In Vivo Imaging of Microglia Turnover in the Mouse Retina After Ionizing Radiation and Dexamethasone Treatment

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Purpose. Gamma irradiation and bone marrow transplantation (BMT) are established clinical procedures for the treatment of hematologic malignancies. The radiation targets cells in the bone marrow, but injury to other tissues, including the central nervous system (CNS), have been reported. Here, we examine if anti-inflammatory treatment can mitigate the radiation-induced turnover of retinal microglia and the replacement by bone marrow–derived cells (BMDCs).

Methods. Two-color chimeric mice were generated by lethal irradiation of heterozygous CX3CR1-GFP mice that express GFP in microglial cells and bone marrow transplantation from universal DsRed donor mice. Mice were treated with the corticosteroid dexamethasone; a control group received no dexamethasone treatment. The populations of resident microglia (GFP+) and BMDCs (DsRed+) were quantified by serial in vivo imaging for 10 weeks after irradiation with a confocal scanning laser ophthalmoscope that we custom-built specifically for multicolor imaging of the murine retina.

Results. Ionizing radiation resulted in loss of 75% of the resident retinal microglia population after 70 days. Recruitment of BMDCs was delayed with respect to the microglia loss, resulting in a transient depletion of the total immune cell number in the retina. With dexamethasone treatment, both the loss of the resident microglia and the infiltration of BMDCs were suppressed by at least 50%.

Conclusions. Anti-inflammatory treatment with the corticosteroidal agent dexamethasone preserves resident microglia and minimizes recruitment of BMDCs after ionizing radiation exposure and BMT.

Keywords: scanning laser ophthalmoscopy, radiation damage, anti-inflammatory agents, microglia, bone marrow–derived cells

Radiation therapy is a clinical tool routinely deployed to target and destroy tumor tissue. In the treatment of hematologic malignancies, such as leukemia and multiple myeloma, the radiation aims to ablate the cancer cells residing in the bone marrow. Because normal hematopoietic cells are also destroyed in the process, the patient is rescued with a bone marrow transplant (BMT) of healthy bone marrow cells. Although cells of the hematopoietic system are the main target of the treatment, damage to the central nervous system (CNS) and the gut have also been reported.[1–4] To protect these tissues from radiation-induced damage, understanding of the cellular effects of ionizing radiation exposure is crucial and intervention strategies need careful evaluation. Here we demonstrate by serial retinal imaging in live mice that treatment with the corticosteroidal anti-inflammatory agent dexamethasone can mitigate cellular radiation effects in the CNS.

Our interest lies in understanding the cellular effects of ionizing radiation in the CNS and the retina, in particular on the population of microglia. Microglia are the resident immune sentinels of the CNS that destroy pathogens and digest necrotic tissue in support of neurons, but also monitor and maintain synaptic function.[5–10] Under homeostatic conditions, the microglial population is thought to be maintained by local proliferation with little or no turnover.[11–13] Their population declines with age, and disease conditions have been described that give rise to recruitment and infiltration of bone marrow–derived cells (BMDCs).[11,14–18] Loss of the neurotrophic resident microglia may lead to changes that are detrimental to the neurological environment long-term.[19,20] Whether recruitment of BMDCs that are typically derived from circulating monocytes[11,13,21] can restore neurotrophic function or adversely affect the CNS remains incompletely understood.

Trafficking of BMDCs to the CNS is frequently studied in chimeric mice. To generate chimeras, recipient mice are exposed to ionizing radiation and receive BMT from a donor carrying a reporter gene that can be traced in the host. BMDCs do not enter the CNS or retina under physiological conditions, but infiltrate when protective barriers are breached and inflammatory chemokines are expressed.[11,12,18,21–23] The idea that microglia turn over and are replenished by BMDC engraftment arose from the observation that some infiltrating BMDCs expressed the microglial marker Iba-1.[24] However, in

the absence of radiation exposure (irradiation with a head shield or generating chimeras by parabiosis), infiltration of BMDCs was minimized.[11,12,25] It has since been suggested that inflammation may play a significant role in the recruitment of BMDCs observed in radiation-induced chimeras.[11,12,21]

If it is true that the observed turnover of the microglial population depends on inflammation, then suppression of the inflammatory response with an anti-inflammatory agent should minimize BMDC infiltration. We have developed methods that enable tracking of resident microglia and BMDCs in the living mouse retina after irradiation and BMT.[26] To this end, whole-body lethal irradiation of $CX_3CR_1^{+/gfp}$ recipients whose microglia express green fluorescent protein (GFP) followed by BMT from a DsRed donor generated two-color chimeric mice. Microglia and BMDCs were tracked by their GFP and DsRed fluorescence by in vivo imaging with our scanning laser ophthalmoscope (SLO) that we custom-built specifically for multicolor confocal imaging in the murine retina.[26] Here, we demonstrate by enumerating the populations of resident microglia and BMDCs by serial live retinal imaging over 70 days, that treatment with dexamethasone minimizes loss of retinal microglia and engraftment of BMDCs.

Methods

Experiments were undertaken in mice engineered to express GFP in microglia under the control of the fractalkine receptor promoter CX_3CR_1 (B6.129P-Cx3cr1tm1Litt/J; Jackson Laboratory, Bar Harbor, ME), which is expressed on microglia, blood monocytes, natural killer, and dendritic cells.[27] Homozygous CX_3CR_1-GFP mice were crossed with the C57BL/6 parental strain to maintain one functional copy of the fractalkine receptor allele in microglia.[28] Heterozygous mice were lethally irradiated with a single dose of 11.5-Gy gamma radiation from a Cesium-137 source (Gammacell 40 Exactor; MDS Nordion, Ottawa, Ontario, Canada). Five hours after the lethal exposure, mice received a whole BMT of 5×10^6 cells harvested from age-matched homozygous actin-DsRed donor mice (B6.Cg-Tg[CAG-DsRed*MST]1Nagy/J; Jackson Laboratory).

To investigate the influence of anti-inflammatory therapy on microglial turnover, six mice were treated with dexamethasone after irradiation and BMT. A dose schedule that tapers the dexamethasone concentration during the treatment time was adopted to minimize the side effects of the steroid[14] (Fig. 1). Mice received initial intraperitoneal (IP) injections of 3 mg/kg dexamethasone (Dexamethasone Sodium Phosphate Injection, USP, 10 mg/mL; West-Ward Pharmaceuticals, Eatontown, NJ) for 2 days, tapering to 1.5 mg/kg IP for 2 more days. A final dose of 0.75 mg/kg was delivered in drinking water for the remainder of the study. Dexamethasone concentration in the drinking water was adjusted twice weekly while monitoring the average daily water consumption and body weight for each mouse. A control group of four mice received no dexamethasone treatment.

The populations of resident GFP+ microglia and DsRed+ BMDCs were tracked by in vivo retinal imaging with our confocal SLO that we developed specifically for multicolor imaging of the murine retina.[26,29] Our custom-built SLO is a confocal microscope that uniquely enables simultaneous excitation and detection of multiple fluorescent markers. To this end, a triple-edge dichroic beam splitter separates the emitted fluorescence from the incident laser light of up to three excitation lasers. Two dichroic beam splitters (FF560-Di01 and FF650-Di01, Semrock, Lake Forest, IL, USA) further split the fluorescence into three spectrally distinct detectors (red = 650–825 nm, green = 550–650 nm, and blue = 500–550 nm). The SLO uses large pinholes (3.2 to 4.1 times the Airy disc

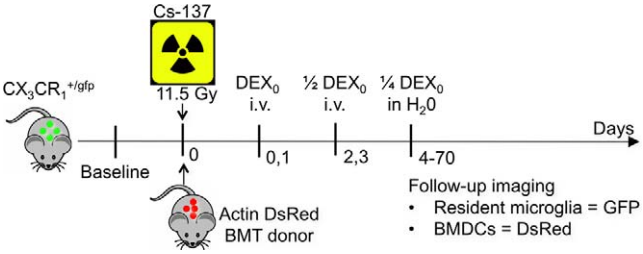

Figure 1. Experimental timeline. Lethally irradiated CX3CR1-GFP mice were rescued by BMT from universal DsRed donors after ionizing radiation exposure. Mice were treated with a tapered dose scheme of dexamethasone. A control group received no dexamethasone treatment. Green fluorescent protein–positive resident microglia and DsRed+ BMDCs were enumerated by serial in vivo imaging for 70 days after the irradiation and BMT.

size, depending on the wavelength), generating an optical section that is approximately 40 μm thick, capturing a thick slice of the retina at once. A source telescope at each excitation laser introduces slight beam divergence to compensate for chromatic aberrations of the mouse eye. This design enables acquisition of up to three channels simultaneously and, thus, allows detection of fluorescence from multiple distinct cell populations. For the experiments described here, GFP fluorescence of microglia was excited with the 491-nm laser, detected through 525/50 bandpass filter and assigned green color. DsRed fluorescence from BMDCs was elicited with the 532-nm laser, detected through a 561 long-pass filter and assigned red color in the RGB images, respectively.

Because an SLO uses the cornea and lens of the eye as the objective, the image quality and resolution depend critically on the optical characteristics of the eye and the incident laser beam. The resolution an SLO can obtain critically depends on the size of the incident laser beam on the cornea and the quality of the eye itself. Although adaptive optics correction has been used to significantly improve image resolution in the aberrated mouse eye,[30,31] we chose an incident laser beam that underfills the mouse eye pupil, aiming for a suitable balance between optical resolution and aberration that both increase with the incident beam diameter.[31] With an incident laser beam of 1.1 mm in diameter, the diffraction-limited resolution is calculated to be 1.3 μm, sufficient to detect cells that are several microns in size, such as microglia and BMDCs.[28,32] To characterize the optical resolution, the intensity profiles of 12 microglial processes and 22 spines from four different mice were fitted to a normal distribution. The full width at half maximum (FWHM) diameter of the measured microglial structure was compared to literature.

A heated holder that integrates a nose cone for inhalation anesthesia (1%–2% isoflurane in oxygen) was mounted on a six-axis stage to position the dilated pupil into the SLO imaging beam. A contact lens (diameter 2.5 mm, base curvature 1.65 mm, power: +12D, material PMMA; Unicon Corporation, Osaka, Japan) was placed on the mydriatic eye and a drop of GenTeal eye gel (Alcon, Fort Worth, TX, USA) prevented the cornea from drying. In vivo images were recorded before the irradiation and BMT (baseline) and at days 7, 14, 28, 42, and 70 after the irradiation and BMT.

At each time point, the numbers of resident GFP+ cells and DsRed+ bone marrow–derived cells were evaluated. The total retinal immune cell population, defined as the sum of remaining microglia and BMDCs, was computed and tracked. To identify extravasated BMDCs, a 10-minute time-lapse stack was generated at each time point, where one 10-frame mean image was taken every 30 seconds. Cells that were stationary for the duration of the time-lapse were considered as

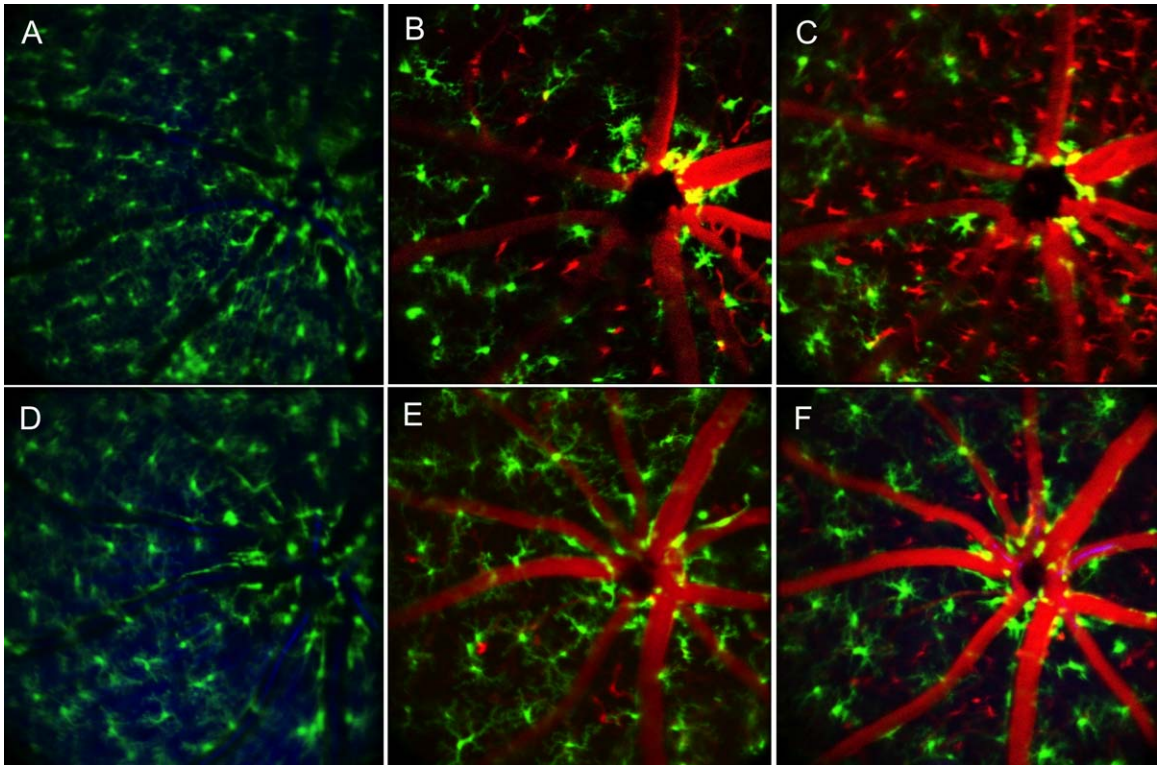

Figure 2. Representative in vivo high-magnification images and timeline of untreated and dexamethasone-treated mice. (**A–C**) Baseline, day 42, and day 70 without dexamethasone treatment show substantial microglia loss and BMDC engraftment. (**D–F**) Dexamethasone treatment demonstrates improved preservation of microglia population and minimized BMDC engraftment. In both cases, surviving microglia display ramified morphology, indicating that they are not activated. Extravasated BMDC morphology resembles that of microglia. Field of view = approximately 20° (≈575 μm).

extravasated BMDCs. All animal procedures were approved by the Massachusetts General Hospital Institutional Animal Care and Use Committee and were consistent with the ARVO statement for the Use of Animals in Ophthalmic and Vision Research.

Results

We developed an SLO capable of resolving cellular features of multiple distinctly fluorescent cell populations simultaneously. Using this instrument, we tracked resident microglia and BMDCs for 70 days after lethal irradiation of $CX_3CR_1^{+/gfp}$ recipients and BMT from actin DsRed donor**.** Resident microglia cells were tracked by their endogenous GFP signal and donor BMDCs by their universal DsRed signal. Without dexamethasone treatment, exposure to ionizing radiation resulted in progressive loss of resident microglia and engraftment of BMDCs. Treatment with the corticosteroid dexamethasone reduced this microglia turnover.

In previous experiments we and others observed that initial BMDC homing occurs near the optic nerve head (ONH) and spreads over time radially from the ONH.[22,26] Microglia loss and BMDC engraftment were therefore enumerated in a field of view that spanned approximately 500 μm central and nasal of the ONH. Figure 2 shows a timeline (baseline, days 42 and 70) of representative images of untreated and dexamethasone-treated mice. Loss of the resident GFP+ microglia with respect to baseline and homing of DsRed+ BMDCs in the vicinity of the ONH are readily apparent (Figs. 2A–C). Dexamethasone treatment (Figs. 2D–F) visibly protected the resident microglia population and minimized the engraftment of BMDCs.

Figure 3A shows a representative image of a live mouse retina injected with Alexa Fluor 647 conjugated dextran to visualize retinal vasculature, GFP microglia, and DsRed BMDCs simultaneously. The morphology of remaining microglia and extravasated BMDCs was readily apparent. Remaining microglia retained ramified morphology and amoeboid microglia were rarely found. Most extravasated BMDCs appeared as dendriform cells. Labeling of the vasculature with Alexa Fluor 647 conjugated to 70 kD dextran in an animal without dexamethasone treatment confirmed that dendriform BMDCs are located outside of retinal vasculature. The resolution of the system was sufficient to detect cellular morphology. Because large pinholes in our SLO generate an approximately 30-μm-thick optical section, each image contains out-of-focus structure alongside morphology situated in the focus. The FWHM of the measured intensity profiles of microglial processes and spines were 2.6 ± 0.7 μm and 2.3 ± 0.3 μm, respectively.

Without dexamethasone treatment, more than 40% of the resident microglia were lost within 7 days of radiation exposure and BMT (Fig. 4A). The microglia population further shrank to 25% of baseline by day 70. Although microglia loss was readily apparent early after irradiation and BMT, engraftment of BMDCs was first observed 6 weeks after the irradiation with approximately 10 cells infiltrating the retina near the optic nerve head (Fig. 4B). At days 56 and 70, the number of engrafting cells increased to the order of 50 cells. In contrast, treatment with dexamethasone preserved the microglia population such that it remained at or above 90% of its baseline level for 4 weeks after irradiation and BMT. More than 60% of the baseline resident microglia population survived until the end of the observation period (day 70). Furthermore, dexamethasone treatment substantially reduced the engraftment of BMDCs. Although initial engraftment of BMDCs was

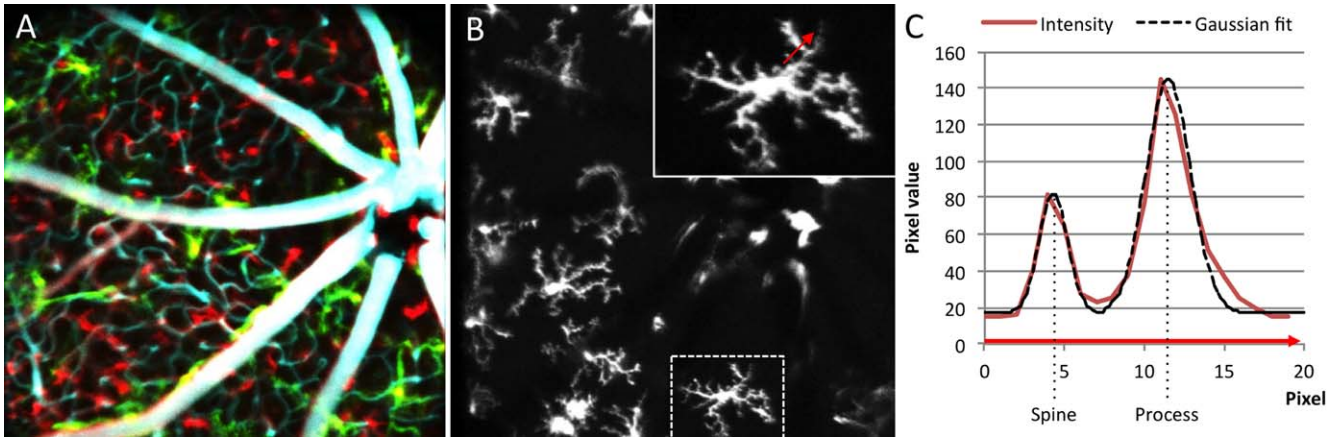

Figure 3. Morphology and location of surviving microglia and engrafting BMDCs. (**A**) Green fluorescent protein–positive resident microglia (*green*), DsRed+ BMDCs (*red*), and Alexa Fluor 647-labeled vasculature (*cyan*) 70 days after irradiation and BMT. Extravasated BMDCs were identified as DsRed+ cells that were stationary during a 10-minute time-lapse acquisition. The vast majority of those cells were characterized by dendriform morphology, a shape not compatible with migratory behavior. Labeling of the vasculature with 70-kD Dextran-Alexa Fluor 647 confirmed that those stationary dendriform cells are located extravascularly. Note that remaining microglia are also ramified; amoeboid microglia, indicating activation, were rarely found. Field of view approximately 20° (≈575 μm). (**B**, **C**) Analysis of system resolution. (**B**) Microglial morphology is apparent. Note that each image contains focused and out-of-focus portions. One well-focused cell is enlarged (*inset*). A *red arrow* marks the location of one intensity profile that intersects a spine and a process. (**C**) Intensity profile of adjacent spine and process with Gaussian fit. The FWHM of this spine and process are 2.1 μm and 3.0 μm, respectively.

also observed at day 42, just as in the untreated group, the number of extravasated cells exceeded 10 cells only at day 70.

In animals that were not treated with dexamethasone, the microglial loss preceded BMDCs engraftment. As a result, the total number of microglia and bone marrow–derived cells fell below the baseline number of resident microglia (Fig. 4C). Without treatment, the number of immune cells in the retina was reduced to 60% of the baseline number in the first week following irradiation and remained at that level for 5 weeks. Dexamethasone treatment, on the other hand, maintained the number of retinal immune cells at or above 80% of the baseline microglial count. In the untreated case, infiltrating BMDCs brought the population up to 80% by day 70, whereas BMDC in treated animals led to a slight increase of cells in the retina above baseline level.

Discussion

The inner retina, as an optically accessible gray-matter compartment of the CNS, is an ideal target to longitudinally track cellular responses to ionizing radiation by in vivo microscopy. We developed two methods that enable tracking of resident microglia and BMDCs in vivo over long periods: an SLO custom-built for confocal retinal imaging in the mouse eye and generation of two-color chimeric mice. Our SLO acquires images at video-rate to minimize motion artifacts and shorten imaging time.[26,29,33] The instrument uses a multicolor excitation and detection scheme so that multiple retinal cells, labeled with spectrally distinct fluorescent markers, can be imaged simultaneously. The resolution of the SLO was characterized by analyzing intensity cross sections of microglial spines and processes. The measured width of the microglial processes from our images (2.6 ± 0.7 μm) matches their published size (2.4 ± 0.5 μm) well. The smallest spine in our images was measured to be 1.8 μm wide, whereas the mean and SD of all measured spines was 2.1 ± 0.3 μm, substantially larger than the reported size of spines that ranges between 0.7 and 1.5 μm (1.2 ± 0.3 μm).[28,32] Thus, without adaptive optics to correct for the aberrations of the mouse eye, the SLO resolves structure on the order of 2 μm in size, approximately 50% larger than diffraction limited, over a large field of view that spans 18 degrees (approximately 575 μm). To place this outcome into perspective, best-corrected resolution of the fully utilized mouse numerical aperture (NA) is approximately 0.7 μm and adaptive optics correction closely obtains that resolution in the mouse eye.[30,31] However, adaptive optics correction is restricted to a field of view of a few degrees, making cell tracking over a large field of view difficult.

Our instrument was used to track the resident microglia and the homing of BMDCs over 70 days by serial in vivo retinal imaging. Our results demonstrate that microglia turnover is minimized with anti-inflammatory treatment. Without treatment, progressive loss of the resident microglia and engraftment of BMDCs was observed. Although loss of microglia was detected within 1 week after irradiation and BMT, initial engraftment of BMDCs was not observed until day 42. As a result, the total retinal immune cell population was relatively depleted for approximately 5 weeks. This transient lack of immune cells may help explain certain retinal complications that BMT patients experience.[2,4]

Treatment with the corticosteroid dexamethasone minimized microglia loss and BMDC infiltration. The population of microglia was preserved at or above 90% of its baseline size for 4 weeks after the irradiation and BMT. After 70 days, the population was maintained at approximately 70% of baseline level, compared with 25% without dexamethasone treatment. With dexamethasone treatment, BMDC infiltration occurred at a substantially reduced rate, although it is interesting to note that the treatment did not further delay initial engraftment. As a result of reduced microglia loss, the total retinal immune cell population was maintained at a level of 80% of the baseline microglia population.

Although treatment with an anti-inflammatory agent can explain the reduction in BMDC engraftment, the preservation of resident microglia is puzzling. Corticosteroids, such as dexamethasone, are thought to switch off inflammatory genes that encode cytokines, chemokines, adhesion molecules, and inflammatory proteins.[34] It was previously shown that irradiation-induced upregulation of adhesion molecules can be controlled by suppression of inflammation response, for example by anti-TNF or dexamethasone treatments.[35–37]

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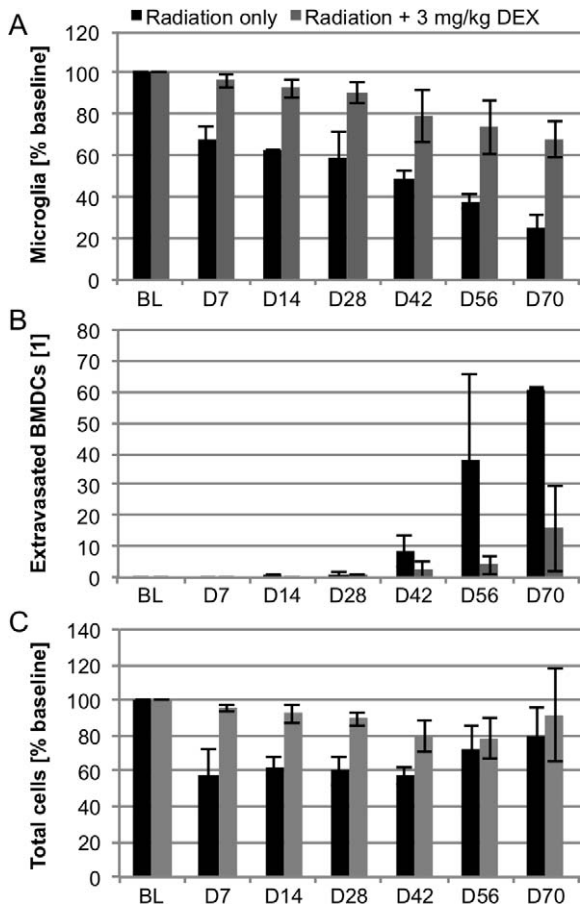

Figure 4. Dexamethasone treatment reduces resident microglia loss and BMDC infiltration after BMT. (**A**) Resident microglia are preserved by dexamethasone treatment (3 mg/kg IP for 2 days, then 1.5 mg/kg IP for 2 more days, followed by 0.75 mg/kg in drinking water for the remainder of the study). Data are average ± SD. The difference in percent GFP cells between dexamethasone and untreated control were statistically significant at all time points ($P < 0.05$). (**B**) Dexamethasone treatment minimizes engraftment of BMDCs. The difference in cell number is statistically significant on and after day 42 ($P < 0.05$). (**C**) Dexamethasone treatment preserves the total number of immune cells in the retina. Without treatment, the total retinal immune cell population is reduced to 60% of the baseline microglia population for 5 weeks. The difference is statistically significant from day 7 up to including day 42. (**A–C**) n (radiation only) = 4 mice; n (radiation +3 mg/kg dexamethasone) = 6 mice.

Furthermore, BMDC recruitment is dependent on monocyte-specific signaling (CCL2).[11,13,21] Thus, modulation of chemokine and adhesion molecule expression combined with impaired ability of circulating monocytes and leukocytes to respond to inflammatory signaling can explain reduced BMDC infiltration.

However, the mechanism by which suppression of inflammation preserves resident microglia that have received a lethal dose of ionizing radiation requires further clarification. To begin with, the mechanism of the microglia cell loss is unknown; microglia may migrate away from the retina or they can succumb to radiation damage. The idea that microglia may migrate away arose from the observation that the vicinity of the ONH was the zone of highest microglial loss and initial BMDC engraftment; the ONH may be an exit and entry point for cells. However, surviving microglia were ramified, indicating that they were "resting." Migrating microglia are expected to acquire an "activated" amoeboid shape, as ramified microglia are not known to migrate. However, two limitations of our imaging protocol may have prevented direct observation of microglial activation and migration. First, the transparency of the mouse cornea decreases with time during anesthesia, limiting the length of each imaging session to approximately 30 minutes. Second, imaging took place every 2 weeks. In contrast, microglia locomotion has been detected on the order of 4 hours after focal laser injury[38] and no migratory behavior has been observed over a time course of 30 minutes after fractalkine ligand treatment.[28] Thus, we may have missed slow migratory events and cannot rule out migration as a reason for microglia loss.

Another possible path of investigation might consider cell death following DNA damage in proliferating cells as the main mode of microglial depletion. Exposure to ionizing radiation induces DNA damage that kills cells when they become mitotically active.[39,40] Dexamethasone has been shown to inhibit division of resident leukocytes after sublethal irradiation.[41] Thus, dexamethasone-induced delay of proliferation in microglia after irradiation may increase their survival by giving the cells time to repair DNA damage.[39] A second path of investigation could aim to establish a causal relationship between endothelial activation and microglia proliferation. If adhesion molecule expression on endothelial cells were to cause microglia proliferation, microglial survival could be improved when dexamethasone modulates endothelial response to damage.

Last, corticosteroids, such as dexamethasone, are associated with significant side effects that require the benefits of the treatment to be considered carefully.[14,41,42] Nonsteroidal anti-inflammatory drugs may serve as therapeutic alternatives with reduced side effects, provided they can be shown to afford benefits comparable to dexamethasone.

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References

1. Sun C-L, Francisco L, Kawashima T, et al. Prevalence and predictors of chronic health conditions after hematopoietic cell transplantation: a report from the Bone Marrow Transplant Survivor Study. *Blood*. 2010;116:3129–3139.
2. Moon SJ, Mieler WF. Retinal complications of bone marrow and solid organ transplantation. *Curr Opin Ophthalmol*. 2003; 14:433–442.
3. Douple EB, Mabuchi K, Cullings HM, et al. Long-term radiation-related health effects in a unique human population: lessons learned from the atomic bomb survivors of Hiroshima and Nagasaki. *Disaster Med Public Health Prep*. 2011;5:S122–S133.
4. Coskuncan NM, Jabs DA, Dunn JP, et al. The eye in bone marrow transplantation. VI. Retinal complications. *Arch Ophthalmol*. 1994;112:372–379.
5. Vilhardt F. Microglia: phagocyte and glia cell. *Int J Biochem Cell Biol*. 2005;37:17–21.
6. Biber K, Neumann H, Inoue K, Boddeke HWGM. Neuronal "On" and "Off" signals control microglia. *Trends Neurosci*. 2007;30:596–602.
7. Wake H, Moorhouse AJ, Jinno S, Kohsaka S, Nabekura J. Resting microglia directly monitor the functional state of

synapses in vivo and determine the fate of ischemic terminals. *J Neurosci*. 2009;29:3974–3980.

8. Petersen MA, Dailey ME. Diverse microglial motility behaviors during clearance of dead cells in hippocampal slices. *Glia*. 2004;46:195–206.
9. Kim SU, de Vellis J. Microglia in health and disease. *J Neurosci Res*. 2005;81:302–313.
10. Kettenmann H, Hanisch UK, Noda M, Verkhratsky A. Physiology of microglia. *Physiol Rev*. 2011;91:461–553.
11. Mildner A, Schmidt H, Nitsche M, et al. Microglia in the adult brain arise from Ly-6ChiCCR2+ monocytes only under defined host conditions. *Nat Neurosci*. 2007;10:1544–1553.
12. Ajami B, Bennett JL, Krieger C, Tetzlaff W, Rossi FMV. Local self-renewal can sustain CNS microglia maintenance and function throughout adult life. *Nat Neurosci*. 2007;10:1538–1543.
13. Davoust N, Vuaillat C, Androdias G, Nataf S. From bone marrow to microglia: barriers and avenues. *Trends Immunol*. 2008;29:227–234.
14. Weissman DE, Janjan NA, Erickson B, et al. Twice-daily tapering dexamethasone treatment during cranial radiation for newly diagnosed brain metastases. *J Neurooncol*. 1991;11:235–239.
15. Priller J, Prinz M, Heikenwalder M, et al. Early and rapid engraftment of bone marrow-derived microglia in scrapie. *J Neurosci*. 2006;26:11753–11762.
16. Djukic M, Mildner A, Schmidt H, et al. Circulating monocytes engraft in the brain, differentiate into microglia and contribute to the pathology following meningitis in mice. *Brain*. 2006;129:2394–2403.
17. Kokovay E, Cunningham LA. Bone marrow-derived microglia contribute to the neuroinflammatory response and express iNOS in the MPTP mouse model of Parkinson's disease. *Neurobiol Dis*. 2005;19:471–478.
18. Müther PS, Semkova I, Schmidt K, et al. Conditions of retinal glial and inflammatory cell activation after irradiation in a GFP-chimeric mouse model. *Invest Ophthalmol Vis Sci*. 2010;51:4831–4839.
19. Schindler MK, Forbes ME, Robbins ME, Riddle DR. Aging-dependent changes in the radiation response of the adult rat brain. *Int J Radiat Oncol Biol Phys*. 2008;70:826–834.
20. Lalancette-Hebert M, Gowing G, Simard A, Weng YC, Kriz J. Selective ablation of proliferating microglial cells exacerbates ischemic injury in the brain. *J Neurosci*. 2007;27:2596–2605.
21. Chen M, Zhao J, Luo C, et al. Para-inflammation-mediated retinal recruitment of bone marrow-derived myeloid cells following whole-body irradiation is CCL2 dependent. *Glia*. 2012;60:833–842.
22. Kezic J, McMenamin PG. Differential turnover rates of monocyte-derived cells in varied ocular tissue microenvironments. *J Leukoc Biol*. 2008;84:721–729.
23. Kezic JM, McMenamin PG. The effects of CX3CR1 deficiency and irradiation on the homing of monocyte-derived cell populations in the mouse eye. *PLoS One*. 2013;8:e68570.
24. Xu H, Chen M, Mayer EJ, Forrester JV, Dick AD. Turnover of resident retinal microglia in the normal adult mouse. *Glia*. 2007;55:1189–1198.
25. Kaneko H, Nishiguchi KM, Nakamura M, Kachi S, Terasaki H. Characteristics of bone marrow-derived microglia in the normal and injured retina. *Invest Ophthalmol Vis Sci*. 2008;49:4162–4168.
26. Alt C, Runnels JM, Teo GSL, Lin CP. In vivo tracking of hematopoietic cells in the retina of chimeric mice with a scanning laser ophthalmoscope. *IntraVital J*. 2012;1:132–140.
27. Jung S, Aliberti J, Graemmel P, et al. Analysis of fractalkine receptor CX3CR1 function by targeted deletion and green fluorescent protein reporter gene insertion. *Mol Cell Biol*. 2000;20:4106–4114.
28. Liang KJ, Lee JE, Wang YD, et al. Regulation of dynamic behavior of retinal microglia by CX3CR1 signaling. *Invest Ophthalmol Vis Sci*. 2009;50:4444–4451.
29. Alt C, Lin CP. In vivo quantification of microglia dynamics with a scanning laser ophthalmoscope in a mouse model of focal laser injury. *Proceedings of SPIE*. 2012;8209:820907.
30. Geng Y, Dubra A, Yin L, et al. Adaptive optics retinal imaging in the living mouse eye. *Biomed Opt Express*. 2012;3:715–734.
31. Geng Y, Schery LA, Sharma R, et al. Optical properties of the mouse eye. *Biomed Opt Express*. 2011;2:717–738.
32. Lee JE, Liang KJ, Fariss RN, Wong WT. Ex vivo dynamic imaging of retinal microglia using time-lapse confocal microscopy. *Invest Ophthalmol Vis Sci*. 2008;49:4169–4176.
33. Veilleux I, Spencer J, Biss D, Cote D, Lin C. In vivo cell tracking with video rate multimodality laser scanning microscopy. *IEEE J Sel Top Quantum Electron*. 2008;14:10–18.
34. Barnes PJ. How corticosteroids control inflammation: Quintiles Prize Lecture 2005. *Br J Pharmacol*. 2009;148:245–254.
35. Yuan H, Goetz DJ, Gaber MW, Issekutz AC, Merchant TE, Kiani MF. Radiation-induced up-regulation of adhesion molecules in brain microvasculature and their modulation by dexamethasone. *Radiat Res*. 2005;163:544–551.
36. Gaber MW, Yuan H, Killmar JT, Naimark MD, Kiani MF, Merchant TE. An intravital microscopy study of radiation-induced changes in permeability and leukocyte-endothelial cell interactions in the microvessels of the rat pia mater and cremaster muscle. *Brain Res Brain Res Protoc*. 2004;13:1–10.
37. Wilson CM, Gaber MW, Sabek OM, Zawaski JA, Merchant TE. Radiation-induced astrogliosis and blood-brain barrier damage can be abrogated using anti-TNF treatment. *Int J Radiat Oncol Biol Phys*. 2009;74:934–941.
38. Paques M, Simonutti M, Augustin S, Goupille O, Mathari El B, Sahel J-A. In vivo observation of the locomotion of microglial cells in the retina. *Glia*. 2010;58:1663–1668.
39. Elshaikh M, Ljungman M, Haken Ten R, Lichter AS. Advances in radiation oncology. *Annu Rev Med*. 2006;57:19–31.
40. Prise KM, Schettino G, Folkard M, Held KD. New insights on cell death from radiation exposure. *Lancet Oncol*. 2005;6:520–528.
41. Nam SY, Chung H-Y The suppression of radiation-induced NF-kappaB activity by dexamethasone correlates with increased cell death in vivo. *Biochem Biophys Res Commun*. 2005;336:603–608.
42. Hempen C, Weiss E, Hess C. Dexamethasone treatment in patients with brain metastases and primary brain tumors: do the benefits outweigh the side-effects? *Support Care Cancer*. 2002;10:322–328.

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