Can dendritic cells see light?

Citation

As Published
http://dx.doi.org/10.1117/12.842959

Publisher
Society of Photo-optical Instrumentation Engineers

Version
Final published version

Accessed
Wed Mar 16 10:21:01 EDT 2016

Citable Link
http://hdl.handle.net/1721.1/57500

Terms of Use
Article is made available in accordance with the publisher’s policy and may be subject to US copyright law. Please refer to the publisher’s site for terms of use.

Detailed Terms

The MIT Faculty has made this article openly available. Please share how this access benefits you. Your story matters.
Can Dendritic Cells See Light?

Aaron C-H Chen, Ying-Ying Huang, Sulbha K Sharma, Michael R Hamblin

a Wellman Center for Photomedicine, Massachusetts General Hospital,
b Department of Dermatology, Harvard Medical School,
c Aesthetic and Plastic Center of Guangxi Medical University, Nanning, P.R China
d Harvard-MIT Division of Health Sciences and Technology,

* corresponding author: BAR414, 40 Blossom Street, Massachusetts General Hospital, Boston, MA02114. email: Hamblin@helix.mgh.harvard.edu. Fax :617-726-8566

ABSTRACT

There are many reports showing that low-level light/laser therapy (LLLT) can enhance wound healing, upregulate cell proliferation and has anti-apoptotic effects by activating intracellular protective genes. In the field of immune response study, it is not known with any certainty whether light/laser is pro-inflammatory or anti-inflammatory. Increasingly in recent times dendritic cells have been found to play an important role in inflammation and the immunological response. In this study, we try to look at the impact of low level near infrared light (810-nm) on murine bone-marrow derived dendritic cells. Changes in surface markers, including MHC II, CD80 and CD11c and the secretion of interleukins induced by light may provide additional evidence to reveal the mystery of how light affects the maturation of dendritic cells as well how these light-induced mature dendritic cells would affect the activation of adaptive immune response.

Keywords: photobiomodulation, low level laser therapy, bone marrow derived dendritic cells, markers of maturation and activation, confocal microscopy, flow cytometry

INTRODUCTION

Dendritic cells are known to be efficient stimulators of T and B-lymphocytes, and they play an important role as antigen presenting cells (APC) in initiating and modulating immune response. Langerhans cells were the first type of dendritic cells discovered in the skin back in 1868, but modern understanding of DC only started about 25 years ago.

A human has about $10^9$ Langerhans cells locating above the proliferating keratinocytes in the skin, and most of DC remain in an immature state, characterized by the lack of migration mobility and their inability to stimulate T cells. Although they lack co-factors for T or B lymphocyte activation, including CD40, CD54 and CD86, they are capable of capturing antigens and expressing them in the context of MHC class II. Only a few DC are necessary to provoke strong T-cell response. With more and more evidence, many immune-related symptoms have revealed the importance of DC, which has been long overlooked before.

Photobiomodulation or low-level light therapy has been proven to have many significant effects in enhancing healing and preventing tissue death [1, 2]. Karu’s laboratory has reported that mitochondria are a principal intracellular target of red and near-infra-red light [3], and cytochrome c oxidase is proposed to be a photoreceptor that absorbs light as far into the infra-red as 1000-nm [4]. There have been reports...
of increased cytochrome c oxidase activity after low level light [5] and by stimulating the mitochondrial electron transport train, increased intracellular ATP after light delivery to isolated mitochondria [6] has been observed.

Many genes have been shown to have their transcription upregulated (or down regulated) after illumination of cells with various wavelengths and fluences of light. For instance, illumination of human fibroblasts with 628-nm light emitting diode led to altered expression of 111 genes of 10 functional groups [7]. Studies have shown that this one mechanism of light-mediated gene regulation is related to the activation of the pleiotropic transcription factor NF-kB [8] probably via generation of mitochondrial reactive oxygen species [9].

Nuclear factor kappa B (NF-kB) is a transcription factor regulating multiple gene expression and has been shown to govern various cellular functions, including inflammatory and stress-induced responses and survival [10]. NF-kB activation is governed by negative feedback by IkB, an inhibitor protein that binds to NF-kB, but can undergo ubiquitination and proteasomal degradation [11] thus freeing NF-kB to translocate to the nucleus and initiate transcription [12].

In addition, NF-kB also plays an important role in activation of inflammation. Many studies have shown that red or near infrared light can reduce inflammatory conditions such as arthritis [13, 14] or gingival inflammation [15].

**MATERIALS AND METHODS**

**Bone Marrow-derived Dendritic Cells Culture**

All animal procedures were approved by the Subcommittee on Research Animal Care of the Massachusetts General Hospital and met the guidelines of the National Institutes of Health. Bone marrow-derived DC were prepared from 5- to 7-week-old male C57 mice purchased from Jackson Laboratories (Bar Harbor, ME).

Femurs from mice were dissected, and muscle and tissue were removed. Cleaned bones were washed twice with Hank’s Buffer Salt Solution, and placed into culture media composed of RPMI-1640 (Gibco) with 1% penicillin–streptomycin (Cellgro), 0.1% 2-mercaptoethanol (Invitrogen), with 10% heat inactivated fetal bovine serum (Invitrogen) (murine DC media). Bones were cut and bone marrow was flushed with at least 5 mL of media. The bone marrow suspension was strained with a 70 mm cell strainer (Becton Dickinson), cells collected by centrifugation at 1500 rpm for 5 min, and erythrocytes were lysed with ammonium chloride (155 mM NH4Cl, 10 mM KHCO3, 0.1 mM EDTA). Bone marrow cell suspension was resuspended at 1.5x10^5 / ml in murine DC media with 20 ng/ mL GM-CSF (Sigma). Cells were plated at 3 ml / well in six well plates (Corning), and incubated at 37°C with 95% relative humidity and 5% CO2. On day 3, cells were fed by exchanging half of the media with fresh murine DC media. Purity and yield of CD3 and CD4 positive cells were inspected by flow cytometry. In addition, cell death was quantified by propidium iodide by flow cytometry. To assure the consistency of cell population, plates with over 85% purity and less than 10% cell death were selected for experiments. On day 8, loosely adherent and non-adherent cells were collected and washed twice in PBS, centrifuged and resuspended at 2 x 10^7/mL in 60mm cell culture treated dishes (Corning), and incubated at 37°C with 95% relative humidity and 5% CO2 over night.
**LPS treatment.**
Lipopolysaccharide from *Escherichia coli* purified by gel chromatography (Sigma-Aldrich, St Louis, MO) was added to DC at a concentration of 1 or 10 μg/mL. Light was delivered at two time points, either immediately after LPS or 12 hours after LPS addition.

**Low Level Light Irradiation**
An 810nm laser (HOYA Conbio) was selected as light source, and light irradiation time was set at 5 minutes. 30, 3 and 0.3 J/cm² fluences were delivered at irradiances of 100 mW/cm², 10 mW/cm² and 1 mW/cm² respectively to individual dishes. Light treated dishes were incubated in the incubator for 24 hours before analysis.

**Cell morphology and Quantification of Protein Expressions**
After 24 hour incubation after light treatment, cell morphology was monitored by using confocal microscopy. Cells were stained with anti-murine fluorescent antibodies against MHC class II, CD80 and CD11c (Invitrogen molecular probes) 15 minutes before microscopy. The same antibodies were used to quantify protein expression in live cells by flow cytometry. Propidium iodide was added to distinguish live cells from dead cells.

**Statistical Analysis**
All chemiluminescence readings were normalized to total protein measured by Bradford’s technique (BCA, Pierce Biotechnology Inc.). All assays were performed in duplicate, and each sample was read twice and took the average. Excel software was used to perform Single-Factor ANOVA to evaluate the statistical significance of experimental results (p < 0.05).

**RESULTS**

**Confocal Microscopy Imaging**
Lipopolysaccharide is a potent stimulator for DC activation and maturation. No other cells exhibit the shape and motility as mature dendritic cells. With proper antigen stimulation, DC display fine long dendrites (>10 μm) and the size of the cell body also enlarges from the immature stage. A fully mature dendritic cell also displays MHCII, CD86 and CD11c on its membrane, which allows it to capture antigens and stimulate antigen-specific T cells. The level of CD11c does not change with levels of maturation and/or activation of DC, thus allowing it to be used as an invariant fluorescent marker of DC purity.

Many studies have shown that low level light has diminishing positive or even negative effects at higher fluence, such as 30 J/cm², due to its biphasic dose response [16]. This high fluence of “low-level” light together with more usual fluences such as 3 and 0.3 J/cm² were selected to test if bone marrow-derived dendritic cells responded to the 810nm continuous wave laser. After 24 hours incubation after light irradiation, which allows dendritic cells to process activation and maturation, confocal microscopy imaging showed that the laser alone did not change either of the membrane markers expression compared with the untreated DC (Figure 1A and B). However, if laser was delivered to the dendritic cells...
continuously incubated with lipopolysaccharide, the surface MHCII was down-regulated while CD86 was up-regulated (Figure 1C and D. Furthermore, the dendrites of DCs with LPS plus light were more developed than those in the LPS only group.

**Figure 1.** Confocal microscopy of membrane protein expressions on DC, Blue, CD11c; red, MHCII and green, CD86.

**FACS analysis of membrane markers**

Confocal microscopy imaging indicated that 24 hours after irradiation was a suitable time point to distinguish changes of membrane marker expression. At the same time point, FACS analysis on the same three markers were quantified as shown in Figure 2. Dexamethasone is a potent synthetic compound of the glucocorticoid class of steroid hormones and acts as an anti-inflammatory and immunosuppressive agent. FACS showed that dexamethasone down-regulated MHCII without significantly affecting expression levels of CD81 and CD11c. On the other hand, lipopolysaccharide up-regulated both MHCII and CD86 after 24 hours. 810nm laser results confirmed the confocal microscopy imaging data that laser alone did not significantly change MHCII or CD86, but in the presence of LPS, 810nm laser reduced levels of expression of membrane MHCII but increased expression of CD86.
DISCUSSION

This study has demonstrated that for the first time that dendritic cells respond to 810nm near infrared laser with significant morphology change and membrane marker regulation. Furthermore, in addition to confirming previous reports and clinical trials from other laboratories that near infrared light indeed has effects on reducing inflammation, this study also supports the fact that near infrared potentially change DC morphology and their membrane markers of maturation, activation and antigen-presenting.

There is evidence that light acts on cells via NF-kB pathway among others and many reports have demonstrated that light could have different impacts on different cells and tissues. The fact that near infrared light alone did not significantly change immature DC but showed significant changes after the addition of LPS had induced maturation might have implied that light acting on DC requires signaling initiation of Toll-like receptors (TLR).

It has been recently discovered that there are (at least) two NF-kB activation pathways [17]. The canonical NF-kB signaling pathway activated in response to infections (toll-like receptor signaling) and cytokines is based on degradation of IkB inhibitors. This pathway depends on the IkB kinase (IKK), which contains two catalytic subunits, IKKα and IKKβ. IKKβ is essential for inducible IkB phosphorylation and degradation, whereas IKKα is not. IKKα is involved in processing of the NF-kB2 (p100) precursor. IKKα preferentially phosphorylates NF-kB2, and this activity requires its phosphorylation by upstream kinases, one of which may be NF-kB-inducing kinase (NIK). IKKα is therefore a pivotal component of a second NF-kB activation pathway based on regulated NF-kB2 processing rather than IkB degradation.
While light irradiation took place immediately after addition of LPS this was an early time point in the process of maturation of dendritic cells, photo-induced signaling could be interfering with signaling from the TLR, and this interference could determine the relative gene expression levels of different DC markers such as MHCII and CD86. We also tested light irradiation at the later time point after LPS stimulation, (12 hours after LPS), and no similar changes in MHCII and CD86 as seen at early time point were observed (data not shown). This observation led us to believe that light did have abilities to change behaviors of DCs, but it requires early irradiation to trigger. It is at present unclear why the levels of MHCII and CD86 moved in different directions after laser treatment. Both these markers are considered to be upregulated during the process of DC maturation and activation. However it is likely that the overall result of unbalancing the coordinated expression levels of MHCII and CD86 would lead to less effective DC function, in other words to lower immune response levels to various stimuli. This may in fact be a partial explanation of the anti-inflammatory that is known to be one of the many benefits of LLLT. Reduced DC activation would tend to lessen the degree of inflammation produced by both the innate and adaptive arms of the immune system in response to diverse insults and injuries. Further work is needed to explore the determinants of LLLT on DC in various states of maturation and activation.

ACKNOWLEDGMENTS
This work was supported by NIH grant R01AI050875, Center for Integration of Medicine and Innovative Technology (DAMD17-02-2-0006), CDMRP Program in TBI (W81XWH-09-1-0514) and Air Force Office of Scientific Research (FA9950-04-1-0079)

REFERENCES


