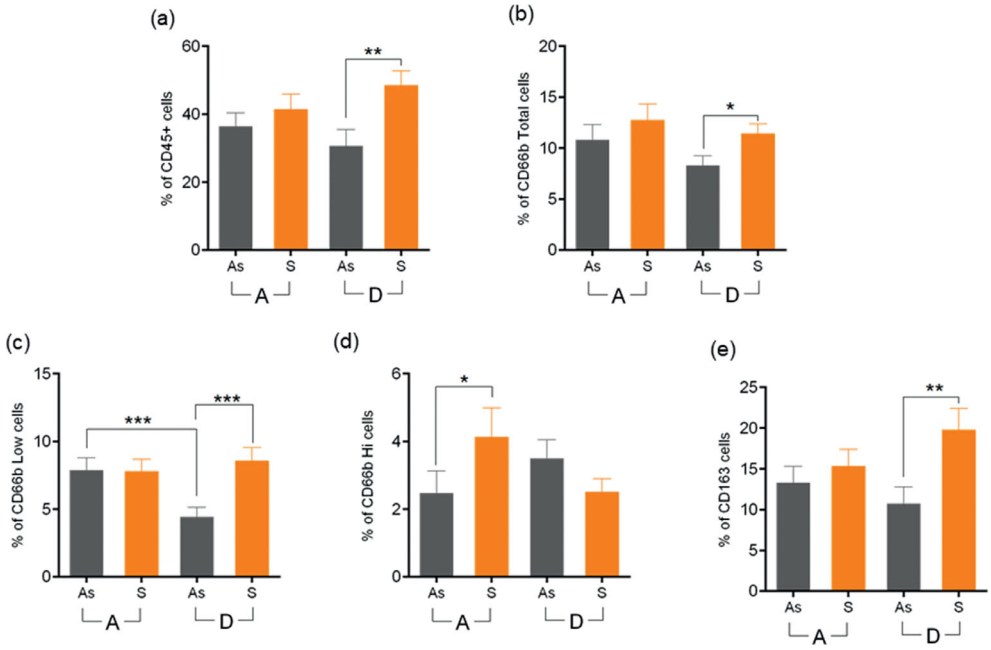

Figure 5. Neutrophil and macrophage profiles for CLD subjects wearing the different lens types. The graphs indicate the percentage of (a) $CD45^+$ (pan leukocyte marker); (b) $CD45^+CD66b^{Total}$ (neutrophils); (c) $CD45^+CD66b^{Low}$ (inactive neutrophils); (d) $CD45^+CD66b^{High}$ (activated neutrophils); and (e) $CD45^+CD163^+$ (macrophages) in the impression cytology samples from subjects without CLD (As) and with CLD (S) as determined by flow cytometry. A, CL-A users; D, CL-D users. CL-A As (20 eyes, $n = 10$), CL-A S (18 eyes, $n = 9$), CL-D As (22 eyes, $n = 11$), CL-D S (20 eyes, $n = 10$). The *bar graph* represents mean ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (Mann–Whitney test).

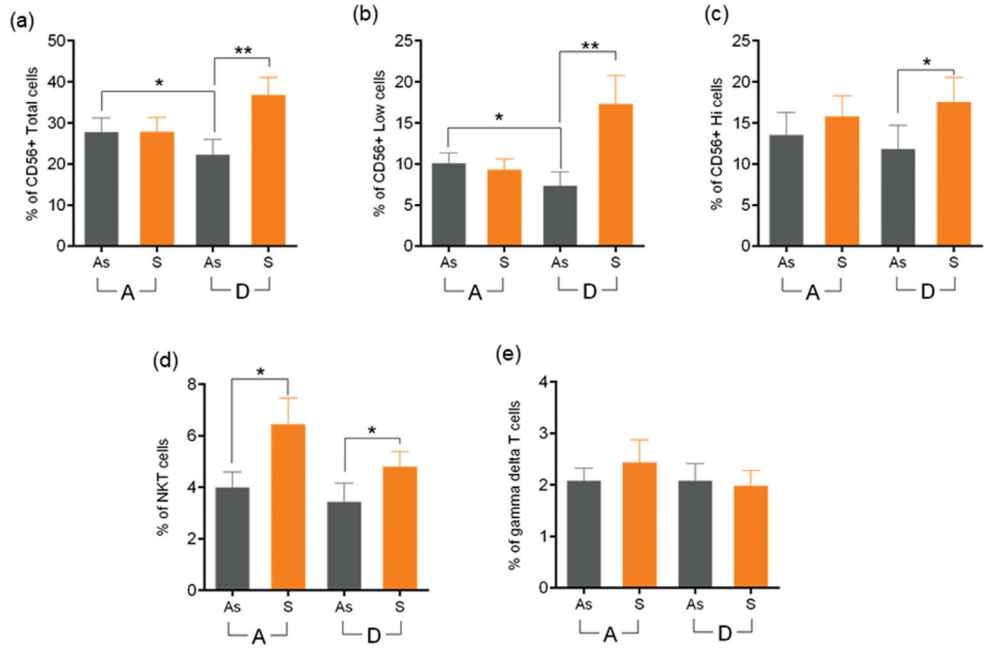

Figure 6. NK cells subsets and $\gamma\delta$T cell profiles for CLD subjects wearing the different lens types. The graph indicates the percentage of (a) $CD45^+CD56^{Total}$ (total NK) cells; (b) $CD45^+CD56^{Low}$ (cytotoxic NK) cells; (c) $CD45^+CD56^{High}$ (cytokine-producing low cytotoxic NK) cells; (d) $CD45^+CD3^+CD56^+$ (NKT) cells; and (e) $CD45^+CD3^+\gamma\delta TCR^+$ cells in the impression cytology samples from subjects without CLD (As) and with CLD (S) as determined by flow cytometry. A, CL-A users; D, CL-D users. CL-A As (20 eyes, $n = 10$), CL-A S (18 eyes, $n = 9$), CL-D As (22 eyes, $n = 11$), CL-D S (20 eyes, $n = 10$). The *bar graph* represents mean ± SEM. *$P < 0.05$, **$P < 0.01$ (Mann–Whitney test).

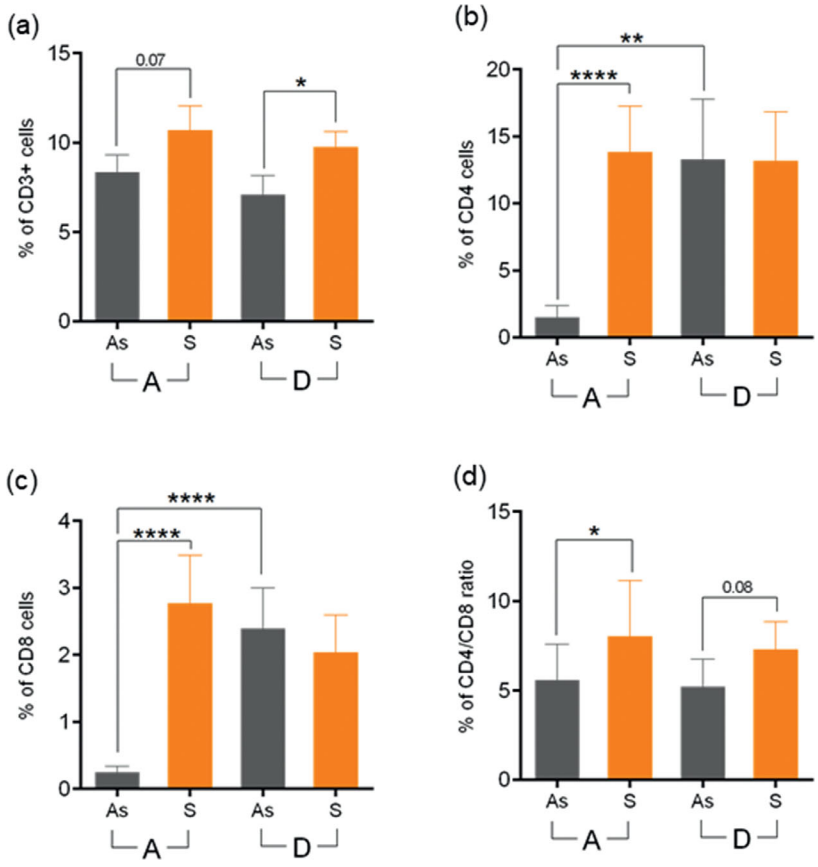

Figure 7. CD4 and CD8 T-cell profiles for CLD subjects wearing the different lens types. The graph indicates the percentage of (a) $CD45^+CD3^+$ (pan T cell marker) cells; (b), $CD45^+CD3^+CD4^+$ (CD4 T helper) cells; (c) $CD45^+CD3^+CD8^+$ (CD8 cytotoxic T) cells; and (d) $CD4^+/CD8^+$ T-cell ratio in the impression cytology samples from subjects without CLD (As) and with CLD (S) as determined by flow cytometry. A, CL-A users; D, CL-D users. CL-A As (20 eyes, $n = 10$), CL-A S (18 eyes, $n = 9$), CL-D As (22 eyes, $n = 11$), CL-D S (20 eyes, $n = 10$). The *bar graph* represents mean ± SEM. *$P < 0.05$, **$P < 0.01$, ****$P < 0.0001$ (Mann–Whitney test).

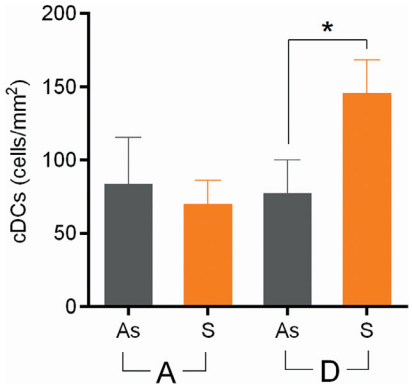

Figure 8. cDCD changes in CLD. The graph indicates the average cDCD (cells/mm^2) in subjects without CLD (As) and with CLD (S). A, CL-A users; D, CL-D users. CL-A As (4 eyes, $n = 2$), CL-A S (8 eyes, $n = 4$), CL-D As (10 eyes, $n = 5$), CL-D S (4 eyes, $n = 2$). The *bar graph* represents mean ± SEM. *$P < 0.05$ (Mann–Whitney test).

Discussion

Inflammatory factors, including immune cells and their secreted mediators, have been found to be associated with an aberrant nociceptive response.[29–31] Modulation of these factors is being explored in the management of pain.[32–34] DED is one of the most important and common ocular surface conditions other than CLD that is associated with discomfort or pain with an aberrant inflammatory and nociceptive component.[27,35–37] The demonstration of a positive correlation between the proportion of ocular surface immune cells and their specific secreted factors in the tear fluid suggests a contribution of immune cells to the ocular surface inflammatory milieu.[19] Recent studies have described the status of ocular surface immune cell subset proportions in DED patients.[18,20,21,27,38,39] Specifically, a direct association between ocular surface discomfort and the proportion of immune cells such

as corneal dendritic cells, total leukocytes, neutrophils, and CD4 T cells has been reported.[18,27,28] An inverse relationship between ocular surface discomfort and the proportion of NK cells was also observed.[18] It is important to note that patients with keratoconus do not commonly complain about ocular surface discomfort, but the proportion of neutrophils and T cells has not been found to be elevated.[19] Hence, ocular surface discomfort may be associated with a distinct immune profile.

Our data demonstrate that the CLD-associated immune cell profile was largely similar to that for patients with DED.[18] However, the proportions of NK and NKT cells were observed to be higher in the CLD patients compared with controls, in contrast to those of patients with DED.[18] The levels of NK cells in the peripheral blood were significantly higher in endometriosis patients with pelvic pain compared with women with stable disease without pelvic pain.[40] In both physiological and pathological conditions, peripheral nociceptive (including pruriceptive) neurons may express a variety of immune-related receptors, such as chemokine receptors and immunoglobulin (Fc) receptors that are usually found on immune cells.[41] Certain ligands such as chemokines and immune complexes may induce abnormal neuronal hyperexcitability and even the discharge of ectopic action potentials, thus producing the sensation of pain, discomfort, or itch in immune-related disease.[41]

Various neuroimmune interactions studied at peripheral, sensory, and central nervous system levels have suggested that innate immunity plays a critical role in central sensitization and in establishing acute pain as a chronic condition.[42] The interactions between the peripheral nervous system and immune system primarily involve pain and discomfort sensitizations.[10] In response to stimuli, nociceptors release various mediators from their terminals that potently activate and recruit immune cells. Infiltrated immune cells further promote the sensitization of nociceptors and the transition from acute to chronic pain by producing cytokines, chemokines, lipid mediators, and growth factors.[43] Such interactions may be the cause of the immune cell alterations we observed in subjects with CLD.

It is interesting to note that the type of CL worn contributed to the immune cell profiles observed in symptomatic wearers. CLD subjects using CL-D lenses demonstrated higher proportions of total leukocytes, total neutrophils, macrophages, NK cell subsets, and cDCs. CLD subjects wearing CL-A lenses demonstrated higher proportions of activated neutrophils, CD4 T cells, and CD8 T cells. However, increases in the proportion of pan T cells and NKT cells and the CD4/CD8 ratio were observed in CLD subjects with either CL-A or CD-D use. This suggests a possible CL-specific immune cell profile imbalance on the ocular surface of CLD subjects. The disruption of tear film dynamics and generation of physical stimuli such as friction following CL wear can induce secretion of cytokines and chemokines on the ocular surface.[6] These may initiate migratory signals that bring immune cells to the ocular surface and possibly activate them, which in turn results in the production of inflammatory factors that can alter nociception.[9,10,44] Therefore, at the ocular surface, enhanced numbers of T cells are a critical immunological factor in CLD. Indeed, in chronic pain, emerging evidence suggests that inhibiting the pro-nociceptive aspects of T cells is a potential management strategy for chronic pain.[34] The CL-A–specific increase in neutrophils remains to be better understood, although neutrophils are associated with increased itch sensations on skin in animal models.[45]

Because we are at the beginning of our investigation into the mechanisms of action for CLD and the patient database is relatively limited at this time, it is possible that additional factors may contribute to the ocular immune cell phenotypes we observed in this study, such as an overlap between CLD and DED[46] or ocular allergy,[47] despite our effort to minimize these influence via stringent exclusion criteria based on clinical presentations. Our findings highlight the urgency and importance of further investigation into immune responses and regulations at the cellular and molecular levels with larger patient populations of varying ethnicity, more diverse contact lens materials, and better differentiation among CLD, DED, and ocular allergy. In conclusion, our study demonstrates, for the first time, to the best of our knowledge, that alterations in immune cell subsets on the human ocular surface due to the use of CLs correspond to subjects' responses to CL usage. The results have important implications for the management of CL intolerance.

Acknowledgments

The authors thank for their contributions Prerna Ahuja and Priyansha Chatterjee, Narayana Nethralaya, Bangalore, India; and Rajeshwari Mahadevan, Sankara Nethralaya, Chennai, India.

Supported by Johnson & Johnson Vision.

Disclosure: **A.P. Nair**, None; **S. Sethu**, None; **H. Nagaraj**, None; **V. Kumar**, None; **S. Nagaraj**, None; **Z. Fadli**, Johnson & Johnson Vision (E); **C. Scales**, Johnson & Johnson Vision (E); **M. Chemaly**, Johnson

& Johnson Vision (E); **X.-Y. Song**, Johnson & Johnson Vision (E); **A. Ghosh**, None; **B. Liang**, Johnson & Johnson Vision (E)

References

1. Dumbleton K, Caffery B, Dogru M, et al. The TFOS International Workshop on Contact Lens Discomfort: report of the subcommittee on epidemiology. *Invest Ophthalmol Vis Sci*. 2013;54:TFOS20–TFOS36.
2. Dumbleton K, Woods CA, Jones LW, Fonn D. The impact of contemporary contact lenses on contact lens discontinuation. *Eye Contact Lens*. 2013;39:93–99.
3. Pritchard N, Fonn D, Brazeau D. Discontinuation of contact lens wear: a survey. *Int Contact Lens Clin*. 1999;26:157–162.
4. Young G, Veys J, Pritchard N, Coleman S. A multi-centre study of lapsed contact lens wearers. *Ophthalmic Physiol Opt*. 2002;22:516–527.
5. Richdale K, Sinnott LT, Skadahl E, Nichols JJ. Frequency of and factors associated with contact lens dissatisfaction and discontinuation. *Cornea*. 2007;26:168–174.
6. Craig JP, Willcox MD, Argueso P, et al. The TFOS International Workshop on Contact Lens Discomfort: report of the contact lens interactions with the tear film subcommittee. *Invest Ophthalmol Vis Sci*. 2013;54:TFOS123–TFOS156.
7. Jones L, Brennan NA, Gonzalez-Meijome J, et al. The TFOS International Workshop on Contact Lens Discomfort: report of the contact lens materials, design, and care subcommittee. *Invest Ophthalmol Vis Sci*. 2013;54:TFOS37–TFOS70.
8. Stapleton F, Marfurt C, Golebiowski B, et al. The TFOS International Workshop on Contact Lens Discomfort: report of the subcommittee on neurobiology. *Invest Ophthalmol Vis Sci*. 2013;54:TFOS71–TFOS97.
9. Belmonte C, Nichols JJ, Cox SM, et al. TFOS DEWS II pain and sensation report. *Ocul Surf*. 2017;15:404–437.
10. Pinho-Ribeiro FA, Verri WA, Jr, Chiu IM. Nociceptor sensory neuron-immune interactions in pain and inflammation. *Trends Immunol*. 2017;38:5–19.
11. Lopez-de la Rosa A, Gonzalez-Garcia MJ, Calonge M, Enriquez-de-Salamanca A. Tear inflammatory molecules in contact lens wearers: a literature review. *Curr Med Chem*. 2020;27:523–548.
12. Panigrahi T, D'Souza S, Shetty R, et al. Genistein-calcitriol mitigates hyperosmotic stress-induced TonEBP, CFTR dysfunction, VDR degradation and inflammation in dry eye disease. *Clin Transl Sci*. 2021;14:288–298.
13. Bron AJ, de Paiva CS, Chauhan SK, et al. TFOS DEWS II pathophysiology report. *Ocul Surf*. 2017;15:438–510.
14. Clayton JA. Dry eye. *N Engl J Med*. 2018;379:e19.
15. Pflugfelder SC, de Paiva CS. The pathophysiology of dry eye disease: what we know and future directions for research. *Ophthalmology*. 2017;124:S4–S13.
16. Reyes JL, Vannan DT, Eksteen B, et al. Innate and adaptive cell populations driving inflammation in dry eye disease. *Mediators Inflamm*. 2018;2018:2532314.
17. Barabino S, Chen Y, Chauhan S, Dana R. Ocular surface immunity: homeostatic mechanisms and their disruption in dry eye disease. *Prog Retin Eye Res*. 2012;31:271–285.
18. Nair AP, D'Souza S, Shetty R, et al. Altered ocular surface immune cell profile in patients with dry eye disease. *Ocul Surf*. 2021;21:96–106.
19. D'Souza S, Nair AP, Sahu GR, et al. Keratoconus patients exhibit a distinct ocular surface immune cell and inflammatory profile. *Sci Rep*. 2021;11:20891.
20. Bose T, Lee R, Hou A, Tong L, Chandy KG. Tissue resident memory T cells in the human conjunctiva and immune signatures in human dry eye disease. *Sci Rep*. 2017;7:45312.
21. Sonawane S, Khanolkar V, Namavari A, et al. Ocular surface extracellular DNA and nuclease activity imbalance: a new paradigm for inflammation in dry eye disease. *Invest Ophthalmol Vis Sci*. 2012;53:8253–8263.
22. Krausgruber T, Fortelny N, Fife-Gernedl V, et al. Structural cells are key regulators of organ-specific immune responses. *Nature*. 2020;583:296–302.
23. Baudouin C, Brignole F, Becquet F, Pisella PJ, Goguel A. Flow cytometry in impression cytology specimens. A new method for evaluation of conjunctival inflammation. *Invest Ophthalmol Vis Sci*. 1997;38:1458–1464.
24. Baudouin C, Brignole F, Pisella PJ, Becquet F, Philip PJ. Immunophenotyping of human dendriform cells from the conjunctival epithelium. *Curr Eye Res*. 1997;16:475–481.
25. Brignole-Baudouin F, Ott AC, Warnet JM, Baudouin C. Flow cytometry in conjunctival impression cytology: a new tool for exploring ocular surface pathologies. *Exp Eye Res*. 2004;78:473–481.

26. Brignole-Baudouin F, Riancho L, Ismail D, Deniaud M, Amrane M, Baudouin C. Correlation between the inflammatory marker HLA-DR and signs and symptoms in moderate to severe dry eye disease. *Invest Ophthalmol Vis Sci*. 2017;58:2438–2448.
27. Khamar P, Nair AP, Shetty R, et al. Dysregulated tear fluid nociception-associated factors, corneal dendritic cell density, and vitamin D levels in evaporative dry eye. *Invest Ophthalmol Vis Sci*. 2019;60:2532–2542.
28. Shetty R, Sethu S, Deshmukh R, et al. Corneal dendritic cell density is associated with subbasal nerve plexus features, ocular surface disease index, and serum vitamin D in evaporative dry eye disease. *Biomed Res Int*. 2016;2016:4369750.
29. Ren K, Dubner R. Interactions between the immune and nervous systems in pain. *Nat Med*. 2010;16:1267–1276.
30. Bethea JR, Fischer R. Role of peripheral immune cells for development and recovery of chronic pain. *Front Immunol*. 2021;12:641588.
31. Calvo M, Dawes JM, Bennett DL. The role of the immune system in the generation of neuropathic pain. *Lancet Neurol*. 2012;11:629–642.
32. Panigrahi T, Shivakumar S, Shetty R, et al. Trehalose augments autophagy to mitigate stress induced inflammation in human corneal cells. *Ocul Surf*. 2019;17:699–713.
33. Periman LM, Perez VL, Saban DR, Lin MC, Neri P. The immunological basis of dry eye disease and current topical treatment options. *J Ocul Pharmacol Ther*. 2020;36:137–146.
34. Laumet G, Ma J, Robison AJ, Kumari S, Heijnen CJ, Kavelaars A. T cells as an emerging target for chronic pain therapy. *Front Mol Neurosci*. 2019;12:216.
35. Wei Y, Asbell PA. The core mechanism of dry eye disease is inflammation. *Eye Contact Lens*. 2014;40:248–256.
36. Enriquez-de-Salamanca A, Castellanos E, Stern ME, et al. Tear cytokine and chemokine analysis and clinical correlations in evaporative-type dry eye disease. *Mol Vis*. 2010;16:862–873.
37. Tan X, Sun S, Liu Y, et al. Analysis of Th17-associated cytokines in tears of patients with dry eye syndrome. *Eye (Lond)*. 2014;28:608–613.
38. Postnikoff CK, Huisingh C, McGwin G, Nichols KK. Leukocyte distribution in the open eye tears of normal and dry eye subjects. *Curr Eye Res*. 2018;43:1253–1259.
39. Postnikoff CK, Held K, Viswanath V, Nichols KK. Enhanced closed eye neutrophil degranulation in dry eye disease. *Ocul Surf*. 2020;18:841–851.
40. He J, Xu Y, Yi M, Gu C, Zhu Y, Hu G. Involvement of natural killer cells in the pathogenesis of endometriosis in patients with pelvic pain. *J Int Med Res*. 2020;48:300060519871407.
41. Wang T, Ma C. Peripheral nociceptors as immune sensors in the development of pain and itch. *Adv Exp Med Biol*. 2016;904:77–85.
42. Verma V, Sheikh Z, Ahmed AS. Nociception and role of immune system in pain. *Acta Neurol Belg*. 2015;115:213–220.
43. Liu JA, Yu J, Cheung CW. Immune actions on the peripheral nervous system in pain. *Int J Mol Sci*. 2021;22:1448.
44. Galletti JG, de Paiva CS. The ocular surface immune system through the eyes of aging. *Ocul Surf*. 2021;20:139–162.
45. Walsh CM, Hill RZ, Schwendinger-Schreck J, et al. Neutrophils promote CXCR3-dependent itch in the development of atopic dermatitis. *Elife*. 2019;8:e48448.
46. Markoulli M, Kolanu S. Contact lens wear and dry eyes: challenges and solutions. *Clin Optom (Auckl)*. 2017;9:41–48.
47. Solomon A. Allergic manifestations of contact lens wearing. *Curr Opin Allergy Clin Immunol*. 2016;16:492–497.