Proceedings of The National Conference On
Undergraduate Research (NCUR) 2018
University of Central Oklahoma, OK
Edmond, Oklahoma
April 5-7, 2018

Analysis of How B Cells Contribute to the Photobiomodulation Technique in Regards to Multiple Sclerosis

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Abstract

Multiple sclerosis (MS) is an autoimmune disease that interrupts the flow of information within the brain and between the brain and the body. Multiple sclerosis is usually diagnosed between the ages of 20-50. Multiple Sclerosis is two to three more times common in women than men. This study aims to explore how B cells contribute to the therapeutic effect of photobiomodulation in an animal model of MS. Photobiomodulation with visible or near infrared light is a therapeutic strategy showing promise for the treatment of chronic inflammation and neurodegeneration. This study looks at how different light intensities impact the disease in the absence of B cells by measuring the amount of the cytokines, Interleukin-10 and interferon-gamma (IFNγ). The cells in this study will come from the lymph nodes of mice lacking B cells, which are referred to as the B cell knockout mice. The ELISA technique, (enzyme-linked immunosorbent assay), using a spectrophotometer, will be used to determine the amount of cytokine in cell culture supernatants. The amount of cytokine, as measured by absorbance of light by the spectrophotometer, is directly proportional to the degree of color change in the assay. The higher the absorbance, the higher the amount of IL-10 or IFNγ in the sample. With performing light treatment on the cells and comparing old data to new data, the anticipated results should demonstrate that B cells produce the IL-10 with the application of different intensities of light. The real-world significance of this study is to understand how photobiomodulation decreases disabilities in MS patients, resulting in an improvement in the quality of life.

Keywords: Multiple Sclerosis, Photobiomodulation, B cells

1. Introduction

According to the National MS society, 1 in 500 people in Wisconsin are affected with multiple sclerosis. Multiple sclerosis is a leading cause in neurologic disability in persons 20 to 40 years of age.[9] MS is an autoimmune disease that impacts the central nervous system (CNS) and interrupts the flow of information within the brain and between the brain and the body. The histopathology of MS is characterized by the presence of the inflammatory, demyelinated lesions in the CNS. The lesions are changing in locations and size over time which leads to the clinical effects associated with MS.[9] Manifestations and symptoms associated with multiple sclerosis are variable and include numbness, tingling, tremor, weakness, paralysis, vertigo, hearing loss, nystagmus, and trigeminal neuralgia.[19] The cause of multiple sclerosis is unknown, but a variety of environmental and genetic factors have been associated with the disease.[9] FDA approved treatments for MS slow disease progression but do not offer neuroprotection. The approved FDA treatments act through immunomodulatory or immunosuppressive mechanisms, aiming at those processes important to the launching of clinical activity and correlated with the relapsing/remitting phase of disease.[4,11,14,1,10,18] My data suggests that B cells have a clinical impact in photobiomodulation.

Photobiomodulation is a therapeutic approach that can be delivered via LED or laser light that acts on the mitochondria to increase energy. Photobiomodulation Therapy (PBMT) with far-red to near infrared (FR/NIR; 630-1110nm) light has developed to be an effectual means of treating neurodegenerative diseases and pro-inflammatory diseases.[21] Photobiomodulation decreases pro-inflammatory factors and increases anti-inflammatory factors to help slow the progression of the disease. Photobiomodulation can resolve chronic inflammation and is effective in wound healing. MS is a combination of two mechanisms of neurodegeneration and chronic inflammation. Kamal A. Muili's work in the Experimental Autoimmune Encephalitis (EAE) model of MS that was performed previously in the lab suggested that photobiomodulation would be an effective treatment strategy for MS. These studies demonstrated that amelioration of clinical disease was associated with a decrease in pro-inflammatory cytokines, (IFNγ and tumor necrosis factor-α) and an increase of the anti-inflammatory cytokine (IL-4, IL-10) in vivo and in vitro.[15] Studies discussed here expand this earlier work to investigate the role of B cells in the clinical improvement noted with PBMT in the EAE model.

2. Methodology

2.1 Animals

Specific pathogen-free female C57BL/6 (B6) mice deficient in B cells were bred in-house from breeding pairs acquired from Jackson Laboratories (Bar Harbor, ME). Mice were managed in micro-isolator cages in an AAALAC-accredited facility in conformance with University and NIH guidelines.[15] All animals in the facility were equipped with food and water and sustained on a 12h light/dark schedule in a temperature and humidity-controlled environment.[15]

2.2 Immunization and LED treatment

For the immunization, 100µg per mouse of myelin oligodendrocyte glycoprotein (MOG) peptide consisting of amino acids 35-55 in 100µL/mouse phosphate buffered saline (PBS) was emulsified with 200µg of heat-killed Tuberculosis strain H37RA per mouse in 100µL Freund's incomplete adjuvant (IFA) per mouse. The mice were injected under the skin (subcutaneously) at each limb with a measurement of 0.05mL per injection for a total volume of 0.2mLof emulsion per mouse. Pertussis toxin (PT; 300ng/mouse/injection in 0.1mL PBS) was injected intraperitoneally at the time of immunization and was given 48 hours later.

For clinical studies, mice were graded for clinical signs every day starting 7 days post immunization. The grading scale is as follows: 0= no clinical signs of disease, 1=loss of muscle tone in the tail (failure to helicopter the tail), 2= failure to right themselves when flipped on their back, 3= 1 back leg paralyzed, 4= both back legs paralyzed, 5= dead or moribund. Treatment began on the day of disease onset. The mice were randomly assigned into the 2 groups. The mice were either treated with the 670nm LED light at 67% intensity for 3 minutes (light treatment group) or treated with restraint stress for 3 minutes (sham treatment group). The mice were treated for 7 days starting at disease onset and then given 7 days of rest with no treatment. This was repeated so that each mouse was treated for a total of 14 days and 14 days of rest.

2.3 Cell Culture and Cytokine ELISA

Lymph nodes were isolated from mice immunized as discussed above. Single cell suspensions prepared from pooled lymph nodes. Cells were cultured in RPMI 1640, L-glutamate (2mM), Sodium pyruvate (0.1mM), 2-mecarptoethanol (50mM) in 96 well flat-bottom plates.[15] Concanavalin A (Con A; 1ug/mL) was stimulated with the cell to serve as a positive control for cell viability.[15] Cells were treated with 670nm light or no light control for 88 seconds at 67% intensity beginning 2 hours post culture and once every 24 hours for a total for 120 hours. Cell culture supernatants were isolated at 48 hours,72 hours, 96 hours and 120 hours.[15] The ELISA method (enzyme-linked immunosorbent assay) was used to measure the IFNγ and IL-10. Quantikine kits were used according to manufacturer's instructions (R&D Systems, Minneapolis, MN).[15] Data is expressed as in the absence of peptide, antigen-specific cytokine secretion.[15] Absorbances following the addition of stop solution were measured using a spectrophotometer. The intensity of color developed is directly proportional to the amount of cytokine present in the sample, which enabled us to compare to the Sham (control) group to see the effect of light treatment on cytokine production.

2.3.1 statistical analysis

Data were analyzed using Prism GraphPad 7.0 by two-way ANOVA or Student's t-test, as indicated. P value of 0.036 was considered significant.

3. Results

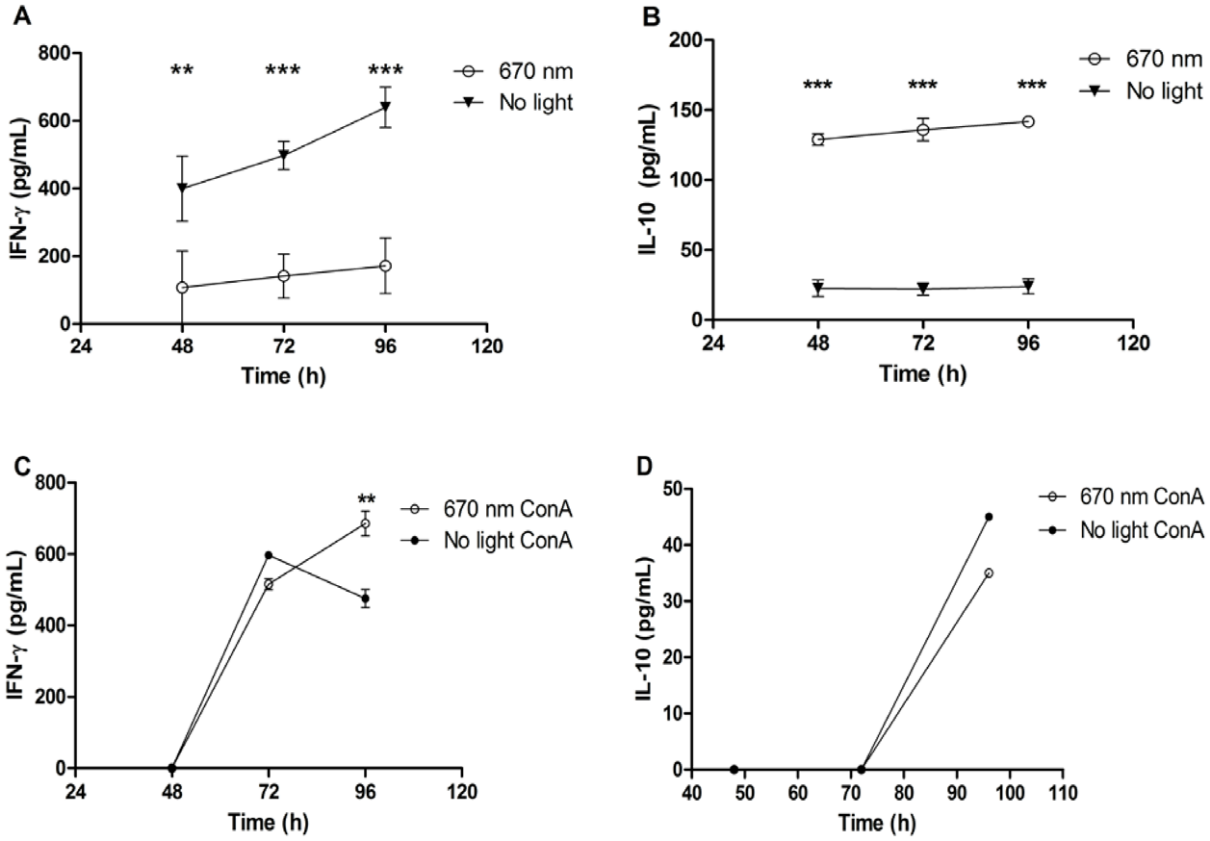
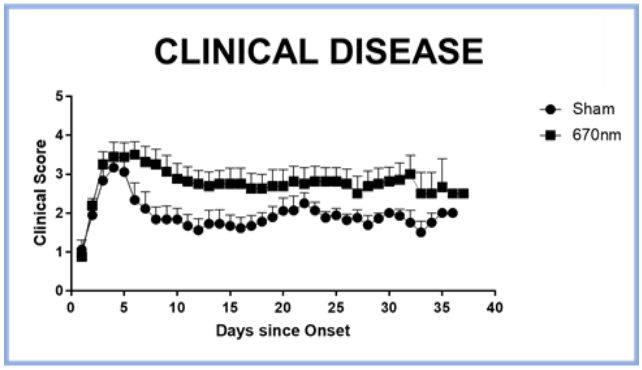
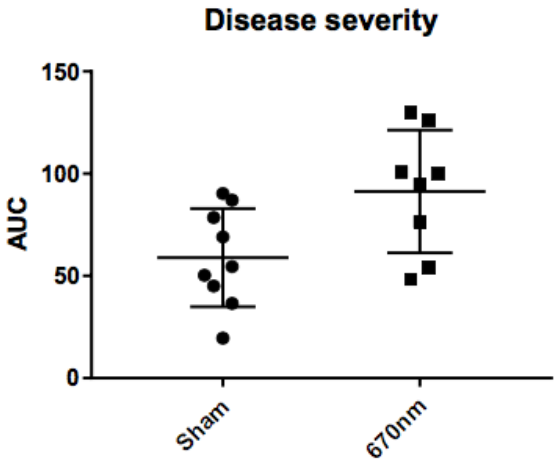


Figure 1. The 670nm light modulates in vitro cytokine production by lymph node cells.[15]

Our previous study demonstrated the therapeutic potential of photobiomodulation with the use of 670nm light in the EAE model through modulation of the immune response.[15] Figure 1 is not data original to this study, but previously published data provided for contrast between effect of PBM in the presence and absence of B cells. According to standard protocol, disease was induced with myelin oligodendrocyte glycoprotein (MOG).[15] The principle findings of this study illustrated that the 670nm light lowered the severity of the disease with both protocols compared to the sham treated mice.[15] Amelioration of the disease was associated with a decrease of pro-inflammatory cytokines (IFNγ) and increase of anti-inflammatory cytokines (IL-10).



a. Average Disease Course



b. Area under the curve of disease severity

Figure 2. Illustration of the Clinical Course of Disease. The B cell deficient mice treated with 670nm light had a more severe clinical disease compared to Sham treated mice.

The goal of the current study was to investigate whether B cells were important in the amelioration of EAE that was previously demonstrated. This was tested using mice genetically deficient in B cells. Unlike wild type mice, the B cell deficient mice treated with 670nm light, had a more severe clinical disease compared to Sham treated mice. The P value was 0.036. This experiment suggests that B cells are critical to the clinical effect afforded by PBMT in WT mice.

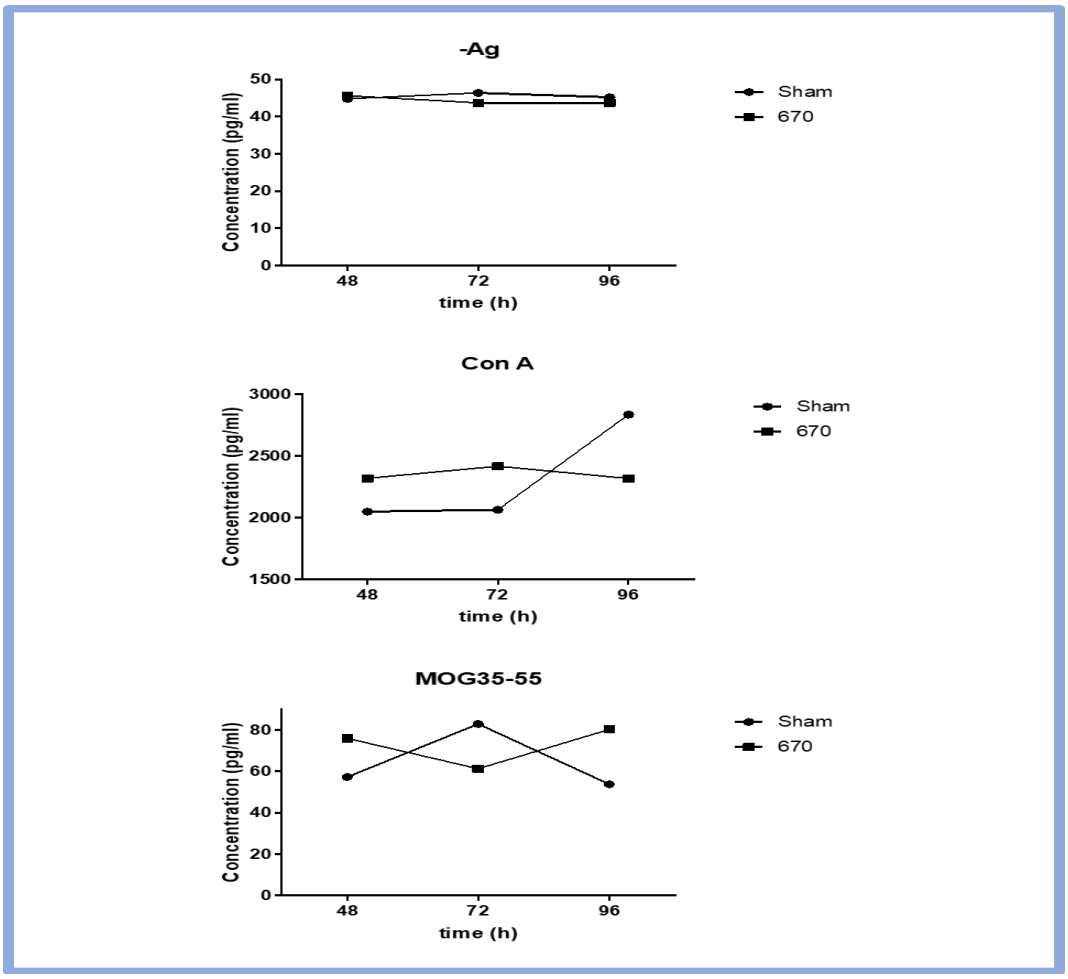


Figure 3. Female Mice deficient in B cells: IFNγ 670nm light vs. Sham

Our previous studies demonstrated the down regulation of inflammatory cytokines and up regulation of anti-inflammatory cytokines by PBMT with 670nm light in WT mice with EAE.[15] Since clinical disease was not ameliorated in B cell deficient mice treated with 670nm light, we investigated the production of pro-inflammatory cytokine, IFNγ following PBMT administered *in vitro.* Unlike previous experiments in WT mice, there was no difference between the cells treated with the 670nm light and those given the sham treatment. Both the sham and 670nm treatment groups had similar production of IFNγ with and without the specific antigen MOG35-55. Con A is a mitogenic lectin that activates T cells in a polyclonal manner by cross-linking of cell surface Beta-glycans. It is used as a positive control for cell viability and it has the ability to respond to appropriate stimulatory signals.[15] -Ag is the no stimulation control.

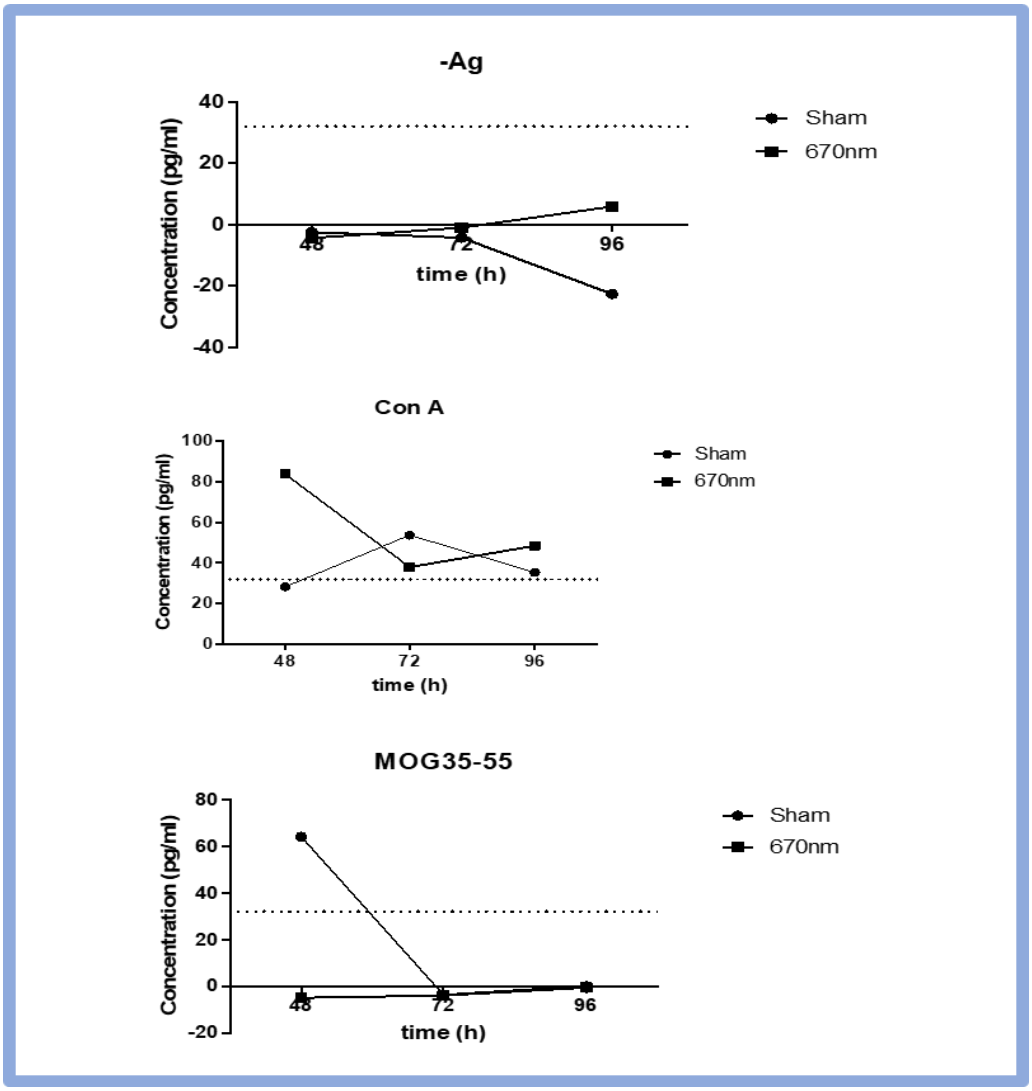


Figure 4. IL-10 Female Mice deficient in B cells: 670nm light vs. Sham

Interleukin 10 (IL-10) is an important anti-inflammatory cytokine that plays a role in the resolution of inflammation and clinical disease.[20] Our previous experiments suggested that IL-10 was important in the clinical effect of PBMT.[15] B cells are an important source of IL-10. To investigate the role of B cells in IL-10 production following PBMT, we measured IL-10 production following *in vitro* PBMT of lymph node cells isolated from B cell deficient mice. There was little to no IL-10 for either treatment. Our data suggest that B cells may be the main producers for IL-10 and are critical to the clinical effect of PBMT in the EAE model of MS.

4. Discussion

An autoimmune disease that is a leading cause of neurologic disability in young adults is Multiple sclerosis (MS).[20] Recent studies indicated that the progression of the disease is due to non-immune mechanism, indicating a role for oxidative stress in the axonal loss, developing late in the disease process.[5,6] None of the current studies help with oxidative stress and axonal loss.

Therapeutic application of light to evoke a biological response has been studied using different wavelengths and modalities.[9] Photobiomodulation induced with FR/NIR light has indicated efficacy in chronic wound healing in experimental and clinical systems.[2,21,22] And for the treatment of neurodegenerative diseases.[3,7,8,23] The diseases

include Retinitis pigmentosa.[8], Parkinson's disease [13,17,23]and stroke.[12,16] Data demonstrates that photobiomodulation promotes down regulation of inflammatory molecules and up regulation of anti-inflammatory molecules.

The pathogenesis of Multiple sclerosis is neurodegenerative and proinflammatory.[15] In WT mice, photobiomodulation induced with 670nm light lead to improvement in clinical signs in an animal model of MS. Protection from disease was associated with down regulation of IFNγ and an up-regulation of IL-10 expression compared to the cells not exposed to light treatment.[15] This shift in the balance of pro-inflammatory and anti-inflammatory cytokines in a way that benefits the clinical course of EAE.[15]

Data presented here demonstrate that 670nm PBMT failed to improve the clinical course of EAE in mice deficient in B cells. Furthermore, PBMT did not impact IFNγ or IL-10 production in B cell deficient mice.

Data presented here suggest that 670nm light induces B cells to produce IL-10 which is important to the improvement of clinical disease in the EAE model of MS. These experiments help us understand the mechanism of protection of PBMT for the treatment of MS, which is important to the development of PBMT as therapeutic approach to MS.

5. Acknowledgements

The author wishes to express their appreciation to Quantum Devices (Barneveld, WI) for providing the LED arrays used in the experiment.

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