Medical records of our first five consecutive eyes of five patients (mean age: 60 ± 9.34 years; three males) with corneal endothelial decompensation and complex anatomic anterior segment disorders (Table S1) in which UT-DSAEK using the SLc Expert Microkeratome® was performed between March and July 2015 were analysed retrospectively.

Targeted central thickness of the resulting donor lamella was defined before preparation ranging from 50 to 80 μm. Central and peripheral corneal thickness (3 mm from centre) was determined by AS-OCT measurements along four meridians before and directly after preparation. Deviations from the targeted central graft thickness as well as differences between peripheral and central graft thickness were calculated. Obtained differences from eight peripheral measuring points to central thickness measurements were divided into two groups (0–135° and 180–315°) to investigate uniformity in thickness relative to cut direction.

Clinical outcome parameters included best spectacle-corrected visual acuity (BSCVA; logMAR), endothelial cell density (ECD; Tomey EM-3000, Erlangen, Germany), central corneal thickness (CCT) and graft thickness measured by AS-OCT (SPECTRALIS® Anterior Segment Module, Spectral-Domain OCT, Heidelberg Engineering GmbH, Heidelberg, Germany) before surgery as well as 1 and 3 months postoperatively.

All five donor preparations were successfully performed. Deviations from the central targeted thickness were below 19.5 μm at any time (Table S1). The difference between peripheral and central graft thickness averaged along eight meridians was 44.1 ± 16.7 μm regardless of the cutting direction.

In two eyes, partial graft detachment necessitated a second intracameral air injection; no further complications occurred.

Mean preoperative BSCVA improved from 1.56 ± 0.67 logMAR to 0.92 ± 0.79 logMAR at 3 months. Endothelial cell density (ECD) of donor buttons was 2804 ± 228.3 cells/mm² preoperatively and 1489.8 ±336.3 cells/mm² at 3 months (Table S1). Central corneal thickness (CCT) was 815.7 ± 222.2 μm preoperatively and 562.8 ± 99.0 μm postoperatively with a hosts' corneal thickness without the donor lamella of 527.9 ±82.5 μm.

Descemet Stripping Automated Endothelial Keratoplasty (DSAEK) grafts prepared using microkeratomes are typically thinner centrally than peripherally, resulting in a hyperopic shift (Dupps et al. 2008; Scorcia et al. 2009). Our results showed comparatively uniformity in graft thickness which may suggest that the uniform profile of UT-DSAEK grafts prepared by the SLc Expert microkeratome may have less impact on sphere and aberrations than 'standard' DSAEK grafts (Rudolph et al. 2012).

Our case series is limited by the small sample size; however, we can conclude that graft preparation with the SLc Expert Microkeratome®, and the subsequent transplantation of these very thin grafts even in eyes with complex anterior segment pathologies seems to be safe and reproducible. Further clinical studies are desirable to evaluate the practicability and the clinical results of this novel technique particular in relation to DMEK.

References

Bachmann B, Schaub F & Cursiefen C (2016): [Treatment of corneal endothelial disorders by DMEK and UT-DSAEK: indications, complications, results and follow-up]. Ophthalmologe **113**: 196–203.

Dupps WJ Jr, Qian Y & Meisler DM (2008): Multivariate model of refractive shift in Descemet-stripping automated endothelial keratoplasty. J Cataract Refract Surg **34**: 578–584.

Fuest M, Salla S, Walter P, Plange N, Kuerten D, Flammersfeld A & Hermel M (2015): Comparison of gebauer SLc and moria CBm carriazo-barraquer ALK microkeratomes for descemet's stripping automated endothelial keratoplasty preparation. Curr Eye Res **41**: 343–349.

Nahum Y, Leon P & Busin M (2015): Postoperative graft thickness obtained with single-pass microkeratome-assisted ultrathin descemet stripping automated endothelial keratoplasty. Cornea **34**: 1362–1364.

Rudolph M, Laaser K, Bachmann BO, Cursiefen C, Epstein D & Kruse FE (2012): Corneal higher-order aberrations after Descemet's membrane endothelial keratoplasty. Ophthalmology **119**: 528–535.

Scorcia V, Matteoni S, Scorcia GB, Scorcia G & Busin M (2009): Pentacam assessment of posterior lamellar grafts to explain hyperopization after Descemet's stripping automated endothelial keratoplasty. Ophthalmology **116**: 1651–1655.

Villarrubia A & Cano-Ortiz A (2015): Development of a nomogram to achieve ultrathin donor corneal disks for Descemet-stripping automated endothelial keratoplasty. J Cataract Refract Surg **41**: 146–151.

Correspondence:
Friederike Schaub, MD
Department of Ophthalmology
University of Cologne
Kerpener Strasse 62 - 50924 Cologne
Germany
Tel: +49 221 478 4300
Fax: + 49 221 478 5094
Email: friederike.schaub@uk-koeln.de

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Demographic data of UT-DSAEK recipients and summary of results.

Th17 and regulatory T cells are increased in blood of patients with birdshot chorioretinopathy

Vincent Daien,[1,2] Frédéric Mura,[1] Geoffrey Martin,[1] Rachel Audo,[3] Sophie Rivière,[4] Amadou Konate,[4] Jacques Morel,[3,5] Max Villain,[1] Michael Hahne,[3] Christelle Schneider[1] and Claire Daien[3,5]

[1]Department of Ophthalmology, Centre Hospitalier Universitaire de Montpellier, Montpellier, France; [2]National Institute of Health and Medical Research (INSERM), Montpellier, France; [3]Molecular Genetic Institute of Montpellier, CNRS, UMR5535, Montpellier, France; [4]Department of Internal medicine, Centre Hospitalier Universitaire de Montpellier, Montpellier, France; [5]Department of Rheumatology, Centre Hospitalier Universitaire de Montpellier, Montpellier, France

doi: 10.1111/aos.12949

Editor,

Birdshot chorioretinopathy (BSCR) represents an intraocular bilateral and chronic inflammation of the pos-

terior segment of the eye. The pathogenesis has not been completely elucidated. Recent publications suggested that T cells of BSCR patients produce IL-17 in response to human retina and choroid lysate (Kuiper et al. 2013, 2014). Moreover, IL-12 and IL-23, two cytokines known to promote Th17 pathway, were shown to be increased in sera of BSCR patients (Yang & Foster 2013). Dagur et al. (2014) described in 11 BSCR patients an increase of CD8+CD146+ T cells, a population producing IL-17 (Tc17).

No data is available on B and NK cells in BSCR. Given the few therapeutic options available for this disease, a better comprehension of BSCR is important. Therefore, we conducted a prospective pilot study to determine Th17, Tc17, Th1 and Treg cell levels and the composition of B and NK cells in peripheral blood of patients with BSCR.

To be included, patients had to meet the BSCR criteria of the 2006 International Consensus Conference. Exclusion criteria were other inflammatory or autoimmune diseases, malignant neoplasm and infection. Patients were divided into those who received a systemic therapy (past or current) and those who were never treated. We recruited controls from the hospital emergency department with a diagnosis of traumatic corneal abrasions. This research was approved by the Institutional Ethics Committee. Peripheral blood mononuclear cells (PBMCs) were isolated by the Ficoll method. B, NK, NKT and Treg cells were assessed without any activation. Treg cells were defined as $CD4^+CD25^{hi}CD127^{lo/-}$ T cells. Th1 (CD4 + IFNg +), Th17 (CD4 + IL-17A +) and Tc17 (CD8 + IL-17A +) cells were assessed after PBMCs culture in RPMI 1640 medium with 10% FCS for 4 hours with PMA (0. mg/ml), ionomycin (0.5mg/ml) and brefeldin A (10mg/ml).

We included 16 controls and 29 patients with BSCR – 16 who received systemic therapy (current or past) and 13 never treated. Patient characteristics are summarized in Table 1. B, Th1, Tc17 and NK cell subsets were similarly distributed in the three groups. Conversely, the proportion of Th17 cells was higher in never treated BSCR than in controls (p = 0.009), with no differ-

Table 1. Patient characteristics and comparison of lymphocyte populations in patients with BSCR and controls.

	Controls	Previous or current systemic therapy	Never treated	Kruskall–Wallis p-value
Number	16	16	13	–
Age (year)	44 (33–54)	56 (49–65)	54 (51–56)	–
Female, *n* (%)	9 (56)	5 (31)	5 (39)	–
Disease duration (year)	NA	8 (4–18)	2 (1–2)	–
Visual acuity (log)	NA	0.26 (0.10–0.70)	0.00 (0.00–0.10)	–
Visual field	NA	5.35 (2.60–9.50)	0.20 (−2.07–4.10)	–
Electroretinogram B (5 dB)	NA	73 (20–120)	238 (141–387)	–
Electroretinogram B (25 dB)	NA	24 (0–71)	98 (73–165)	–
Log-CRP (mg/dl)	NA	0.13 (−0.51–0.61)	0.34 (−0.04–0.60)	–
Cell populations				
Leucocytes (/mm^3)	6.4 (5.2–7.7)	7.1 (5.7–7.9)	5.7 (4.5–6.8)	0.096
$CD19^+$ B cells	27 (23–48)	22 (12–39)	28 (20–42)	0.306
$CD8^+$ T cells	497 (400–977)	516 (319–621)	540 (408–610)	0.847
$CD4^+$ T cells	573 (486–829)	493 (371–706)	512 (294–661)	0.152
NK cells	193 (135–562)	237 (192–655)	203 (172–275)	0.619
NK T cells	124 (102–208)	80 (52–201)	161 (130–198)	0.194
Naïve B cells (% $CD19^+$) IgD^+ $CD27^-$	64 (49–76)	65 (49–74)	65 (63–72)	0.815
Transitional B cells (% $CD19^+$) $CD24^{hi}$ $CD38^{hi}$	5.0 (3.9–6.6)	4.8 (3.5–7.1)	5.5 (4.6–10.7)	0.265
Memory B cells (% $CD19^+$)				
$CD27^+$	37 (31–44)	35 (24–44)	34 (29–37)	0.747
IgD^+ $CD27^+$ unswitched	15 (13–19)	14 (11–25)	17 (13–21)	0.626
IgD^- $CD27^+$ switched	17 (12–25)	13 (11–19)	14 (9–18)	0.133
T cells (% $CD4^+$)				
Th1 ($CD4^+$ IFNγ+)	14.55 (11.33–18.9)	12.75 (8.56–17.10)	10.90 (9.19–19.10)	0.648
Th17 ($CD4^+$ IL-17A+)	**0.92 (0.84–1.07)**	**1.10 (0.81–1.47)**	**1.28 (0.97–1.71)**	**0.032** [a,b,c]
Tc17 ($CD8^+$ IL-17A+)	0.35 (0.16–0.59)	0.36 (0.32–0.63)	0.38 (0.30–0.62)	0.592
T regulatory cells ($CD25^{hi}$ $CD127^{lo}$)	**1.34 (0.95–1.59)**	**1.76 (1.49–2.38)**	**2.52 (1.37–2.75)**	**0.003** [d,e,f]
NK cells				
$CD56^{dim}$ $CD16^{bri}$	2.2 (1.3–2.5)	1.5 (1.1–2.4)	1.9 (1.5–2.6)	0.567
$CD56^{bri}$ $CD16^{dim}$	60 (50–68)	56 (50–63)	57 (51–75)	0.858

Results are expressed in medians (IQR25-75). Cells were assessed without any stimulation, except for Th1, Th17 and Tc17 cells that were measured after ionomycin + PMA + brefeldine A. Visual acuity, visual field and electroretinogram of right eye are given.
n = number; y = years.
[a]p value comparing control versus previous or current systemic therapy = 0.336; [b]p value comparing control versus never treated = 0.009; [c]p value comparing previous or current systemic therapy versus never treated = 0.081; [d]p value comparing control versus previous or current systemic therapy = 0.014; [e]p value comparing control versus never treated = 0.001; [f]p value comparing previous or current systemic therapy versus never treated = 0.280.

ence between treated BSCR and controls ($p = 0.08$). The proportion of $CD4^+CD25^{hi}CD127^{lo/-}$ T cells was higher in treated BSCR and untreated BSCR than in controls ($p = 0.003$). Levels of Th17 and $CD4^+CD25^{hi}CD127^{lo/-}$ T cells were not significantly different between treated and untreated BSCR. All the results are summarized in Table 1. We did not find any association between levels of Th17 and $CD4^+CD25^{hi}CD127^{lo/-}$ T cells and clinical data such as visual field ($p = 0.30$ and $p = 0.91$, respectively), visual acuity ($p = 0.12$ and $p = 0.18$, respectively) or electroretinogram data ($p = 0.83$ and $p = 0.71$, respectively).

In conclusion, this study is one of the first to broadly explore lymphocytes in BSCR patients. Whereas we did not find significant abnormalities in proportions of Th1, Tc17, B and NK cells, we found an increase of Th17 and $CD4^+CD25^{hi}CD127^{lo/-}$ T cells. The increase proportion of Th17 cells is concordant with previous results (Kuiper et al. 2011). The increase proportion of Treg cells was unexpected since there is usually an inverse correlation between Th17 and Treg cells. Foster et al. (2013) did not find any difference in CD4 + CD25hi Treg cells between BSCR patients ($n = 5$) and controls but a decreased expression level of FoxP3. The present study explored a larger number of BSCR patients but further studies are required to confirm this Treg cell increase. The increased proportion of Treg cells, however, may not reflect their function, which remains to be explored.

References

Dagur PK, Biancotto A, Stansky E, Sen HN, Nussenblatt RB & McCoy JP (2014): Secretion of interleukin-17 by CD8+ T cells expressing CD146 (MCAM). Clin Immunol **152**: 36–47.

Foster CS, Siddique SS, Amorese L, Mulki L & Suelves A (2013): Regulatory T cells in blood of patients with birdshot retinochoroidopathy. Ophthalmology **120**: 430.e1.

Kuiper JJW, Mutis T, de Jager W, de Groot-Mijnes JDF & Rothova A (2011): Intraocular interleukin-17 and proinflammatory cytokines in HLA-A29-associated birdshot chorioretinopathy. Am J Ophthalmol **152**: 177–182.e1.

Kuiper JJW, Emmelot ME, Rothova A & Mutis T (2013): Interleukin-17 production and T helper 17 cells in peripheral blood mononuclear cells in response to ocular lysate in patients with birdshot chorioretinopathy. Mol Vis **19**: 2606–2614.

Kuiper JJW, Rothova A, Schellekens PAW, Ossewaarde-van Norel A, Bloem AC & Mutis T (2014): Detection of choroid- and retina-antigen reactive CD8(+) and CD4(+) T lymphocytes in the vitreous fluid of patients with birdshot chorioretinopathy. Hum Immunol **75**: 570–577.

Yang P & Foster CS (2013): Interleukin 21, interleukin 23, and transforming growth factor β1 in HLA-A29-associated birdshot retinochoroidopathy. Am J Ophthalmol **156**: 400–406.e2.

Correspondence
Vincent Daien
Service d'ophtalmologie
Hôpital Gui de Chauliac
CHU de Montpellier
80, avenue Augustin Fliche 34295
Montpellier Cedex 5
France
Tel: + 33 673 055877
Fax: + 33 467 337557
Email: vincent.daien@gmail.com

We would like to thank La Fédération des Aveugles de France for its financial support and to Charlotte Hua and Jamila El Hmioui for technical support.