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Ocular Dysfunction in a Mouse Model of Chronic Gut Inflammation

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Abstract

Background—Ocular pathology is well known to occur in a subset of patients suffering from the inflammatory bowel diseases (IBD). Although this extraintestinal manifestation has been recognized for a number of years, the pathogenetic mechanisms responsible for this distant organ inflammatory response are unknown.

Methods—In the current study, we used a T-cell transfer model of chronic colitis in mice in which we quantified colonic inflammation, ocular function (electroretinography), ocular blood flow (intravital microscopy of the retina), intraocular pressure, and retinal hypoxia.

Results—Ocular function in colitic mice was significantly impaired, with decreases in retinal b-wave amplitudes and oscillatory potentials. Moreover, retinal a-waves and oscillatory potentials were delayed. Retinal blood flow was significantly reduced in the colitic mice, and this decrease in perfusion coupled with significant decreases in hematocrit would decrease oxygen delivery to the eye. Accordingly, mice with severe colitis had increased levels of immunostaining for the hypoxia-dependent probe pimonidazole. Finally, intraocular pressures were found to be reduced in the colitic mice.

Conclusions—Ocular pathology occurs in a mouse model of chronic colitis, with retinal dysfunction appearing to be related to insufficient perfusion and oxygen delivery.

Keywords

Crohn's disease; colitis; eye; retina; blood flow; anemia

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Introduction

Ocular pathology is one of several extraintestinal manifestations of the inflammatory bowel diseases (IBD), with others including hematologic, rheumatologic, hepatobiliary, pulmonary, and dermatologic pathologies.^{1, 2} Ocular manifestations of IBD can include inflammation throughout the eye, including blepharitis, conjunctivitis, eyelid edema, scleritis, episcleritis, iridocyclitis, macular edema, choroiditis, optic neuritis, keratopathy, and cataracts.^{3, 4} Effects on ocular blood vessels include a spectrum of vasculitis, ischemia, hyperemia, neovascularization, and hemorrhaging.⁵ The disturbances in the eye can occur early, and ophthalmic symptoms will occasionally even precede the diagnosis of IBD.¹

Treatments aimed at reducing the primary gut inflammation can sometimes provide relief from the coexisting ocular maladies; unfortunately, many of the systemic and local treatments are not sufficiently effective, and can introduce undesired side effects (e.g., cataract formation and elevated intraocular pressure with steroid treatment¹). Developing the most appropriate therapy should begin with knowledge of the precise mechanisms causing the ocular pathology, however virtually nothing is known at the current time. Investigations into the pathogenetic mechanisms responsible for the ocular manifestations observed in IBD would be accelerated through the use of appropriate animal models of chronic intestinal inflammation that display this type of pathology; however no such studies have been reported. Data presented in the current study demonstrate, for the first time, that significant ocular dysfunction occurs in a well-defined, T cell-dependent model of chronic gut inflammation. Furthermore, we present data suggesting that this pathology may be related to tissue hypoxia produced by reductions in blood flow as well as decreased hematocrit in colitic mice. The implications of these novel findings are discussed.

Materials and Methods

Animals

Interleukin-10-deficient (IL-10^{-/-}), recombina-activating gene-1 deficient (RAG^{-/-}), and C57BL/6 mice were purchased from Jackson Laboratories (Bar Harbor, ME). The mice were housed on a 12-hour light/12-hour dark cycle in standard animal cages with filter tops under specific pathogen-free conditions in our animal care facility at Louisiana State University Health Sciences Center in Shreveport (LSUHSC-S). The mice were provided water and standard laboratory rodent chow ad libitum. All experimental procedures involving the use of animals were reviewed and approved by the Institutional Animal Care and Use Committee of LSUHSC-S and performed according to the criteria outlined by the National Institutes of Health.

T-cell transfer model of chronic colitis

Chronic colonic inflammation was induced in mice by the adoptive transfer of IL-10^{-/-} CD4⁺ T-cells into RAG^{-/-} recipients as previously described.⁶⁻⁹ Briefly, RAG^{-/-} recipient mice at an age of 2-3 months were injected with 10⁶ CD4⁺ T cells obtained from IL-10^{-/-} donor mice, with the T cells enriched (>90%; from single cell suspensions of splenocytes) by negative selection using a commercially available kit (Dyna; Invitrogen; Carlsbad, CA). Additional age-matched RAG^{-/-} mice and C57BL/6 mice were treated identically except for the injection of vehicle alone (phosphate-buffered saline, PBS) instead of T cells. The three groups of mice are abbreviated as IL-10^{-/-}CD4⁺→RAG^{-/-}, PBS→RAG^{-/-}, and PBS→C57BL/6. At 7-8 weeks post-injection, the mice were subdivided into 3 separate measurement protocols as described below, including studies of retinal function, blood flow, and hypoxia.

Retinal function - electroretinography

Mice were dark-adapted overnight, with the experimental procedures performed in a dark room with minimal red lighting (scotopic measurements). The mice were anesthetized with i.p. injections of 150 mg/kg ketamine and 10 mg/kg xylazine and the body maintained warm with a 38°C heating pad (Physitemp, Clifton, NJ). The pupil of the left eye was dilated with tropicamide ophthalmic solution (1%; Bausch & Lomb; Tampa, FL) and phenylephrine hydrochloride ophthalmic solution (2.5%; Bausch & Lomb) prior to placement of a contact lens electrode (LKC Technologies, Gaithersburg, MD) and a drop of Gonak (Akorn, Lake Forest, IL) on the cornea. The ERG ground electrode was a subcutaneous needle in the lower back, and the reference electrode was a subcutaneous needle placed along the jaw of the eye being measured. Recordings taken with a Biopac system (MP100 with Acknowledge software; Goleta, CA) were differentially amplified at $\times 1000$ and filtered 0.3-1000 Hz using a Grass P55 Preamplifier (Grass Technologies, West Warwick, RI). A Hum Bug noise eliminator (Quest Scientific, North Vancouver, BC) subtracted 60 Hz noise.

The mouse's left eye was positioned 10 inches from a light source (Grass PS33 photic stimulator, Grass Technologies, West Warwick, RI) capable of emitting light of various intensities. We used three light intensity settings on the stimulator (1, 4, and 16), with an additional lower intensity (0.1) obtained using an instrument setting of 1 in combination with neutral density glass (66ND9, Tiffen, Hauppauge, NY) placed immediately in front of the light source. These four light intensities were calibrated with an International Light Technologies meter (ILT1700, with SED033/ZCIE detector, Peabody, MA) as the following: $-3.3 \log \text{cd}\cdot\text{s}/\text{m}^2$ (setting 0.1); $-2.3 \log \text{cd}\cdot\text{s}/\text{m}^2$ (setting 1); $-2.0 \log \text{cd}\cdot\text{s}/\text{m}^2$ (setting 4); and $-1.4 \log \text{cd}\cdot\text{s}/\text{m}^2$ (setting 16). A white ping-pong ball cut in half was positioned over the eye to aid light dispersion. The time interval between flashes was 15 sec for the lower three intensities (performed first; starting with the lowest intensity) and 30 sec for the highest intensity (performed last). For all intensities, 10 measurements were recorded and averaged.

Figure 1 provides an example of an ERG measurement, from a control $\text{PBS} \rightarrow \text{RAG}^{-/-}$ mouse (flash intensity setting = 1). In the ERG signal, the voltage initially decreases (a-wave) followed by a positive voltage wave (b-wave), typically with four prominent oscillations (oscillatory potentials, OPs) found on the b-wave. ERG signals typically are analyzed for the wave amplitudes and implicit times, with the latter being defined as the time delay between the light flash and peak amplitude of the wave or oscillation. The b-wave amplitude is defined as the voltage difference between the peak and the a-wave minimum, and OP amplitudes are defined as the voltage difference between each peak and the preceding minimum (trough). The oscillatory potential waveforms are of a higher frequency than the a- and b-waves, and were separately analyzed after filtering the ERG signal with a bandpass filter (40-300 Hz for intensity settings 0.1 and 1; 50-300 Hz for settings 4 and 16). As presented by others,^{10, 11} we combined the oscillatory potential data into a summation of the first four oscillations on the b-wave. Once the ERG measurements were completed, intraocular pressure (IOP) was measured by tonometry (Tonopen Vet; Reichert Inc.; Depew, NY).^{12, 13} At the conclusion of the experiment, a blood sample was drawn from the carotid artery for measurements of hematocrit, and the mouse was euthanized with 150 mg/kg pentobarbital. The hematocrit and other measurements obtained from the blood samples have been reported as part of a separate study.¹⁴

Retinal blood flow – intravital microscopy

Intravital microscopy was used to measure retinal hemodynamics as we have published previously.^{13, 15-17} The mice were anesthetized with i.m. injections of 50 mg/kg pentobarbital and 50 mg/kg ketamine, and the right femoral vein was cannulated for

vascular infusions. The mouse was placed on the stage of a Nikon Eclipse E600FN microscope (Nikon Instruments Inc.; Melville, NY), with the left eye under the objective at a position that allowed visualization of the retinal arterioles and venules extending out of (and into) the optic disk. Through the femoral vein cannula, a 40-50 μ l bolus of 4.5 mg/kg fluorescein isothiocyanate (FITC)-dextran was infused and the retinal vasculature was observed under 4 $\times$ magnification. The retinal arterioles were identified as the vessels filling first with the dye, with the venules filling after the dye traveled through the capillary bed. Approximately 2-4 minutes later, the diameters of the retinal arterioles and venules were video-recorded with a CoolSNAP ES digital camera (Photometrics, Tucson, AZ) using a 10 $\times$ objective and fluorescein filter. Red blood cell (RBC) velocities were measured using fluorescently labeled (1,1'-dioctadecyl-3, 3,3',3'-tetramethyl-indocarbocyanine perchlorate; DiI; Invitrogen Molecular Probes, Eugene, OR) erythrocytes obtained from donor C57BL/6 mice as we have described previously in greater detail.^{13, 15} The DiI-labeled RBCs appeared as fluorescent streaks in the vessels, with the length of the streak proportional to RBC velocity, with velocity calculated as the streak length divided by the camera exposure time (10 ms). Measurements of the diameters (D) and RBC velocities were performed using NIS Elements Basic Research software (Nikon Instruments, Melville, NY). Retinal blood flow was calculated as $V\pi D^2/4$, with V being the mean RBC velocity obtained from ~ 10 fluorescent RBC streaks per vessel. Blood flow for the entire retina was obtained by summing the flows in each of the arterioles (and separately, venules) and averaging the total arteriolar and venular flows. Each retina had 4-7 arterioles and 4-7 venules. At the conclusion of the experiment, the mice were euthanized with 150 mg/kg pentobarbital.

Retinal hypoxia – immunostaining of retinal cross-sections

Mice were injected i.p. with 60 mg/kg pimonidazole (Hypoxyprobe; National Pharmacia International; Burlington, MA). One hour later, the eyes of the mice were excised under deep anesthesia with 150 mg/kg ketamine and 10 mg/kg xylazine, and a blood sample was obtained via the right carotid artery, with hematocrit and other blood measurements also reported in a separate study.¹⁴ The left eye was placed in 4% paraformaldehyde for 24-48 hr prior to embedding in paraffin and cross-sectioning at 5 μ m thickness. Sections were labeled with Hypoxyprobe rabbit antiserum as a primary antibody (1:3000 dilution); the secondary antibody was anti-rabbit immunoglobulin conjugated to horseradish peroxidase (1:50 dilution; DakoCytomation, Carpinteria, CA). Other sections were labeled with HIF-1 α rabbit polyclonal primary antibody (1:500 dilution; LifeSpan BioSciences; Seattle, WA); the secondary was rabbit polyclonal antibody immunoglobulin conjugated to horseradish peroxidase (1:500 dilution; Abcam; Cambridge, MA). Negative controls were performed in the absence of the primary antibody, with no staining present. Staining intensities of various layers of the retina were quantified with Image J software (v. 1.4; National Institutes of Health), using the tissue approximately halfway between the optic disk and the peripheral termination of the retina.

The colons were excised and fixed in 10% buffered formalin for 10 min, embedded in paraffin, and later sectioned at 5 μ m thickness. For histological scoring of pathology, the sections were stained with hematoxylin and eosin (H&E). The slides were scored by a blinded pathologist (S.Z.) using our previously published scoring criteria,⁸ based on the degree of inflammatory infiltrate in the lamina propria (0-3 scale), Goblet cell loss (0-2), neutrophils per 40 $\times$ field (0-4), abnormal crypt architecture (0-3), number of crypt abscesses (0-1), mucosal to frank erosion (0-1), and submucosal to transmural involvement (0-3) for a total score ranging from 0-17. As published previously,⁸ a total score in the range of 1-5 is classified as mild colitis, 6-10 is moderate colitis, and 11-17 is severe colitis.

Statistics

Groups were compared using analysis of variance (ANOVA) with Student-Newman-Keuls post-hoc corrections (GraphPad software, San Diego, CA). Values are presented as means $\pm$ standard error.

Results

Blinded histopathological evaluation revealed that reconstitution of RAG^{-/-} mice with IL10^{-/-}CD4⁺ T-cells induced consistent colonic inflammation ranging from mild to severe disease (Figure 2). Of the 26 RAG^{-/-} mice injected with IL10^{-/-}CD4⁺ T cells, 12 mice were found to have severe colitis, with 6 and 8 mice exhibiting moderate and mild disease, respectively, as assessed by the blinded score (Fig 2). No significant inflammation was observed in the two PBS-injected control groups.

We determined that colitis in the IL10^{-/-}CD4⁺→RAG^{-/-} mice was associated with a decrease in retinal function, as assessed by electroretinography (ERG). Figure 3 provides example ERG signals from a PBS→RAG^{-/-} mouse and an IL10^{-/-}CD4⁺→RAG^{-/-} mouse, with the signals from each mouse obtained at four flash intensity settings (0.1, 1, 4, and 16, as described in the Methods). ERG data from all three groups of mice (N=10-12 per group) are compiled into Table 1, with these data demonstrating significant retinal changes in the colitic mice.

As shown in Table 1, the ERG a-waves were delayed in the IL10^{-/-}CD4⁺→RAG^{-/-} mice, as indicated by the increase in a-wave implicit times ($p < 0.05$ vs PBS→RAG^{-/-} and PBS→C57BL6 mice at two of the four flash intensities). However, a-wave amplitudes were not different among the three groups. The ERG b-wave amplitudes were significantly reduced by about 30% in the IL10^{-/-}CD4⁺→RAG^{-/-} mice compared to the two control groups ($p < 0.05$ at two of the four flash intensities), although the b-wave implicit times were similar among the three groups. Finally the oscillatory potentials were not only lower in amplitude (by ~25-40%) in the IL10^{-/-}CD4⁺→RAG^{-/-} mice, but were also delayed (increased implicit times) with respect to the two control groups. No differences were observed in the ERG parameters between the two PBS-injected control groups, indicating that the non-colitic lymphocyte-deficient RAG^{-/-} mice do not have any apparent retinal dysfunction.

Deficient retinal signaling can result from a number of causes, one of which is insufficient retinal perfusion. Our intravital microscopic measurements of the retina indicated that both arteriolar and venular RBC velocities decreased by ~30% in the IL10^{-/-}CD4⁺→RAG^{-/-} mice compared to the two PBS-injected control groups ($p < 0.01$; see Table 2), but with no change in arteriolar or venular diameters. The decrease in the calculated retinal blood flow rates in the IL10^{-/-}CD4⁺→RAG^{-/-} mice (shown in Figure 4) was therefore similar to the decrease in RBC velocity, that is, ~30% compared to the two PBS-injected controls. No differences in any of the microvascular parameters were observed between the two PBS-injected control groups.

Retinal perfusion is proportional to the hydrostatic gradient between the arterial pressure head and the pressure of the intraocular fluid. With respect to the latter, elevated intraocular pressure (IOP) can decrease retinal perfusion. However, the decrease in retinal perfusion observed in the IL10^{-/-}CD4⁺→RAG^{-/-} mice does not seem to be attributable to changes in IOP: as shown in Figure 5, IOP is decreased in colitic IL10^{-/-}CD4⁺→RAG^{-/-} mice ($p < 0.01$ vs both control groups), and also slightly decreased in non-colitic PBS→RAG^{-/-} mice (by ~2 mmHg).

As reported elsewhere,¹⁴ the circulating hematocrit differs among these groups of mice, with the value in the IL10^{-/-}CD4⁺→RAG^{-/-} mice (33±1%) significantly lower than in either the PBS→RAG^{-/-} mice (41±1%) or PBS→C57BL6 mice (41±1%). With this 20% decrease in the circulating RBC concentration in the colitic mice, combined with a 30% decrease in retinal perfusion, we hypothesized that retinal oxygenation could be diminished in the IL10^{-/-}CD4⁺→RAG^{-/-} mice. Figure 6 presents oxygen-dependent measurements in the retina, that is, immunostaining intensities for the hypoxia-dependent probe pimonidazole (Hypoxyprobe) and the hypoxia-dependent factor HIF-1α. Increases in Hypoxyprobe staining in the IL10^{-/-}CD4⁺→RAG^{-/-} mice were observed in three out of the four retinal layers analyzed (inner plexiform layer, outer nuclear layer, and the inner segment of the photoreceptors), and an increase in HIF-1α staining was also observed in the inner segment of the photoreceptors. However, this evidence for hypoxia was only observed in the subset of IL10^{-/-}CD4⁺→RAG^{-/-} mice with more severe colonic inflammation (pathology scores between 13-16; see Figure 2), with no significant changes in these indicators of retinal hypoxia in the mice with mild-to-moderate inflammation.

Discussion

To our knowledge, this is the first report describing ocular dysfunction in an animal model of chronic intestinal inflammation. In this study, we determined that chronic colitis, induced by the transfer of IL10^{-/-}CD4⁺ T cells into lymphopenic mice, was associated with remarkable decreases in retinal blood flow, retinal function, and intraocular pressure, and increases in retinal hypoxia.

Decreases in retinal blood flow rate can be the consequence of either decreased ocular perfusion pressure (OPP), or increased resistance to flow of the retinal microcirculation. OPP is defined as mean arterial pressure (MAP) minus intraocular pressure (IOP),¹⁸ with the value of MAP sometimes adjusted in the calculation (lowered by 33%) to allow for the drop in pressure prior to the entrance into the optic disk.¹⁹ We do not believe that there was a substantial change in OPP, as defined by (0.67×MAP)-IOP, with a small decrease in MAP (not statistically significant, in our previous study of this model⁶) offset by a small decrease in IOP. However, we cannot exclude the possibility that perfusion pressure entering the optic disk could have been diminished by any potential obstruction of the retinal artery associated with colitis (not evaluated in this study).

In addition to the effect of ocular perfusion pressure, retinal blood flow also can be influenced by microvascular resistance, with a decrease in flow induced by either microvascular constriction, a decrease in capillary density, or an increase in blood viscosity. The latter is not likely to have occurred in this model, with hematocrit decreasing significantly by ~20%. In addition, arterioles and venules on the inner retinal surface were not found to be smaller than normal (Table 2), although our intravital microscopy techniques did not allow us to evaluate any potential constriction downstream of these vessels toward the mid-retina. Therefore, the cause of the decrease in retinal blood flow still needs further evaluation.

ERG a-waves are derived primarily from photoreceptors, b-waves primarily from bipolar and Müller cells, and oscillatory potentials primarily from amacrine cells. In the colitic mice, we observed alterations in either the amplitude and/or implicit time of each measured ERG wave/oscillatory potential, indicating possible neuronal dysfunction in multiple layers of the retina. A decrease in retinal blood flow can hamper visual function, as detected by attenuated ERG signals.^{20, 21} In fact, the b-wave (significantly attenuated in our study; Table 1) has been used as an index of retinal ischemia.²⁰ In addition, visual function is impaired by a drop in hematocrit, with reports of decreased ERG signaling with anemia,²²⁻²⁵ a condition

affecting 40% of adults with IBD and 70% of children with IBD.²⁶ The importance of a good examination of the posterior eye in these patients has been emphasized by Felekis et al.,²⁷ who reported 8/26 IBD patients with posterior segment manifestations (either choroiditis, retinal vasculitis, optic neuritis, retinal pigment epithelium disturbances, or serous retinal detachment).

A combination of a 20% drop in hematocrit with a 30% drop in retinal blood flow rate could be expected to decrease retinal arterial oxygen delivery by up to 40-50% ($0.8 \times 0.7 = 0.56$). This decrease could significantly hamper the function of retinal neurons, which are highly active and consume large quantities of oxygen. To supply the retina with sufficient oxygen, the tissue is fed by two separate capillary systems, the retinal microcirculation (for the inner retina) and the choroidal microcirculation (for the outer retina). Yu & Cringle²⁸ have measured oxygen levels throughout the mouse retina using microelectrodes, showing that oxygen levels drop precipitously from the choroid (40 mmHg) to below 10 mmHg toward the middle of the retinal tissue, with pO_2 returning to > 20 mmHg at the inner retinal surface (the location of the arterioles and venules studied in our intravital microscopy experiments). The inner plexiform layer (IPL) and the inner segment of the photoreceptors (PR-IS) normally have oxygen tensions just above 10 mmHg, which was useful in our Hypoxyprobe assay, inasmuch as pimonidazole sensitivity to oxygen begins with oxygen tensions below that value. In the event that oxygen delivery to the retina decreases, the inner plexiform layer and inner photoreceptor segment would be highly sensitive areas to detect increased hypoxia with the probe, and in fact, Figure 6 shows the largest two increases in staining in those two layers. Although the pattern of changes in HIF-1 α was reasonably similar to that of Hypoxyprobe, the changes in the hypoxia inducible factor did not reach statistical significance in most retinal layers. However, it should be mentioned that changes in HIF-1 α are time-dependent, and the timing of our data collection might not have coincided with a peak change in the protein's expression. For example, the peak increase in HIF-1 α has been reported to occur at approximately 12 hours post-occlusion of the central retinal artery, with the acute upregulation of the molecule 3-fold higher than at the 21-day time point post-occlusion.²⁹

The current study focused primarily on the retinal dysfunction associated with colitis, but it is likely that pathologies could be detected in this mouse model in other areas of the eye. Further use of this model may help determine the mechanisms of the ocular manifestations of colitis, with potential applications to human IBD patients.

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Figure 1. Example of an electroretinogram (ERG) signal obtained from a dark-adapted mouse. ERG parameters evaluated in this study included the initial negative deflection (a-wave), the large positive amplitude (b-wave), and the voltage oscillations, called oscillatory potentials (OP), on the b-wave.

Figure 2. Blinded pathology scores of colitis in the three experimental groups. The IL10^{-/-}CD4⁺→RAG^{-/-} mice had significantly (p<0.001) higher indices of pathology than either of the control groups.

Figure 3.

Example ERG signals obtained from a PBS→RAG^{-/-} mouse and an IL10^{-/-}CD4⁺→RAG^{-/-} mouse. The ERG signals were obtained using four increasingly brighter series of light flashes, with the flash intensity settings described in the Methods. The ERG signals (especially the b-waves and oscillatory potentials) were attenuated in most of the colitic IL10^{-/-}CD4⁺→RAG^{-/-} mice of the study.

Figure 4.

Retinal blood flow rates were significantly attenuated in the colitic IL10^{-/-}CD4⁺→RAG^{-/-} mice compared to the two non-colitic control groups. Data are provided as means ± SE. **p<0.01 vs PBS→C57BL6 and ++p<0.01 vs PBS→RAG^{-/-}. N=8, 7, and 10 mice per group from left to right.

Figure 5.

Intraocular pressures (IOP) were significantly attenuated in the colitic IL10^{-/-}CD4⁺→RAG^{-/-} mice compared to the two non-colitic control groups. In addition, IOP was slightly decreased in PBS→RAG^{-/-} mice compared to PBS→C57BL6 mice. Data are provided as means ± SE. **p<0.01 and ***p<0.001 vs PBS→C57BL6 and ++p<0.01 vs PBS→RAG^{-/-}. N=12, 11, and 11 mice per group from left to right.

Figure 6.

Immunostaining analysis of retinal cross-sections for Hypoxyprobe and HIF-1α, with the staining intensities quantified in four layers of the retina: the inner plexiform layer (IPL), the inner and outer nuclear layers (INL, ONL) and the inner segment of the photoreceptors (PR-IS). As indicated on the figure, the data from the colitic IL10^{-/-}CD4⁺→RAG^{-/-} mice were segregated according to the severity of histologic pathology scores. Data are provided as means ± SE. *p<0.05 vs PBS→C57BL6 and +p<0.05 vs PBS→RAG^{-/-}. N 9 mice per group.

Table 1

Scotopic electroretinogram data from the left eye of the indicated groups. Data from the first four oscillatory potentials on the ascending b-wave are summed (Σ OP). N=10-12 per group.

Flash intensity	PBS→C57BL6	PBS→RAG ^{-/-}	IL10 ^{-/-} CD4 ₊ →RAG ^{-/-}	Statistics
a-wave amplitude (mV)				
0.1	0.10 ± 0.004	0.08 ± 0.006	0.08 ± 0.011	NS
1	0.19 ± 0.008	0.18 ± 0.011	0.17 ± 0.016	NS
4	0.28 ± 0.012	0.27 ± 0.014	0.25 ± 0.019	NS
16	0.39 ± 0.015	0.39 ± 0.018	0.34 ± 0.027	NS
b-wave amplitude (mV)				
0.1	0.32 ± 0.03	0.31 ± 0.03	0.21 ± 0.04	* +
1	0.42 ± 0.02	0.43 ± 0.03	0.30 ± 0.05	NS
4	0.52 ± 0.03	0.55 ± 0.04	0.38 ± 0.06	* +
16	0.65 ± 0.03	0.68 ± 0.04	0.49 ± 0.08	NS
Σ OP amplitudes (mV)				
0.1	0.35 ± 0.05	0.30 ± 0.03	0.22 ± 0.04	NS
1	0.56 ± 0.04	0.54 ± 0.06	0.35 ± 0.06	* +
4	0.70 ± 0.05	0.72 ± 0.08	0.47 ± 0.08	* +
16	0.79 ± 0.05	0.84 ± 0.08	0.64 ± 0.09	NS
a-wave implicit time (ms)				
0.1	16.2 ± 0.6	16.8 ± 0.5	18.0 ± 0.8	NS
1	12.8 ± 0.3	13.1 ± 0.4	14.6 ± 0.5	** +
4	12.2 ± 0.1	12.6 ± 0.2	13.3 ± 0.4	** +
16	11.0 ± 0.2	11.4 ± 0.1	11.6 ± 0.3	NS
b-wave implicit time (ms)				
0.1	51.8 ± 1.7	53.8 ± 1.7	56.4 ± 2.3	NS
1	41.5 ± 1.7	43.0 ± 1.9	45.9 ± 2.2	NS
4	49.0 ± 2.2	49.7 ± 2.5	49.0 ± 2.8	NS
16	51.9 ± 0.8	53.8 ± 0.9	54.3 ± 2.0	NS
Σ OP implicit times (ms)				
0.1	173 ± 6	180 ± 6	194 ± 9	NS
1	156 ± 3	164 ± 4	179 ± 8	** +
4	150 ± 2	157 ± 4	172 ± 7	** +
16	143 ± 1	149 ± 3	157 ± 4	** +

Statistical symbols:

NS; no statistically significant differences between groups

*
p < 0.05;

**
p < 0.01 vs PBS→C57BL6

⁺
p < 0.05 vs PBS → RAG^{-/-}

Table 2

Intravital microscopy measurements of retinal RBC velocities and diameters in the indicated groups of mice (N=7-10 mice per group). Data are provided for both arterioles and venules.

	PBS→C57BL6	PBS→RAG ^{-/-}	IL10 ^{-/-} CD4 ⁺ →RAG ^{-/-}	Statistics
Arteriolar RBC velocity (mm/s)	32 ± 1	33 ± 3	24 ± 1	*** ++
Venular RBC velocity (mm/s)	29 ± 1	28 ± 2	22 ± 1	** ++
Arteriolar diameter (μm)	61 ± 2	59 ± 2	59 ± 1	NS
Venular diameter (μm)	71 ± 2	71 ± 3	70 ± 2	NS

Statistical symbols:

NS; no statistically significant differences among groups.

**
p < 0.01;

p < 0.001 vs PBS→C57BL6

++
p < 0.01 vs PBS→RAG^{-/-}