In Vivo Visualization of Dendritic Cells, Macrophages, and Microglial Cells Responding to Laser-Induced Damage in the Fundus of the Eye

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PURPOSE. To study the in vivo response of mononuclear phagocytes (i.e., dendritic cells [DCs] and macrophages [Mφs]) in the posterior eye segment after laser-induced injury, and to gain a better understanding of the role of these cells in inflammatory eye disease.

METHODS. CX3CR1GFP/+ knockin mice were used, in which DCs, Mφs, and microglia cells (μGCs) are constitutively fluorescent. These reporter mice were examined by a confocal scanning laser ophthalmoscope (cSLO) after argon laser coagulation. cSLO was complemented by fluorescence microscopy of retinal flatmounts and eye cryosections, to study cell morphology and location, and by multicolor flow cytometry, to determine the number and identity of the fluorescent cells.

RESULTS. The retina of healthy reporter mice featured abundant fluorescent μGCs. After laser injury to the fundus, these cells accumulated and migrated laterally toward injury after 60 minutes. Distinctly shaped fluorescent cells accumulated within laser spots and were identified by flow cytometry and immunofluorescence microscopy as DCs and Mφs in the retina and choroid. The DCs rapidly disappeared from the retina, whereas the Mφs stayed longer. Choroidal infiltrates were detectable even 35 days after laser injury, in particular in larger spots resulting from higher laser intensity. In addition, nonfluorescent granulocytes were detected in the choroid.

CONCLUSIONS. The synergistic use of ophthalmoscopy, flow cytometry, and immunofluorescence microscopy allows detailed dissection of the in vivo response of mononuclear phagocytes to laser injury of the fundus. The number of μGCs increased in the retina. DCs and Mφs were present in the retina and choroid infiltrate. Mφs and granulocytes persisted in the choroid infiltrate longer than previously thought. (Invest Ophthalmol Vis Sci. 2008;49:3649–3658) DOI:10.1167/iovs.07-1322

Virtually all tissues contain a network of mononuclear phagocytes capable of phagocytosis and antigen presentation, consisting of resident dendritic cells (DCs) and macrophages (Mφs).1,2 These cells are thought to be derived from precursors such as monocytes circulating in the blood.3 They serve sentinel purposes in infection and inflammation and contribute to tissue homeostasis by removing cellular debris. The latter function is usually ascribed to Mφs, whose phagocytic abilities exceed those of DCs.4 Specialized Mφ subpopulations reside in various organs, such as Kupffer’s cells in the liver. During anti-infectious immune responses, Mφs contribute to the early innate defense by phagocytosing pathogens and by secreting toxic mediators. Later, in the healing phase after infectious or inflammatory injury, they are involved in the removal of tissue debris and tissue remodeling. Evidence suggests that distinct Mφ subsets are involved in these two particular tasks, as selective depletion of macrophages revealed special, opposing roles during liver injury and repair.5 Different subsets of Mφs have been shown to be recruited in response to ischemia-induced retinopathy.6,7 In experimental autoimmune uveitis, Mφs generated tissue damage in the eye.8 They are found primarily in the choroid and have been described as poor presenters of antigen, yet they may augment the antigen-presenting function of DCs.9,10 DCs bridge innate and adaptive immunity11 and are considered the chief inducers of adaptive immune responses.12,13 To this end, DCs that have captured infectious pathogens migrate to draining lymph nodes to activate T cells.1,12 Some DCs remain in tissues, to modify the response of infiltrating T cells, which has also been observed in the eye.10 The presence of DCs in the ocular environment appears paradoxical, especially because elaborate systems exist to maintain immunoprivilege.14 Nevertheless, DCs have been demonstrated also in the eye by histology and immunohistochemistry in the cornea, iris, ciliary body, and choroid of different species.15-21 Their exact role in the healthy and diseased eye remains largely unresolved, partially due to the lack of adequate experimental systems to study these rare cells. Forrester et al.10 identified two types of DCs in the rat choroid: major histocompatibility complex (MHC) IIlo nontranslocating cells and MHC class IIhi rapidly translocating cells, the latter of which probably represent matured DCs.5,9 Under physiological conditions, tissue-traversing DCs have been proposed to prevent T-cell activation and thereby to maintain immune privilege and homeostasis in the eye.19 DCs have been detected in the peripheral margins and juxtapapillary areas of the retinas of healthy mice, but not in inflammatory conditions.20,22 In contrast, microglial cells (μGCs) are abundant in this site and form a dense network. These cells have been shown to be involved in the inflammatory response for example in ischemia- and kainate-induced retinal damage.7,23 Their exact role as immune cells is still under intense investigation.24

DCs and Mφs have also been shown to play a critical role in age-related macular disease. Injured retinal pigment epithelial (RPE) cells attract dendrites from DCs in the choroid which constitute the “core” of approximately 40% of drusen.25,26 DCs then attract Mφs that subsequently provoke local inflammation, which commonly is thought to represent one cause of late-stage AMD.26 μGCs have also been proposed to be in-
volved in this condition. All these cell types express the fractalkine receptor (CX3CR1), which plays a role in their infiltration of the eye and also has been described to be involved in AMD.

CX3CR1-GFP/+ knockin mice have greatly facilitated the study of mononuclear phagocytes in vivo. In these animals, one copy of the fractalkine receptor (CX3CR1) gene has been replaced by green fluorescent protein (GFP), leaving the endogenous promoter intact. This modification renders cells expressing the fractalkine receptor, such as blood monocytes, μGCs, DCs, and MΦs, intrinsically fluorescent, and does not result in immunologic alterations in these animals. Fluorescence microscopy of the organs of these animals has demonstrated, for example, that renal DCs and their dendrites form an extensive network around the tubules that alter the immune system in response to ischemia/reperfusion injury. Moreover, these mice have revealed that intestinal DCs extend dendrites into the gut lumen to capture bacteria. However, investigating these organs necessitates killing the animals to obtain tissue. In the eye, Paques et al. have shown the presence of μGCs in the retina of CX3CR1-GFP/+ mice by using scanning laser ophthalmoscopy. This technique may also permit visualizing DCs and MΦs in the fundus of viable mice and thereby allow kinetic studies in the same animals. However, ophthalmoscopy does not allow immunostaining and multiparameter analysis of fluorescent cells. This problem can be overcome by flow cytometry, which facilitates simultaneous detection of more than six parameters per cell in a quantitative fashion and also convenient cell enumeration. However, this technique requires ex vivo material and does not provide anatomic information. The individual disadvantages of these techniques can be overcome by their combined application. In this study, we did so to investigate the response of phagocytic immune cells to local laser-induced injury.

**METHODS**

**Mice and Reagents**

All mice used had been backcrossed >10 times to a C57BL/6 background and were bred and kept under SPF conditions. CX3CR1GGFP/+ mice were provided by Frederic Geissmann (Université Rene Descartes Paris V, France). All experiments were conducted in accordance with local animal ethics procedures. Animals were kept and experiments were performed in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Unless indicated otherwise, all reagents were obtained from Sigma-Aldrich (Taufkirchen, Germany).

**Scanning Laser Ophthalmoscopy**

For examinations mice were anesthetized by inhalation of isoflurane. One eye per mouse was treated with an argon green laser with a laser spot size of 50 μm, duration of 0.1 second, and energy of 50 mW. The left eyes were chosen for convenience. The pupils were dilated with tropicamide eye drops. The mice were anesthetized by inhalation of isoflurane. In addition, the cornea was anesthetized with lidocaine (Xylocaine; Astra, Berlin, Germany) before the mouse was placed in front of the slit lamp.

**Fluorescence Microscopy of Retinal Flatmounts and Eye Cryosections**

Lasered and control animals were killed by cervical dislocation or by enflurane treatment. After enucleation, the eyeballs were fixed in 1% phosphate-buffered paraformaldehyde for 1 hour. The retinas were extracted microsurgically, wholemounted on microscope slides, and fixed in mounting medium (DakoCytomation, Hamburg, Germany) for fluorescence microscopy (filter λ = 488–520 nm; model X71; Olympus, Tokyo, Japan).

**Isolation and Analysis of Cells by Flow Cytometry**

Retina and choroid were extracted from the eyes microsurgically. The tissue was digested for 30 minutes at 37°C with 0.5 mg/mL collagenase and 100 µg/mL DNase I in RPMI 1640 medium (Invitrogen, Karlsruhe, Germany) containing 0.5% heat-inactivated FCS (PAO Laboratories, Pasching, Austria) and 20 mM HEPES. Cell suspensions were filtered through a 100-μm nylon mesh, washed with HBSS without Ca²⁺ and Mg²⁺ containing 10 mM EDTA, 0.1% BSA, and 20 mM HEPES. The number of viable cells was determined by trypan blue staining. FC receptors were blocked with 24G2 culture supernatant. Titrated amounts of the following antibodies from BD-PharMingen were used for staining of 1 × 10⁶ cell samples: anti IAb-FITC (clone 25-9-3), anti CD11b-PerCp/Cy5.5 (M1/70), anti CD11c-APC (HL-3), and anti F4/80-biotin (Cl:A3-1) revealed by streptavidin-Alexa568. The cells were analyzed on a cytometer (LSR II; BD Bioscience, Heidelberg, Germany; Flow Jo software; Tristar, Phoenix, AZ). The abundance of different cell populations was calculated by adding a defined number of 10 million PerCP/Cy5.5-labeled microbeads (BD Bioscience). Flow cytometric classification of immune cells was performed in accordance with established criteria, which are summarized in Table 1. When we compared the flow cytometric analyses of perfused and nonperfused mice, we found no significant differences in NΦG, MΦ, and DC content (data not shown), indicating that intravascular cells represented only a minor leukocyte subset.

**RESULTS**

**In Vivo Visualization of Fluorescent Mononuclear Phagocytes in the Posterior Eye Segment**

Examination by cSLO with an excitation wavelength of 488 nm showed a dense network of fluorescent cells in the fundus of heterozygous CX3CR1GGFP/+ knockin mice. These cells were most easily detectable when the system was focused on the level of the retina. The cells featured highly complex and long arborescent protrusions, consistent with previous studies identifying these cells as μGCs. They were located unrelated to retinal vessels. In comparison, nontransgenic mice showed no fluorescent cells in the fundus at all, confirming specificity for GFP fluorescence.

**Laser-Induced Posterior Eye Damage Attracts Mononuclear Phagocytes**

Sixty minutes after laser coagulation, the fluorescent cells migrated toward the laser spots. The highest accumulation of fluoro...
rescent cells in the laser spots was seen 1 to 4 days after laser treatment. Thereafter, the intensity in the laser spots faded slowly (Fig. 1). An accumulation of fluorescent cells was still visible 4 to 7 weeks after laser- ing. The intensity and duration of cell infiltration was dependent on laser intensity and spot size, where larger spots with higher intensity (200 μm, 200 mW) showed more cell accumulation, which was clearly visible even 9 weeks after laser- ing (Fig. 2). Cells within the laser spots showed a different shape compared to μGCs: Whereas some displayed dendritic features, others appeared rather round and somewhat larger than μGCs. In the day 1 and 4 images, the regular dense network of original dendrite-shaped cells was thinned out in areas adjacent to laser spots with an accumulation of migrated fluorescent cells within the laser spots (Fig. 1). Wild-type mice did not show any fluorescence within the laser spots, therefore, any background fluorescence through window defects of destroyed RPE could be excluded as the source of the fluorescence.

Fluorescence Microscopy of Retinal Flatmounts and Cryosections of the Eye

The highly arborescent shape of μGCs in the untreated retina was confirmed by fluorescence microscopy of retinal flat- mounts (Fig. 3A). As previously described, these cells form a dense network across the entire retina. In the laser-treated retina, fluorescence microscopy confirmed the decrease in the number of fluorescent cells in areas close to laser injury and the accumulation of fluorescent cells right next to and within the laser spots (Fig. 3C), a phenomenon we also found by scanning laser ophthalmoscopy (Fig. 1). Cryosections of the nonlased eyes demonstrated that GFP+ μGCs were located in the inner and in the outer plexiform layer of the retina (Fig. 3B). Some GFP+ cells were also seen in the choroid (Fig. 3B, top right corner), which did not show the arborescent shape of μGCs. After laser injury, GFP+ cells accumulated at the site of the laser burn in the retina and choroid (Fig. 3D, red arrow).

A magnified image of this laser burn showed that most GFP+ cells infiltrated the choroidal layer, but some were also located in the retina (Figs. 4A–C). Consistent with SLO findings (Fig. 1). Most GFP+ cells coexpressed the marker F4/80 (Figs. 4A–C) expressed by MΦ, DCs, and μGCs (for expression patterns, see Table 1). In contrast, distinct GFP-negative cells in the infiltrate expressed the Ly6G marker restricted to granulo- cytes (Figs. 4D, 4F), consistent with the absence of GFP expression by these cells in CX3CR1GFP+/- knockin mice.

Classification of the Fluorescent Cells in the Choroid of Healthy Eyes by Flow Cytometry

To characterize further the fluorescent cells detected by ophthal- moscopy and microscopy, we prepared single-cell solu- tions from separated retinas and choroids of CX3CR1GFP+/− knockin mice, stained them with fluorochrome-labeled antibodies against leukocyte surface molecules, and performed analysis by six-color flow cytometry. When we examined expression of GFP versus the murine DC marker CD11c on choroidal cells (Fig. 5A), we noted two populations of fluo-resent cells that expressed either low or high levels of CD11c (Fig. 5A, top row, regions R2 and R3). Plotting GFP versus CD11b demonstrated that both populations expressed this myeloid cell marker (Fig. 5B). The GFP-expressing CD11cHI subset in R3 expressed high levels of MHC II (Fig. 6A, top row) and of the common leukocyte marker CD45 (Fig. 6B) and intermediate levels of F4/80 (Fig. 6C, top row), which is characteristic of conventional CD8-negative tissue DCs. The GFP-expressing CD11cLO cell population in R2 expressed high levels of CD11b (Fig 4B) and F4/80 (Fig 6C), either lower levels of or no MHC II (Fig. 6A), and high levels of CD45, albeit not as high as the DCs did (Fig. 6B), identifying them as MΦs.

Plotting GFP against CD11b (Fig. 5B) revealed an additional small population of infiltrating leukocytes, which were non- fluorescent and expressed high levels of CD11b (Fig 5B, top row, region R4), which is typical of NΦGs.40 This was con- firmed by their expression of the granulocyte marker Gr1 and by their lack of MHC II and of F4/80 expression (data not shown). Of interest, we noted within the region for MΦs a CD45+ choroidal cell subset lacking MHC II expression. These cells may present immature precursors of mononuclear phago- cytes, such as monocytes. The absolute number of cells is given in Table 2.

Classifications of the Fluorescent Cells in the Retinas of Healthy Eyes by Flow Cytometry

In the retinas of healthy mice, very few cells were detected in the regions with the immunoprofile of DCs, MΦs, and NΦGs. Instead, a distinct cell population expressing even higher GFP amounts (region R1) was noted (Fig. 5C, top row), which we will refer to as GFPHI cells, as opposed to GFPHIL DCs and MΦs. These cells in R1 expressed CD11b (Fig. 5D); low levels of CD11c (Fig. 5C), MHC II (Fig. 6A) and CD45 (Fig. 6B); and intermediate levels of F4/80 (Fig. 6C). This staining pattern is characteristic of μGCs, which was consistent with the microglia-typical morphology of GFP-expressing cells observed by ophthalmoscopy (Figs. 1, 2) and immunohistology (Figs. 3, 4) in the retina of nonlasered mice and in previous reports.59 The absolute number of cells is given in Table 3.

### Table 1. Criteria for Phagocyte Classification by Cell Surface Markers

<table>
<thead>
<tr>
<th>Leukocyte Subset</th>
<th>Conventional Dendritic Cells (DCs)</th>
<th>Macrophages (MΦs)</th>
<th>Microglia Cells (μGCs)</th>
<th>Polymorphonuclear Phagocytes</th>
<th>Neutrophilic Granulocytes (NΦGs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CX3CRI</td>
<td>Int</td>
<td>Int</td>
<td>High</td>
<td>High</td>
<td>Neg</td>
</tr>
<tr>
<td>MHC II</td>
<td>High</td>
<td>Int or low</td>
<td>Low</td>
<td>Low</td>
<td>Neg</td>
</tr>
<tr>
<td>CD11c</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>Low</td>
<td>Neg</td>
</tr>
<tr>
<td>CD11b</td>
<td>Int or neg</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>Neg</td>
</tr>
<tr>
<td>CD45</td>
<td>Highest</td>
<td>High</td>
<td>Int</td>
<td>Int</td>
<td>Neg</td>
</tr>
<tr>
<td>Ly6G</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>F4/80</td>
<td>Int</td>
<td>High</td>
<td>Int</td>
<td>Int</td>
<td>Neg</td>
</tr>
</tbody>
</table>

Int, intermediate; neg, negative.
FIGURE 1. Infrared and 488-nm fluorescence pictures of one representative heterozygous CX3CR1<sub>GFP<sup>+</sup></sub> knockin mouse taken with the scanning laser ophthalmoscope before and at different time points after laser injury. Results are representative of three mice investigated in three individual experiments.
Flow Cytometric Analysis of Fluorescent Cells in Retina and Choroid 2 Days after Laser-Induced Damage

Two days after laser application to the eyes of CX3CR1GFP/+ mice, the choroid contained a higher number of DCs (Figs. 5A, second row, 5E, Table 2). MHC II and CD45 expression by these cells remained unaltered and high (Figs. 6A, 6B). Also MΦs were more abundant compared with healthy eyes (Figs. 5A, second row, 5E, Table 2). The proportion of MΦs with higher MHC II expression increased from 29% to 42% (Fig. 6A), whereas CD45 remained high (Fig. 6B). F4/80 expression levels did not change in these two cell types (Fig. 6C). Granulocytes were markedly increased (Figs. 5B, 5E). GFP+/H9262 μGCs were absent from the lasered choroid (Figs. 5A, 5E).

Also, the retina of lasered mice on day 2 contained DCs and MΦs (Figs. 5C, 5F, Table 3), whereas the increase in granulocytes was not significant (Figs. 5D, 5F). MHC II and CD45 levels on retinal DCs and MΦs were comparable to those in the choroid (Figs. 5A, 5F, Table 2). MHC II levels on retinal DCs and MΦs were comparable to those in the choroid (Figs. 5A, 5F). Granulocytes were also increased (Fig. 5A, 5F). GFP+/H9262 μGCs were absent from the lasered choroid (Figs. 5A, 5E).

The number of retinal μGCs increased significantly with time (Figs. 5C, second row, 5F, Table 3), whereas the expression of MHC II, CD45, and F4/80 on these cells were not significantly changed (Figs. 6A–C). These findings confirmed the histologic and ophthalmoscopic detection of a strong inflammatory response in the retina and choroid, and identified leukocyte subsets participating in this response.

Flow Cytometric Analysis of Fluorescent Cells in Retina and Choroid 9 and 35 Days after Laser-Induced Damage

The number of DCs, MΦs, and granulocytes in the choroid remained increased at 9 days and, surprisingly, had increased even further at day 35 after lasering (Figs. 5, third and bottom rows, 5E, Table 2), consistent with their ophthalmoscopic
detection within lasered areas at these time points (Fig. 1). MHC II and CD45 expression did not change on these cells (data not shown). In the retina, the DCs within the infiltrate on day 2 had disappeared by day 9, whereas the number of M/H9021s and M/H9262 GCs remained elevated, even after 5 weeks (Figs. 5C, 5F, Table 3). These findings indicate that a chronic inflammatory response was detectable even 5 weeks after laser injury, as evidenced by the persistent infiltration of Mφs and μGCs remained elevated, even after 5 weeks (Figs. 5C, 5F, Table 3).

These findings indicate that a chronic inflammatory response was detectable even 5 weeks after laser injury, as evidenced by the persistent infiltration of Mφs and granulocytes, but not of DCs, located within the laser spots, according to our microscopic and ophthalmoscopic findings (Figs. 1, 2, 3, 4).

**DISCUSSION**

Many diseases of the posterior pole of the eye and also laser therapy are associated with the influx of inflammatory cells, some of which actively participate in the inflammatory response.8,44 Most previous experimental studies on the role of these immune cells relied on histology, which reveals their exact location and morphology, but requires killing the experimental animals. The development of transgenic mice producing intrinsically fluorescent cell subsets, such as in CX3CR1 reporter mice, permitted circumventing of this problem by scanning laser ophthalmoscopy. However, this technique does not allow further cell phenotyping by multiparameter analysis. This deficiency can be overcome by flow cytometry, which delivers detailed information on the cellular subsets present in an organ. Such subsets have often been defined based on expression of cell surface markers, although it has to be kept in mind that the cell surface phenotype does not always indicate cell type or functionality. Nevertheless, flow cytometry represents an ideal supplement to ophthalmoscopy and histology, which reveals the tissue location of these cells. The present experimental study is the first to combine cSLO in vivo observation of μGCs, DCs, and Mφs in the fundus of the eye with histologic and flow cytometric ex vivo analysis. These three techniques synergized to permit in-depth analysis of these cells and revealed their number, identity, location, and functional state in homeostatic conditions and in response to local injury.

**FIGURE 4.** Costainings of GFP<sup>−</sup> cells with NφG/Mφ markers in cryosections. (A) GFP<sup>−</sup> cells accumulate within the laser spot in deep retinal layers (PR) and choroid (CH). Overlay of CX3CR1-GFP (green) and nucleus (blue) and F4/80 (red) staining. (B) Same section, showing only CX3CR1-GFP (green). (C) Same section, showing only F4/80 expression (red). ON, outer nuclear layer; PR, photoreceptor layer; CH, choroid; SC, sclera. (D) Overlay of nuclear staining (blue), CX3CR1 (green), and Ly6G (red) of a different lasering site. (E) Same section, showing only GFP<sup>−</sup> cells (green) and cell nuclei (blue). (F) Same section, showing only Ly6G expression on NφGs in red. Results were representative of two individual experiments.
A kinetic analysis of immune cell influx into the retina and the choroid after laser-mediated damage demonstrated striking differences between these two compartments. In the retinas of healthy mice, all three techniques confirmed the well-known constitutive presence of H9262 GCs. Our findings were consistent with the recent description of these cells in CX3CR1 reporter mice by Paques et al. We extended that study by investigating the response of phagocytes to laser-induced cell injury. Of interest, flow cytometric analysis revealed an accumulation of cells exhibiting the typical H9262 GC phenotype in the retina (shape, high CX3CR1 reporter expression, intermediate levels of CD11b and F4/80, and low levels of CD11c, CD45, and MHC II). Possible explanations of this novel phenomenon are the local proliferation of H9262 GCs or the recruitment of their precursors. Both possibilities may have contributed, as recent studies have described recruitment of H9262 GC precursors to areas of axonal degeneration and subsequent proliferation of H9262 GCs within the central nervous system. Multicolor flow cytometry revealed infiltrating MΦs and DCs in the retina after laser injury, which to our knowledge represents the first clear evidence of DCs in the injured retina. In the healthy retina, there is one previous study, in which DCs may have been detected: It showed in rats a very minor MHC II-positive retinal population distinct from H9262 GC, which expressed the rat marker molecule ED2 and was therefore classified as an MΦ subset. Theoretically, these few retinal cells may have been DCs, since subsequent work by the same group showed that choroidal rat DCs can express ED2. In our study, DCs rapidly disappeared from this compartment, in contrast to MΦs, which persisted in the retina. Possibly, the retina represents an unfavorable environment for DCs, at least when compared to the choroid, where these cells were abundant. MΦs lingered in both compartments, perhaps because their function in tissue remodeling and repair was needed for a longer time after injury.

The exact location of the infiltrating cells of the retina cannot be determined by flow cytometry. This question could be answered by scanning laser ophthalmoscopy and immunofluorescence, both of which demonstrated infiltration of immune cells, primarily MΦs, within the laser spots. These findings are consistent with previous histologic studies demonstrating infiltration of laser spots by immune cells, primarily MΦs, and DCs, which also must have been located within laser spots, because we did not detect significant infiltrates of GFP-expressing cells in other parts of the retina by histology or ophthalmoscopy. Although the detection of DCs and MΦs in the normal choroid and the recruitment of MΦs was consistent with results in earlier studies, the infiltration by DCs and the persistence of DCs and MΦs in response to laser injury has not been reported yet.

The parallel use of flow cytometry also revealed that infiltrating DCs expressed the CD11b molecule and therefore belonged to the conventional CD8-negative tissue DC subset according to the classification by Shortman and...
Naik. These DCs had been referred to as myeloid DCs, a term now considered obsolete by authorities in the field. Our findings are consistent with those in a study that demonstrated that DCs in experimental autoimmune uveitis show this subtype. Thus, DC infiltration is not only a feature of uveitis as shown by Jiang et al. and Forrester et al., but also occurs in response to laser-induced damage. In contrast, μGCs were absent from the choroid at any time point.

**TABLE 2. Number of Infiltrating Immune Cells in the Two Choroids of a Mouse**

<table>
<thead>
<tr>
<th>Time Point</th>
<th>Mononuclear Phagocytes</th>
<th>Polymorphonuclear Phagocytes</th>
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<tbody>
<tr>
<td></td>
<td>DCs</td>
<td>MΦ</td>
</tr>
<tr>
<td>d0</td>
<td>None</td>
<td>191 ± 38</td>
</tr>
<tr>
<td>d2</td>
<td>720 ± 191</td>
<td>2470 ± 488</td>
</tr>
<tr>
<td>d9</td>
<td>435 ± 193</td>
<td>1521 ± 661</td>
</tr>
<tr>
<td>d35</td>
<td>497 ± 65</td>
<td>2702 ± 567</td>
</tr>
</tbody>
</table>

**TABLE 3. Number of Infiltrating Immune Cells in the Two Retinas of a Mouse**

<table>
<thead>
<tr>
<th>Time Point</th>
<th>Mononuclear Phagocytes</th>
<th>Polymorphonuclear Phagocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DCs</td>
<td>MΦ</td>
</tr>
<tr>
<td>d0</td>
<td>None</td>
<td>341 ± 97</td>
</tr>
<tr>
<td>d2</td>
<td>438 ± 267</td>
<td>1086 ± 330</td>
</tr>
<tr>
<td>d9</td>
<td>None</td>
<td>367 ± 134</td>
</tr>
<tr>
<td>d35</td>
<td>None</td>
<td>637 ± 115</td>
</tr>
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The nature of the transgenic GFP reporter did not allow direct visualization of granulocytes by ophthalmoscopy and fluorescence microscopy. Nevertheless, flow cytometry permitted detecting and enumerating these cells, and immunohistochemistry of cryosections confirmed these results. Of note, DCs, MΦs, and NΦGs remained present in choroid laser spots for at least 35 days. Thus, laser-induced inflammation persisted much longer than clinically thought and previously described, implying that the healing process after such injury may take longer. The persisting immune cells were mostly MΦs, which may be involved in the removal of cell debris. Furthermore, granulocytes were found, which was surprising, since bacterial pathogens, which would have required immune effector functions of these cells, were absent in our study. Thus, the role of granulocytes in laser-induced damage remains speculative.

In conclusion, we found that cSLO in CX3CR1-GFP mice is an easy, fast, and reliable method of studying the response of μGCs, DCs, and MΦs in the retina and choroid in vivo. The combination with histology and flow cytometry permitted the detailed description of the location and composition of the infiltrate at various time points. We demonstrated early infiltration and rapid departure of DCs and a persistent increase in μGCs. In long-term follow-up studies, we found that laser spots have much longer periods of infiltration by MΦs and granulocytes than was ever expected from past clinical impressions. Although it has to be kept in mind that a single laser spot affects a relatively larger area of a murine eye compared with humans, multiple laser spots in patients may jointly occupy a comparable injured area. These findings indicate that laser coagulation of the posterior pole of the eye is not an inert procedure that rapidly heals, but instead may result in a chronic inflammatory response that may have adverse effects on the underlying condition.

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References


