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The Role of CB2 Receptors in Proliferative Vitreoretinopathy

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Abstract

Proliferative vitreoretinopathy (PVR) is known to be the most common sight-seeing complication of retinal detachment. The pathogenesis of PVR is characterized by the proliferation and migration of retina pigmented epithelial cells, which leads to formation of contractile cellular membranes and retinal breaks, as well as immune cell infiltration. The Endocannabinoid system modulates immune response; cannabinoid receptor 2 (CB2) is expressed in the cells of the immune system, but also found in the retina. The activation of the CB2 receptor by endogenous or exogenous ligands produces biological activities of immune function; the expression of this receptor is up-regulated by the activation of various inflammatory triggers. The purpose of this study is to further understand what role the CB2 receptor plays during inflammation in the retina and if it is involved in the mediation of immunosuppressive effects.

An animal model (mice) was used to induce PVR with an intraocular injection (0.2μl) of 0.1U dispase, a proteolytic enzyme. The severity of PVR in wild type (WT) and CB2−/− saline and dispase groups were evaluated through the observation of cross-sectioned H&E stained ocular tissues. IL-1β cytokine concentrations were captured using an enzyme linked immunosorbent assay with IL-1β sensitive antibodies.

Histological H&E staining demonstrated pronounced ocular damage in CB2−/− animals compared to WT animals. IL-1β cytokine concentrations showed no significant difference (p>0.05) between WT and CB2−/− animals. From the results, the ocular damage was more pronounced in CB2−/− mice as compared to WT mice, which suggests an immunodulatory role for CB2 receptors.

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**Introduction**

The eye is a complex organ with many structures that are ultimately responsible for vision (Figure 1). It is covered by three layers: the outer layer consists of the anterior transparent cornea and the posterior white fibrous protective sheet known as the sclera. The middle layer, choroid, is found between the sclera and the retina and is the vascular layer of the eye which provides nutrients to the outer retina (Lens et al., 2008). The most inner layer is the retina, the site of transformation of light energy into an electrical neural signal. The retina provides an output of information or stimuli to the brain via axons of ganglion cells which is commonly known as the optic nerve (Jenkins, 2010). Before light reaches the retina, it enters the eye through the pupil, and then through the lens, which focuses images from different distances on retina. The vitreous humor which is a clear gel-like substance, composing approximately 80% of the volume of the eye, is positioned between the lens and the retina.
Figure 1: (a) Sagittal section of the whole eye and (b) magnification of retinal layers (www.nei.nih.gov, 2010).
Proliferative Vitreoretinopathy

Three decades ago what was termed massive periretinal proliferation was known to be a serious complication of post-retinal surgery with severe ocular trauma or intraocular inflammation. The owl monkey was used in experimental studies to demonstrate that massive periretinal proliferation was due to retinal detachment (Canto Seoler et al., 2002). Following further studies, this condition was modified away from the Machemer classification in 1978, and in 1983 the condition was coined as proliferative vitreoretinopathy (PVR) by the Retina Society Terminology Committee (Pastor, 1998). Today, PVR is known to be the most common sight-seeing complication of retinal detachment (Pastor, 1998) that may lead to blindness. The pathogenesis of PVR is characterized as the growth and contraction of cellular epiretinal membranes within the hyaloids, on the inner and outer retinal surfaces and within the vitreous humor (Limb, 1991; Pastor, 1998). As the cellular membranes employ traction, this can create retinal breaks. This in turn cause a traction retinal detachment, macula distortion and impaired vision. Canataroglu (2005) and Nagasaki et al. (1998) add to Pastors characterization by indicating that the contraction of cellular membranes cause retinal detachment. This results from the proliferation and migration of trans-differentiated retina pigmented epithelial (RPE) cells and the breakdown of the blood-ocular barrier. In addition, the pathology of PVR involves immune cell activation and their infiltration of ocular tissues. The infiltrating immune cells include glia cells (Müller cells, astrocytes, and microglia) and inflammatory cells (macrophages and lymphocytes) (Nagasaki et al., 1998).
**Inflammation**

Acute inflammation is a natural and essential response to injury, stress and infection. This innate process provides protection, tissue maintenance and homeostasis by recruiting several immune cells from local vasculature (Gronert, 2010). The cell types that are typically involved in the early onset of an inflammatory response are macrophages, central nervous system microglia and phagocytic cells (neutrophils) which release pro-inflammatory cytokines, such as tumour necrosis factor alpha (TNF-\(\alpha\)) and interleukin 1 beta (IL-1\(\beta\)), amongst others. These cells are responsible for maintaining the inflammatory response by up-regulating the secretion of immune cells through autocrine signaling and self-synthesizing (Kaiser, 2012; Collins, 2012).

Chronic or exaggerated inflammation on the other hand is detrimental as extensive tissue damage and scarring can occur. With prolonged permeability of vasculature to cytokines and neutrophils, blood will accumulate at the site of injury. Lysosomal contents from neutrophils will be discharged affecting surrounding healthy tissue to be destroyed and eventually turn to scar tissue (Kaiser, 2012).

**Macrophages, Microglia and Cytokines**

Macrophages are mediators in innate and adaptive immunity. Their effects occur through recognition, phagocytosis and destruction of cellular and tissue debris and foreign agents (Croxford and Yamamura, 2005; Pastor et al., 1998). Macrophages secrete inflammatory mediators, such as nitric oxide (NO) to kill bacteria, growth factors and cytokines to carry signals to other cells (Croxford & Yamamura, 2005). Microglia cells are phenotypically and functionally related to macrophages (Croxford & Yamamura,
2005). Microglia normally reside in a quiescent state in the healthy central nervous system (CNS) and make up 10% of the total glial adult CNS cell population. In response to injury or inflammation microglia undergo morphological changes to become amoebalike and proliferate. Once activated, they produce proinflammatory factors including various cytokines, chemokines, prostaglandins and nitric oxide and up-regulate surface antigens (Town, 2005).

Cytokines are known as local signaling molecules that are released in responses to inflammation in an autocrine, paracrine and endocrine fashion. One method of cytokine classification is dividing them into pro-inflammatory and anti-inflammatory groups. Pro-inflammatory cytokines are those that amplify inflammation responses due to infection or injury, whereas, anti-inflammatory cytokines are those that inhibit or limit inflammation responses. The growth factors and cytokines secreted by microglia include pro-inflammatory cytokines such as interferon gamma (IFN-γ), IL-1β and TNF-α (Nagasaki, 1998; Town, 2005).

IFN-γ possesses antiviral activity and plays a central role in immunoregulatory processes. This cytokine is involved in host defenses against unwarranted intracellular organisms and has the ability to activate macrophages and enhance their ability of phagocytosis. IL-1β is produced by activated macrophages and is an important mediator during an inflammatory response. This cytokine is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis. However, if there are high concentrations of this cytokine, it can cause increased inflammation and degeneration of neurons (Russell, 2004). Lastly, TNF-α is also secreted primarily by macrophages. It is a key player in the local chronic inflammatory immune response and
initiates a cascade of cytokines and increases vascular permeability, thereby recruiting macrophage and other immune cells to the site of infection (Goldsby, 2003).

While microglia play an important role in host defense and tissue repair, the chronic activation of these cells results in deleterious effects or extensive “bystander damage” on the surrounding neurons depending on the intensity and temporal activation of microglia. The destructive properties of the long-term activation of microglia have been demonstrated in neurodegenerative diseases such as, but not limited to, multiple sclerosis, Alzheimer’s and Parkinson’s (Russell, 2004). Microglia and the IL-1β cytokine are the main focus in this research due to their assisting and degenerative properties during an immune response to inflammation. These assumptions are based on a growing body of evidence that suggests the endocannabinoid system modulates the activity of microglia and cytokines and can be neuroprotective in a variety of inflammatory conditions.

**Endocannabinoid System**

*Cannabis sativa* (marijuana plant) has been used by humans for thousands of years due to its psychoactive and medicinal properties. The main psychoactive component of the cannabinoid plant Δ⁹-tetrahydrocannabinol (Δ⁹-THC) has been identified (Gaoni and Mechoulam 1964; Mechoulam 1970), allowing rapid advancement in cannabinoid research.

The endocannabinoid system is known as the “late comer” of the neuromodulatory family (Pacher, 2011). This system is composed of neuromodulatory lipid based endogenous ligands, enzymes responsible for their synthesis and degradation,
and cannabinoid receptors 1 and 2 (CB1 and CB2) (Svizenska, 2008). The endogenous ligands are arachidonoylethanolamide (AEA) (also known as anandamide) and 2-arachidonyl-glyceryl (2-AG), both of which are physiological agonists for the cannabinoid receptors. The CB1 receptor was identified and cloned in 1990 from a clone DNA library. This receptor codes for 473 amino acids, and is a G-protein coupled receptor (Klein, 1998). The CB1 receptor is the most abundant G-protein coupled receptor within the nervous system, primarily within the brain on presynaptic neurons. AEA has high binding affinity to the CB1 receptor and is mainly synthesized (on demand) by postsynaptic neurons which act as retrograde messengers to modulate neurotransmitter release from CB1-expressing presynaptic terminals. The binding of AEA to the CB1 receptor controls and mediates the behavioural, pharmacological and psychoactive effects of cannabinoid usage, as well as pain and synaptic neurotransmission (Tanasescu 2010; Croxford and Yamamura 2005; McAllister 2002). The activation of the CB1 receptor can be thought of as protective to the nervous system from over-activation or over-inhibition of neurotransmitters.

The CB2 receptor was identified by cloned DNA using the polymerase chain reaction and showed a 44% similarity to the CB1 receptor. Unlike CB1, the CB2 receptor only has 360 amino acids but also uses G-proteins (Klein, 1998; Svizenska et al., 2008). The CB2 receptor is predominantly expressed in the cells of the immune system, including microglia cells, but is also found in the retina (Svizenska, 2008). In microglia cells, the expression of the CB2 receptor is upregulated by the activation of various inflammatory triggers such as damage and invasion of bacteria or a virus. Therefore, decreases the microglia reactivity (Pacher, 201; Croxford and Yamamura, 2005). The 2-
AG endocannabinoid ligand exhibits high affinity for the CB2 receptor and when bound together show biological activities of immune function, cell proliferation, neuroprotection, neuromodulation, and inflammatory responses (Svizenska 2008; Tanasescu 2010). It is therefore possible that the CB2 receptor plays an immunomodulatory role in the retina.

Δ⁹-THC has been known to have effects on immune function and the suppression and inhibition of macrophage secretion of the pro-inflammatory cytokines such as TNF-α, IFN-γ and IL-1β (Tanasescu, 2010; Eisenstein, 2007).

For the purposes of this research, PVR will be induced by the intravitreal injections of dispase in the eyes of adult mice in order to observe if the CB2 receptor has immunomodulatory effects against the IL-1β cytokine. Dispase is a proteolytic enzyme derived from *Bacillus polymyxa* that cleaves fibronectin and collagen IV, and therefore results in cleavage of the basement membrane in various tissues (Tan 2012; Valeria Canto Soler 2002). More important for this research, dispase also creates an inflammatory response causing ocular changes in the vitreous, retina and anterior chamber where several immune cells aggregate and are actively involved in the site of injection. Therefore, dispase serves as a useful model for our study.

*Hypothesis and Objectives*

The overall objective of my research is to investigate the role of CB2 receptor modulation in the animal model of PVR. I will use wild type (WT) and CB2 receptor knockout (CB2-/−) mice to examine the role of the endocannabinoid system and its receptors during an inflammatory response in the retina. The activation of the CB2
receptor decreases the inflammatory response associated with PVR and therefore preserves retinal integrity. Animals lacking the CB2 receptor should show no defense or down regulation of harmful cytokines and microglia. To observe the behavior of the CB2 receptor, this study will:

(1) compare ocular pathology in a WT vs. CB2-deficient mouse model of PVR and,

(2) examine the expression of the pro-inflammatory cytokine, IL-1β, in WT vs. CB2-deficient mouse model of PVR.

The significance of this research is to further understand what role the CB2 receptor plays during inflammation in the retina and if it is involved in the mediation of immunosuppressive effects.

Methods

Animals

C57BL/6 male mice (20-25 g; Charles Rivers, QC, Canada), and CB2 receptor knockout animals (20-25g; in-house breeding) were used for experiments. The animals were housed on a 12 hour light/dark cycle, with unrestricted access to food and water. All experiments were conducted in accordance with the standards and procedures of the Canadian Council on Animal Care and the Dalhousie University Animal Care Committee.
Experimental groups and Injections

The mice were divided into four groups: WT injected with saline, WT injected with 0.1U dispase, CB2\(^{-/-}\) injected with saline and CB2\(^{-/-}\) injected with dispase. Each group contained three mice, therefore a sample size of 12 mice in total. PVR was induced in CB2\(^{-/-}\) mice and wild type (Blk57) animals with an intraocular injection of dispase (Sigma), a neutral protease, which cleaves the basement membrane of the dorso-lateral quadrant of the left eye which is the active site of this enzyme. Dispase was diluted to the concentration of 0.1U \(\mu l^{-1}\) in a sterile Ringer saline solution. Intraocular injections (2\(\mu l\)) were made using a microscope with a Hamilton syringe attached to a 30 G needle. Control animals received 2\(\mu l\) of sterile Ringer saline solution.

Twenty four hours following the intraocular dispase or saline injection mice were sacrificed with an intraperitoneal injection of 0.2ml euthanyl (pentobarbital sodium) and the left eye was enucleated from each animal. Each left retina was either placed in a numbered Eppendorf tube suspended in Dulbecco’s phosphate buffered saline (PBS) and then used for protein preparation, or the whole eye was removed, and prepared for histological staining.

Tissue Digestion and Protein Extraction

A preparation of standard proteins of known concentrations and proteins from retinal tissue were used to quantify the amount of protein present in each retina in order to make a protein standard curve. Protein extraction was carried out by homogenizing the retinal tissue in a RIPA solution containing protease inhibitors. The RIPA solution comprises of approximately 400mL distilled water, 5mL Triton X-100, 0.5g 20% SDS,
4.39g NaCl, 1.21g Tris-HCl base and 2.5g deoxycholic acid (pH 8 must be reached in order for deoxycholic to dissolve).

The protease inhibitors used and mixed into the RIPA solution (RIPA+) above are 1μl/ml Pepstatin A, 1 μl/ml Leupeptin, 5 μl/ml PMSF and 2mg/ml Iodoacetamide. These contents are only added to RIPA on the day that it is needed.

Once the RIPA+ solution was made, the Dulbeccao’s phosphate buffered saline solution that the mouse retinas were suspended in was replaced with 100μl of RIPA+. Tubes were then incubated on ice for 20 minutes and centrifuged for 15 minutes at 4°C at 13000rpm. The supernatant was transferred to fresh Eppendorf tubes and stored at -80°C.

Protein Quantification Assay

Thermo Scientific Pierce® BCA Protein Assay Kit was used to create a Bovine Serum Albumin (BSA) standard curve based on absorbance and known concentrations. This curve was then used to estimate the amount of protein present in each of our retina samples based on their absorbance reading at 562nm. Briefly, each sample with an unknown protein concentration had 5μl pipetted from the original tube to a clean Eppendorf tube which contained 45μl of deionized water and 50μl of 1% SDS. A volume of 1ml of BCA working reagent was added to each tube and incubated for 30 minutes. Samples and standards were transferred to a microplate for the absorbance to be read at 562nm. A standard curve was made with the seven standards and subsequently the protein concentrations of the samples were calculated based on this standard curve.
Enzyme-Linked Immunosorbent Assay (ELISA)

The ab100690 Interleukin-1 beta Mouse ELISA Kit from Abcam was performed using retinal tissue lysates from individual mice.

ELISA is used to detect and measure an antibody or antigen of interest. The most commonly used ELISA is the antibody-sandwich. The Abcam ELISA kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of mouse interleukin-1 beta (IL-1β) in cell lysate and tissue lysate. This assay employs an antibody specific for Mouse IL-1β coated on a 96-well plate. Standards and samples prepared by the protocol of Abcam were pipetted (100µl) into the wells and if any IL-1β was present in a sample it bound to the wells by the immobilized antibody after being incubated at room temperature for 2.5 hours. The wells were washed and biotinylated anti-Mouse IL-1β antibody was added (100µl) and incubated for one hour at room temperature. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin was pipetted (100µl) to the wells and incubated at room temperature for 45 minutes. The wells were again washed and 100µl of a TMB (3,3’,5,5’-tetramethylbenzidine) substrate solution was added to the wells and colour developed in proportion to the amount of IL-1β bound. The Stop Solution was added (50µl) to each well and is responsible for changing the colour from blue to yellow, depending on the IL-1β concentration. The intensity of colour was measured immediately by a spectrophotometer at 450nm.
**Histological Samples**

Animals were sacrificed 24 hours after the intraocular injection, eyes were enucleated and inserted into a cassette and immersed in 10% formalin for 24 hours to preserve the tissue. The formalin was then washed out with 70% ethanol three times and eyes were suspended and dehydrated in a container of ethanol then embedded in paraffin wax to be cut later for slide preparation. Each paraffin wax block was cut (sagittal section) with a microtome at 5μm thick and sections were placed on eight slides with five sections per slide to be stained with hematoxylin and eosin (H&E).

H&E staining is a common technique used in histology and pathology and is essential for recognizing tissue types and morphological changes within a tissue (Fischer, 2008). This staining method comprises of two main dyes: Haematoxylin and Eosin. Haematoxylin is a basic dye responsible for staining acidic structures within a cell creating a purple/blue colour; nuclei, ribosomes and rough endoplamic reticulum have high affinity to this dye due to their high concentrations of DNA and RNA found. Eosin is an acidic dye responsible for staining basic structures within a cell and turning these structures pink in colour. The cytoplasm, intracellular membranes and extracellular fibers will stain pink upon application. The H&E protocol used was responsible for rehydrating the cells in order for successful adherence of the stains to the optic cells and then dehydrated for preservation. Rehydration is comprised of xylene and decreasing concentrations of ethanol (100%-70%) allowing water to enter the cells. Staining included single washings of Heamatoxylin and Eosin Y to adhere on presenting cells and lastly, dehydration included several washings of increasing ethanol concentration (70%-100%) and xylene for permanent preservation. The ocular sections were photographed.
with the Nikon Digital DXML200F microscope camera at 2.5x and 20x magnification for an overall morphology of the eye and retina in order to observe and score (scale 0-4) pathological changes characteristic of PVR. For PVR scoring please see Table 1.

Table 1: Clinical evaluation of PVR (Retina & Optic Nerve Laboratory, Dalhousie University)

<table>
<thead>
<tr>
<th>PVR Histopathology</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No disease, normal retinal architecture</td>
</tr>
<tr>
<td>0.5</td>
<td>Mild inflammatory cell infiltration in the retina, no tissue damage</td>
</tr>
<tr>
<td>1</td>
<td>Infiltration, retinal folds and focal retinal detachments, few small granulomas in choroid &amp; retina</td>
</tr>
<tr>
<td>2</td>
<td>Moderate infiltration, retinal folds, detachment, focal photoreceptor damage, granulomas, perivascularitis</td>
</tr>
<tr>
<td>3</td>
<td>Moderate to marked infiltration, extensive photoreceptor damage. Exudate with hemorrhage (opaque anterior chamber), completely obscured pupil</td>
</tr>
<tr>
<td>4</td>
<td>Severe inflammation and/or full thickness retinal damage with serous exudates and subretinal neovascularization, large granulomatous lesions</td>
</tr>
</tbody>
</table>

Statistical Analysis

The histological scorings were analyzed by one-way ANOVA using GraphPad Prism 5 (GraphPad Software, Inc.). Analysis of cytokine expression, detected by ELISA, was performed using Student’s t-test. Data are presented as mean ±SEM. A statistical significant difference was assumed at p<0.05.
Results

External Anatomy

Figure 2: External anatomy of CB2+/ mouse eye (a) before and (b) after injection of dispase (0.1U).

Figure 2 shows the external anatomy of a mouse eye before and after injections of dispase shows the retracted retinal layers characteristic of PVR.

Histology

Figure 3: PVR severity scoring by comparison of mean scores based on table 1 between WT and CB2+/ animals treated with saline or dispase (0.1U).
Figure 3 graphically represents results of PVR severity scoring for WT and CB2\textsuperscript{−/−} groups. The more damage that the retina sustained the higher the score it received, whereas, if the retina sustained no or little damage a lower score was received (Table 1). There was no significant difference in histological scoring (p>0.05) between the WT mice injected with saline (n=9) and WT animals injected with dispase (n=9). However, there was a significant difference (p<0.05) between the saline treated (n=9) and dispase injected (n=9) CB2\textsuperscript{−/−} animals. There was no significant difference between WT animals and CB2\textsuperscript{−/−} animals injected with saline (p>0.05); however, there was a significant difference (p<0.05) between WT and CB2 animals injected with dispase (p<0.05), which suggests the involvement of CB2 receptors in PVR.
Figure 4: H&E histology staining on cross sections of WT and CB2$^{-/-}$ mice retinas at 2.5x and 20x magnification. (a & b) WT dispase mouse retina, (c & d) WT saline mouse retina, (e & f) CB2$^{-/-}$ dispase mouse retina and, (g & h) CB2$^{-/-}$ saline mouse retina.
The stained photomicrograph cross sections highlight the morphology of the whole eye and retina in WT and CB2⁻/⁻ animal models (Figure 4). Inflammatory cells (purple) and blood accumulation (pink/red) can be seen in dispase injected models while in saline injected models few to no immune cells are seen with an intact retina. Missing parts of the retina are due to mechanical errors, not pathological characteristics of PVR.

**IL-1β ELISA**

![Figure 5: ELISA IL-1β cytokine concentrations in both control (saline) and experimental (dispase) models.](image)

An ELISA was performed along with quantifying the concentration of IL-1β with the use of a spectrophotometer. To see if there were any significant differences between and within groups t-tests were performed (Figure 5). A t-test was used to compare between WT dispase and CB2⁻/⁻ dispase models and showed no significant difference (p > 0.05). T-tests were also performed within WT and CB2⁻/⁻ groups. There was no
significant difference (p>0.05) between saline and dispase models within the WT group, but there was a significant difference (p<0.05) between saline and dispase models within the CB2<sup>+/−</sup> group. Standard error bards were added to indicate the range of error of performed t-tests.

**Discussion**

The present study supports a growing body of evidence that the CB2 receptor has an immunomodulatory and neuroprotective role during inflammatory conditions. Histology stains (Figure 4: E and F) demonstrate pronounced ocular damage in CB2<sup>+/−</sup> animals through activation and recruitment of immune cells causing retinal folds and dispersion of retinal layers which are characteristics of PVR. This indicates that animals without the CB2 receptor do not have the protective mechanisms to modulate or ward off damaging effects of immune cells. On the other hand, WT animals (Figure 4 A-D) have an intact retina with few or no immune cells present at the site of inflammation due to the presence of the CB2 receptor. The photomicrograph that best demonstrates the modulatory role of the CB2 receptor is Figure 4B as there is a dense population of immune cells and some bleeding, however, the retina shows no damage indicating that the receptor does seem to protect and down-regulate the number of cytokines and other immune cells at the site of inflammation.

An important role of the endocannabinoid system is to maintain homeostasis in health and disease. Interestingly, some diseases such as Parkinson’s, Alzheimer’s and Multiple Sclerosis seem to trigger an up-regulation of cannabinoid receptors selectively in cells or tissues for symptom relief and inhibition of disease progression (Pertwee,
A study based on CB2 receptors within the gastrointestinal tract and their regulatory systems during states of inflammation was conducted by Wright et al. (2009). In this study, it was also found that the CB2 receptor down-regulated leukocyte infiltration during inflammation through the inhibition of cytokine and chemokine production. This supports the findings that the CB2 receptor has immunomodulatory pathophysiology properties during a response to inflammation (Wright et al., 2009). The histological results clearly demonstrate that the CB2 receptor has protective properties within the eye, in which case being able to medicinally target this receptor and its respective endogenous ligands in patients with such diseases could be beneficial in disease management.

Since this study looked at the cytokine profile 24 hours after injection, it is appropriate to mention that IL-1β is released by activated macrophages within 1-3 days of inflammation recognition (Kaiser, 2012). Therefore, we should see relatively high concentrations of this cytokine in both WT and CB2−/− mice due to the chosen time point. The results of the targeted cytokine (IL-1β), a pro-inflammatory cytokine, were to see a higher concentration in CB2−/− mice, especially in the dispase treated mice and lower concentrations in WT mice as this group has the CB2 receptor to modulate cytokine migration and activation. However, after surgically removing the retinas at a 24 hour time point, there were no significant differences of IL-1β concentrations between or within WT and CB2−/− groups (Figure 5) after induction of PVR. Since IL-1β is known to be one of the first cytokines to reach a site of inflammation, it is thought that the initial needle prick to the mouse eye was enough to cause an inflammatory response that at 24 hours a large and almost equal concentration of IL-1β was found in all control and experimental
models (Figure 5). This level is expected to decrease in saline animals after 72 hours and should exacerbate in dispase models. Other possibilities as to why we did not see any significant differences in IL-1β between groups could be due to low protein levels in the retinas, not having enough CB2−/− animals and technical difficulties running the ELISA. If these variables are corrected in future studies, a concentration difference of IL-1β could be significant between WT and CB2 knockout models.

In future research, looking at cytokine profiles at a longer or shorter time points would be beneficial in order to demonstrate if it is to be expected that cytokine-mediated pathways of chronic inflammation are involved in the pathogenesis of PVR. Identification and expression of other specific cytokines causing ocular pathology involved in PVR immune responses could be of therapeutic and pathogenic importance for this debilitating disease. To accurately identify the immune cells involved in PVR, immunohistochemistry staining should be conducted as this stain displays cell morphology.

Due to limited time to conduct this research, a small sample size was used therefore, for further investigation into this topic a larger sample size within groups should be used for more robust statistical analysis.
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