Aqueous Cell Differentiation in Anterior Uveitis Using Fourier-Domain Optical Coherence Tomography

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PURPOSE. The differential diagnosis of a patient presenting with anterior uveitis is broad and can present a diagnostic challenge. In this study, we evaluate the characteristic findings of inflammatory cells on optical coherence tomography (OCT) both in vitro and in vivo.

METHODS. Blood from two healthy volunteers was prepared using standardized methods for cell sorting with a flow cytometer (FACSAria). Neutrophils, lymphocytes, monocytes, and red blood cells were placed in suspension and scanned with a 26-kHz Fourier-domain OCT system (RTVue) with 5-μm axial resolution. Custom software algorithms were used to identify cells based on their reflectance distribution. These algorithms were then applied to OCT images obtained from uveitis patients with active anterior chamber inflammation.

RESULTS. On OCT images the cells appeared as hyperreflective spots. In vitro, cell reflectance was statistically significantly different between all of the cell types (neutrophils, monocytes, lymphocytes, and red blood cells, \( P < 0.001 \), Mann-Whitney test). In vivo, the relationship between underlying disease and cell type imaged on OCT was highly statistically significant, with human leukocyte antigen (HLA)-B27-associated uveitis patients having a predominantly polymorphonuclear pattern on OCT and sarcoidosis and inflammatory bowel disease patients having a predominantly mononuclear pattern on OCT (\( P < 0.001 \), Fisher’s exact test).

CONCLUSIONS. These in vitro and in vivo data demonstrate the potential of OCT to evaluate cells in the anterior chamber of patients noninvasively. Optical coherence tomography may be a useful adjunct to guide diagnosis and treatment of ocular inflammatory conditions.

Keywords: Fourier-domain optical coherence tomography, anterior uveitis, polymorphonuclear leukocytes, mononuclear leukocytes, image analysis

The differential diagnosis of a patient presenting with anterior uveitis is broad and includes infectious etiologies, systemic autoimmune disease, and malignancy. Histologically, different types of uveitis show distinct patterns of inflammation. For example, sarcoidosis, tuberculosis, and Vogt-Koyanagi-Harada are characterized by a mononuclear infiltrate, while infectious endophthalmitis and human leukocyte antigen (HLA)-B27-associated acute anterior uveitis are associated with a predominantly polymorphonuclear leukocyte response.

Currently, treatment of uveitis is guided by clinical characteristics of the uveits using the Standardization of Uveitis Nomenclature (SUN) criteria. The true composition of cells within the anterior chamber of a patient is unknown without a diagnostic aqueous tap. This is an invasive procedure with risk of sight-threatening complications such as endophthalmitis.

There has been recent interest in using noninvasive imaging to guide the diagnosis and treatment of uveitis (Lowder CY, IOVS 2004;45:ARVO E-Abstract 3372). Anterior segment optical coherence tomography (OCT) is a particularly appealing option for imaging cells within the anterior chamber given its high resolution, safety, and availability at many centers. Several groups, including ours, have investigated its potential in grading anterior chamber inflammation. The imaged cells were seen as reflective spots within the anterior chamber, and the cell count correlated well with clinical grading.

In this study, we evaluated the characteristic findings of inflammatory cells on Fourier-domain OCT both in vitro and in patients with active anterior chamber inflammation. Our goal was to determine whether OCT may be a noninvasive way of determining the composition of cells within the anterior chamber and therefore be a useful adjunct to guide diagnosis and treatment of uveitis.

METHODS

The study protocol was approved by the institutional review board of Oregon Health and Science University (OHSU). This study followed the tenets of the Declaration of Helsinki and was in accord with the Health Insurance Portability and Accountability Act of 1996. Written informed consent was obtained from all human subjects after explanation of the nature of the study, as well as the risks and benefits of participating.

In Vitro Cell Preparation

Blood was drawn from two healthy volunteers. A sample of the blood from each subject was set aside for complete blood cell count with differential. The remaining blood samples were prepared for cell sorting using standardized methods. The blood samples were centrifuged at 1200g for 4 minutes, and the
three resulting layers corresponding to the plasma, white blood cells, and red blood cells were separated and placed into labeled tubes. Three different validated and titered antibodies were used as cell markers: CD33-percp Cy5.5 (BD Biosciences, San Jose, CA, USA) for neutrophils, CD45-Pacific orange (Invitrogen, Carlsbad, CA, USA) for lymphocytes, and CD14-APC H7 (BD Biosciences, San Jose, CA, USA) for monocytes. A flow cytometer (FASCAria; BD Biosciences, San Jose, CA, USA) was used for cell sorting. Approximately 1,000,000 cells of each type were placed in suspension in glass tubes for OCT scanning. For the second volunteer, only lymphocytes and neutrophils were separated by flow cytometry and placed in suspension for OCT scanning. A sample of each was also plated on a glass slide for histologic evaluation.

OCT Imaging

A 26,000-Hz Fourier-domain OCT system (RTVue; Optovue, Inc., Fremont, CA, USA) with a corneal adaptor module (CAM) was used to image the blood cells in vitro. It has an axial (depth) resolution of 5 μm in tissue and a transverse resolution of approximately 20 μm. The depth-dependent spectral-domain OCT signal roll-off curve was acquired from the manufacturer (Optovue, Inc.) and was used to compensate the OCT sensitivity drop when the signal intensity was measured. The cell suspensions were placed in test tubes and scanned three times each. A Pachymetry+CPwr scan pattern (6-mm scan diameter, 8 radials, 1024 axial scans each radial, 640 pixels each axial scan, 2-mm scan depth in tissue, repeated five times) was used for OCT scans. The zero delay line was placed above the top of the OCT image with the CAM mode. The tube wall was positioned at approximately the same depth position in OCT scans (Fig. 1). Custom-designed software algorithms, previously described, automatically identified cells as hyper-reflective spots from the OCT images. In brief, to be differentiated from OCT speckle noise, a cell had to be larger than 2 pixels in the image based on 4-connected pixel connectivity, and each adjoining pixel had a signal level higher than a preset reflectance threshold. The OCT imaging resolution was better axially than transversely. Therefore, the cell size was measured along the axial direction and designated cell axial width. Once a cell was identified, its axial OCT signal intensity profile was up-sampled, and a Gaussian function was fitted to the cell axial profile. The cell axial width was calculated by taking the full-width half-maximum of the fitted Gaussian function. The cell reflectance (brightness) was calculated as the maximum signal intensity of the cell divided by the maximum signal intensity of a mirror (reflectance = 1.0). The average cell axial width, as well as the cell reflectance of each cell type, was calculated. Signal and image processing were performed with MATLAB software version 7.10.0 (The MathWorks, Inc., Natick, MA, USA).

In Vivo Cell Imaging

Patients from OHSU uveitis clinics with active anterior chamber inflammation were identified, and the anterior chamber of their affected eyes was imaged with OCT using the same scan pattern as used for the in vitro study. In order to standardize the images, the scans were centered over the pupil. The cornea was positioned at approximately the same depth position in OCT scans, and the posterior cornea boundary had to be clearly visible and continuous. Scans that did not meet these criteria were eliminated and reacquired. The aqueous cells were identified in the OCT images using our custom computer algorithm. The average anterior chamber cell axial width and cell reflectance were calculated for each study eye.

Assuming that the cells present in the anterior chamber of an eye were polymorphonuclear (PMN), or mononuclear, or a combination of PMN and mononuclear cells, we developed an OCT cell differentiation algorithm to categorize aqueous cell types in vivo based on their reflectance patterns. The composition of the aqueous cells in an eye was determined by fitting the cell reflectance distribution measured in vivo to a linear combination of PMN (represented by neutrophils, \( t \)% and mononuclear (represented by lymphocytes, \( 100 - t \)%) cell reflectance distributions acquired in vitro. The predominant cell type, representing greater than 50% of the cells in the anterior chamber, was determined for each study participant.

Statistical Analysis

The axial width and cell reflectance measurements obtained during the in vitro study were not normally distributed (\( P < 0.001 \), Kolmogorov-Smirnov test). Pairwise comparisons were performed with the nonparametric Mann-Whitney test between:

1. Cells of the same type from the two healthy donors (neutrophils and lymphocytes only); and
2. Each of the cell types (neutrophils and lymphocytes including cells acquired from both healthy donors).

For the in vivo study, participants were divided into two groups based on clinical diagnosis and prior histologic studies: those expected to have predominantly PMN cells (endophthalmitis, HLA-B27 sudden onset) and those expected to have predominantly mononuclear cells (sarcoidosis, inflammatory bowel disease). Idiopathic cases were excluded from this analysis. Pairwise comparisons on OCT cell axial width and reflectance measurements between the two groups (PMN predominant versus mononuclear predominant) were performed with the nonparametric Mann-Whitney test. Moreover, the predominant cell type determined by the OCT cell differentiation algorithm was compared to the expected predominant cell types based on clinical diagnosis using a Fisher’s exact test.

All analyses used a two-sided \( \alpha \) of 0.05 to define significance. The statistics were performed using MedCalc 12.0 software (MedCalc Software bvba, Mariakerke, Belgium).

RESULTS

In Vitro Cell Imaging

The complete blood cell count and differential of the volunteers were normal. Histologic examination of the flow-sorted samples confirmed relatively pure populations of each cell type. On static OCT images the cells appeared as hyperreflective spots (Fig. 1), which could be identified by our custom computerized algorithm. No statistically significant differences were detected from the average neutrophil axial width (6.5 μm, \( P = 0.53 \)) and reflectance (2.39 \( \times 10^{-7}, P = 0.88 \)) of the two healthy volunteers. The lymphocytes from one donor were slightly bigger (axial width 6.5 vs. 6.2 μm, \( P = 0.015 \)) and slightly brighter (reflectance 0.87 vs. 0.81, \( P < 0.0001 \)) than those of the other donor (Table 1). Table 2 summarizes the average axial width and cell reflectance (brightness) of each of the cell types. No statistically significant differences were found between the average axial width of the neutrophils, lymphocytes, monocytes, or red blood cells. However, the average reflectance of each cell type was statistically significantly different (\( P < 0.001 \)). The histogram distributions of in vitro cell reflectance are shown in Figure 2. The neutrophil reflectance distribution demonstrated more
FIGURE 1. Optical coherence tomography (OCT) images of (A) neutrophils, (B) lymphocytes, (C) monocytes, and (D) red blood cells in suspension. The cells, identified by customized software, are marked in OCT images (circles) and magnified images (arrows). The locations of the right-side magnified images are marked in left-side OCT images with squares. The bright bands on top of the OCT image are artifact from the tube wall.
heterogeneity (longer tail, wider distribution) compared with those of the lymphocytes, monocytes, and red blood cells.

**In Vivo Cell Imaging**

Nineteen eyes of 14 anterior uveitis patients were included in the study. Table 3 outlines their underlying diagnosis, slit-lamp grading using the SUN criteria, and the predominant cell type identified on OCT. All patients had active inflammation clinically graded at between 0.5+ and 4+ using the SUN criteria. There were seven patients with HLA-B27-associated uveitis who had a predominantly PMN pattern on OCT. There was one study participant with bilateral HLA-B27-associated uveitis who had a predominantly PMN pattern in the symptomatic eye and mononuclear pattern in the asymptomatic eye. In the one patient with inflammation related to inflammatory bowel disease and the two patients with sarcoidosis uveitis, a mononuclear cell pattern was predominant as determined by OCT. In the one case of endophthalmitis there was mostly a PMN pattern on OCT, and this was confirmed histologically from a sample provided during his tap and inject. Finally, in two patients with idiopathic anterior uveitis, one had a PMN pattern while the other had a mononuclear pattern.

The aqueous cells of the PMN-predominant cases had statistically significant higher cell reflectance than those of the mononuclear-predominant cases (P = 0.0018). Figure 3 shows in vivo OCT images of the aqueous cells and the plots of corresponding cell reflectance distribution in two patients (one PMN-predominant case and one mononuclear-predominant case). The cell reflectance plots show that the PMN-predominant case had a longer tail and wider distribution than the mononuclear-predominant case, similar to the characteristics observed in neutrophil and lymphocyte in vitro plots. No statistically significant differences were detected between the cell axial widths of PMN-predominant and mononuclear-predominant cases in vivo (P = 0.82).

The average in vivo cell reflectance was lower than the in vitro cell reflectance measurements for both PMN-predominant (in vivo 1.70 × 10^{-7}; in vitro neutrophils 2.39 × 10^{-7}; P < 0.0001) and mononuclear-predominant cases (in vivo 0.56 × 10^{-7}; in vitro lymphocytes 0.83 × 10^{-7}; P < 0.0001). The average in vivo cell axial widths were slightly larger than in vitro cell size measurements (in vivo 6.7–6.8 versus in vitro 6.3 μm; P < 0.001).

**DISCUSSION**

Cells in suspension, both in vitro and in the anterior chamber of patients, appear as hyperreflective spots on OCT and can be characterized by cell reflectance. The differences in cell reflectance between groups of PMN and mononuclear blood cells shown in this study suggested that OCT could potentially...
### Table 3. Clinical and Optical Coherence Tomography Characteristics of Patients With Active Anterior Uveitis

<table>
<thead>
<tr>
<th>Study ID</th>
<th>Age, y</th>
<th>Sex</th>
<th>Eye</th>
<th>Diagnosis</th>
<th>Slit-Lamp AC Grades by SUN Criteria</th>
<th>Cell Reflectance, % Bright</th>
<th>Predominant Cell Type</th>
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<tr>
<td></td>
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<td></td>
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<td></td>
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<td>RBC</td>
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<tr>
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<td>F</td>
<td>OS</td>
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<td>2</td>
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<td>M</td>
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<td>3</td>
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<td>M</td>
<td>OS</td>
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<td>F</td>
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<td>6</td>
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<tr>
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<tr>
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<td>Idiopathic uveitis, chronic inflammation but with recent flare-up</td>
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<td>0</td>
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<tr>
<td>13</td>
<td>36</td>
<td>F</td>
<td>OS</td>
<td>Idiopathic uveitis, chronic inflammation but with recent flare-up</td>
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<td>F</td>
<td>OS</td>
<td>Crohn’s disease, recent flare-up</td>
<td>2+</td>
<td>0</td>
<td>Some</td>
</tr>
</tbody>
</table>

AC, anterior chamber; F, female; M, male; WBC, white blood cell; RBC, red blood cell; AAU, acute anterior uveitis.
be useful for discriminating between different types of cells in the anterior chamber.

Our results suggest that cell reflectance and axial width measurements are derived from the cell nucleus rather than the whole cell. In order for the cells to be visible on OCT, the refractive index has to be sufficiently different from the surrounding media to create optical transition zones. The refractive index of cytoplasm $n = 1.376$ does not differ significantly from that of balanced salt solution ($n = 1.3345$) while the refractive index of cell nuclei $n \approx 1.5$ does, thus creating optical index contrasts that are visible on OCT. Consistent with this theory, the axial width we measured was similar to the diameter of the neutrophil and mononuclear cell nucleus in suspension.19–22 Due to similarities in size of nuclei between the leukocytes, we were not able to distinguish between them based on axial width.

Mononuclear and PMN cells were, however, readily distinguishable based on their cell reflectance. Neutrophil nuclei are multilobed and therefore quite different from the other cell types. In suspension, each lobe is approximately 3.6 to 4.35 μm in size, with each neutrophil having between three and five lobes.21 The more complicated nuclear structure in PMN leukocytes in general and neutrophils in particular could give rise to interference effects between multiple backscattering foci (refractive index transition surfaces between nuclear lobes and the cytoplasm). This could in turn manifest in a wider distribution of reflectance amplitudes and directional anisotropy (variation in reflected signal strength with the direction of the angle with which the OCT beam strikes the multilobed nucleus). This is the likely etiology of the wider distribution of cell reflectance seen in Figure 1. For example, if an individual neutrophil had four strong scattering foci within the coherent length of OCT light (5 μm) and the backscattered light from all four foci were in phase (constructive interference from backscattered light with phases that are integer multiples of 2π radians in round-trip delay), the backscattered intensity would be 16 times that of a single index transition. This is due to the fact that reflections add in amplitude when they are in phase, and intensity is the square of amplitude. In contrast, mononuclear cell nuclei have only a front and back surface to generate backscattering. According to this theory, other PMN leukocytes such as basophils and eosinophils should also generate a wider distribution of reflectivity.

We also found a highly statistically significant relationship between pattern of inflammation seen on OCT and underlying uveitis diagnosis in our small series of uveitis patients with active inflammation. These findings have potential clinical applications since different types of uveitis show distinct patterns of inflammation.1,2 We had histologic confirmation of the anterior chamber cells in one patient with endophthalmitis who showed a predominantly PMN pattern on OCT and was found to have neutrophils on Gram stain of his tap and inject specimen. Although we did not have histologic confirmation for the other patients in our study, it is remarkable that our findings are quite consistent with prior histopathologic studies showing that HLA-B27 uveitis is associated with a predominantly PMN infiltrate while sarcoidosis and inflammatory bowel disease have a predominantly mononuclear infiltrate.3–6

Red blood cells, which do not have nuclei, derive their axial width and cell reflectance measurements from the entire cell. In this case the hemoglobin in the cytoplasm makes the refractive index quite different from that of balanced salt solution and more similar to the index of refraction of other cell nuclei.10 Although we may be able to distinguish between red blood cells and leukocytes based on their cell reflectance, this may not translate into a clinically useful measurement because the differences are small and clinicians are able to differentiate these cells by their pigmentation at the slit lamp.

The aqueous cells in vivo appeared relatively larger (6.7–6.8 μm) and dimmer (cell reflectance PMN 1.70 ± 0.56 vs. 0.83 ± 0.56/C0) than those of in vitro cell measurements. One explanation is that the in vitro cell experiment imaged relatively pure cell populations. In contrast, it is possible that more than one type of inflammatory cell were involved in the aqueous of an eye with anterior uveitis. Cell types other than neutrophils or lymphocytes, such as macrophages, may also be involved. Another possible reason is that the inflammatory cells present in the anterior chamber might not have the exact same size and shape as those acquired from peripheral blood. More study is needed to further investigate these differences.

One limitation of our study was the overlap between cell measurements because the coherent light reflecting off the cell
may not be coming from the center of the cell but may be off axis. Therefore, at this time we are not able to identify an individual cell as a PMN versus a mononuclear cell. However, by taking the average measurements of cells within the anterior chamber, it is feasible to differentiate groups of cells. These findings should also be confirmed in a larger series of uveitis patients and ideally with histologic confirmation from anterior chamber paracentesis. Other clinical applications, such as the ability of OCT to differentiate malignant cells in the anterior chamber, also warrant future investigation. 18

These in vitro and in vivo data demonstrate the potential of OCT to evaluate cells in the anterior chamber of patients noninvasively. Therefore, OCT may be a useful adjunct to guide the diagnosis and treatment of ocular inflammatory conditions.

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References