The Wavelength of Light Governing Intraocular Immune Reactions

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Injection of antigen into the anterior chamber of the eye results in the induction of suppressed systemic cell-mediated responses as measured by delayed-type hypersensitivity or contact hypersensitivity (CHS). Previous studies from the authors' laboratories have determined that this response is governed by exposure of the eye to visible light during the initial intraocular encounter between T cells and antigen. To more fully understand the role of light, as well as to begin to understand the molecular mediators involved, the authors chose to explore the properties of light governing the effect. Neutral density filters were used to demonstrate that the minimum amount of light required to induce suppression of CHS following anterior chamber injection of antigen is 1–2 lux (lumens/meter²). With narrow band filters, the wavelengths responsible for suppression were shown to be 500–510 nm. The results show that the effect of light extends beyond the hapten-derivatized spleen cell system to other antigens placed in the anterior chamber of the eye. Studies also show that the retina and the pineal gland, two light absorbing structures, may not be involved. The results in this report show that light of very restricted wavelengths controls intraocular immune reactions. Invest Ophthalmol Vis Sci 33: 1788–1795, 1992

We have been studying the consequences of presenting antigen to the immune system via the anterior chamber of the eye. The results of our studies,1–5 with those of other laboratories,6–9 suggest that a unique environment exists in the eye that modulates the pattern of responses generally observed when antigen is encountered subsequently at other sites. An important model for studying intraocular immune reactions is anterior chamber associated immune deviation (ACAID).4 In this system, the injection of antigens into the anterior chamber of the eye induces a consistent pattern of systemic responses characterized by the induction of antigen specific suppression for delayed type hypersensitivity (DTH) or contact hypersensitivity (CHS).1–9 Simultaneously, antibody production is normal or elevated.5

We previously showed that down regulation of CHS following anterior chamber injection of 2,4,6-

trinitrophenol-coupled spleen cells (TNP-Spl) is initiated by the activation of certain T cells within the eye,2 leading to the elaboration of an inhibitory molecule from the eye that causes the activation of T cells in the spleen. By using dark-reared and light-reared mice (Balb/c), we established that visible light directly affects this intraocular phenomenon.3 If light was prevented from reaching the eye by dark-rearing, by placing light-reared animals in the dark post anterior chamber injection, or by closing the eyelid of light-reared animals post anterior chamber injection, ACAID is not established. Furthermore, we demonstrated that visible light—not ultraviolet (UV) or infrared—was responsible for the observed effects and that the effect of light on the eye was not developmentally mandated (as is visual development), but was inducible in adult dark-reared animals. These results suggest that light entering the eye establishes the conditions necessary for the induction of suppressed CHS by influencing the intraocular T cell reactions that initiate suppression.

To further examine the effect of light on the eye, we sought to determine the amount of light required, as well as an action spectrum, for the observed effects. Studies also were done to evaluate a role for the retina and the pineal gland, as well as to extend the phenomenon to other antigens. These studies have important implications for understanding intraocular immune regulation and determining the molecular mediators that regulate intraocular immune responses.
Materials and Methods

Mice

Balb/cByJ, C3H/HeJ (rd/rd; [rd for retinal degeneration]), and CBA/J mice were purchased from Jackson Labs, Bar Harbour, ME. In all in vivo experiments, groups consisted of five or more animals. Animals were used when they were 6–10 wk old. Balb/c mice that were neonatally pinealectomized and sham-operated mice were purchased from Taconic Farms (Germantown, NY) when they were 4–6 wk old. Animal experiments were performed in compliance with the ARVO Resolution on the Use of Animals in Research.

Construction of Boxes for Wavelength Determinations

Boxes were constructed and fitted with special filters to control the delivery of light to animals in our experiments. Wratten photographic filters were purchased from Kodak Co., Rochester, NY. Two x two inch filters that cover the visible spectrum from 400–700 nm were purchased from Oriel Corp., Stratford, CT. Each filter transmits 95% of its light within 5 nm of the optimum wavelength. The light sources were halogen beam automobile headlights (Western Auto, Fenton, MO) powered by a standard 12 V power source equipped with a rheostat. The power source was connected to a timer switch so the appropriate wavelength was delivered on a 12 hr light/12 hr dark cycle. Light was focused through the 2 x 2 inch filters fitted in the top of a box. Each light illuminated a cage containing five mice. The amount of light was adjusted with the rheostat on the power source and was standardized for each experiment with a photometer so all mice received the same amount of light regardless of the filter used.

Photometric Measurements of Light

Because our phenomenon was governed by the response to light in the visible range, we chose to study light using photometric measurements and to standardize our experiments using a photometer. Photometry measures the visible light as the eye can respond to it. The measurement is expressed in lumens/meter² or lux. Measurement in lux gives the amount of power emitted from a source per unit of surface area. Because different wavelengths have different energies, we tried to standardize our light source and the number of lumens the eye received in the visible range. A mouse cage was centered in the box 3-1/2 inches below the filter. The photometer then was placed on the bottom of the cage and zeroed. The amount of light was adjusted with the rheostat on the power source. We do not believe that the effect of light on ACAID was related solely to the amount of light energy. The reason is that the action spectrum falls in the visible range, with UV and blue (lower energy) and infrared and red (higher energy) having no effect.

Antigens

2,4,6 Trinitrobenzene sulfonic acid (TNBS), pigeon cytochrome-C, and hen egg lysozyme (HEL) were obtained from Sigma Chemical Co. St. Louis, MO.

Preparation of Hapten-Modified Cells

Erythrocyte-free (treated with .83% Tris/NH₄Cl) spleen cells (1 x 10⁷/ml) were mixed 1:1 (volume:volume) with 10 mM TNBS for 8–10 min at room temperature. Following incubation, cells were washed 3 x with HBSS, counted, and resuspended at the appropriate concentration for use.

Anterior Chamber Inoculations

Cells were delivered to the anterior chamber in 0.005 ml volume using a Hamilton Microliter syringe (Hamilton Co., Reno, NV) fitted with a 33 G needle. Mice were lightly anesthetized with Metofane (methoxyflurane; Pitman-Moore, Mundelein, IL) and injections were done under a dissecting microscope. Anterior chamber injection was done 1–24 hr prior to immunization. For TNP-spl, 5 x 10⁵ cells and for primed T cells, 1 x 10⁵ cells were injected in .005 ml.

In Vivo Assays

CHS to TNP: Mice were injected subcutaneously with 0.2 ml of a 10 mM solution of TNBS in phosphate buffered saline (PBS). Seven days later, animals were ear challenged with 0.1% 2,4,6-trinitrochlorobenzene (TNCB) in acetone/olive oil (3:1). The right ear received 0.015 ml of TNCB, while the left ear was unchallenged. Values are expressed as units of swelling with one unit = 10⁻³ cm. Background values obtained from challenged, naive mice were subtracted from each group.

DTH to proteins: Animals were injected subcutaneously with 100 µg of protein in 0.1 ml of PBS mixed with .1 ml of 10 mg/ml Al(OH)₃ and 10 mg/ml Mg(OH)₂. Seven days later, mice were challenged in the right footpad with 0.033 ml antigen in PBS. The left footpad received 0.033 ml of PBS. Values are expressed in units of swelling with one unit = 10⁻³ cm. Background values obtained from challenged, naive mice were subtracted from each group.
Immunization for Primed T Cells:

Primed T cells were obtained from the spleen and draining lymph nodes of immune mice. Mice were injected subcutaneously with 100 µg of antigen in 0.1 ml PBS emulsified 1:1 with complete Freund's adjuvant (Sigma). Ten to fourteen days later spleen and draining lymph nodes were removed, single cell suspensions prepared, and T cells purified.

Purification of T Cells:

T cells were prepared from single cell suspensions of erythrocyte free spleens and lymph nodes. Five milliliter Econo columns (BioRad, Richmond, CA) were filled with glass beads (mean diameter = 0.2 mm) coated with normal mouse immunoglobulin, followed by the addition of a 1/5 dilution of rabbit antimouse immunoglobulin. After 1 hr of incubation, columns were washed 3x with HBSS, and cell suspensions were added at a concentration of 2 x 10^8 cells per column (add 2 ml of 10^8/ml). The flow rate was adjusted to 1 drop per 6-10 sec and cells were collected as they emerged from the column. Yield was typically 25-30% of added cells that were >98% T cells.

Statistical Analysis

Significant group differences were evaluated using Student's t-test at \( P < 0.05 \).

Results

Amount of Light for Suppression

To better understand the mechanisms by which light effects suppressed CHS, we sought to determine the properties of light. In our previous report, \(^3\) we determined that only visible light was responsible for the observed effects. Therefore, we focused our attention on visible wavelengths. Because it was not possible to get high amounts of light (e.g., room light levels) through all wavelength filters, it was necessary to determine the minimum level of light needed to standardize our experiments. Initially, we determined the amount of light required to induce suppression of CHS to TNP (TNP-ACAID).

The apparatus described in Materials and Methods was fitted with neutral density filters. These filters allow precise reductions in the amount of light without eliminating wavelengths in the visible range. The starting point for these studies was 20 lux. Because we had no a priori reason to know an appropriate starting point, we measured the amount of light received by the bottom shelf on the mouse cage rack in the shadow cast by the shelf immediately above. Many studies \(^1\)\textsuperscript{-}\textsuperscript{5} in our laboratories had demonstrated that our experiments work if the mice are placed in this position. (As a point of reference, the top shelf, which is about 2–3 ft from the light source, receives 600–800 lux). Balb/cByJ mice were injected in the anterior chamber with TNP-spl and placed in boxes fitted with neutral density filters that reduce light by 10%, 20%, 50%, 90%, and 99%. Twenty-four hours later, they were immunized with TNBS and maintained in the boxes for the remainder of the experiment. Six days later, the animals were challenged with TNCB and CHS that was measured 24 hr later. The data in Figure 1 show that a 99% reduction (0.2 lux) in light is required to abolish suppression, while a 90% reduction (2 lux) did not. The remainder of the experiments in this report were performed with light at 2 lux.

![Figure 1. Amount of light for suppression. Balb/cByJ mice were AC-injected with TNP-T cells and immediately placed in boxes fitted with neutral density filters that reduce light from 0%–99%. Twenty-four hours later the mice were immunized with 0.2 ml of 10 mM TNBS SC, and 5 days later ear-challenged with TNCB. Ear thickness was measured 24 hr later. Values are expressed as units of CHS with 1 unit = 10^{-3} cm (* denotes significant suppression vs. immune control).](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933167/ on 11/13/2018)
Determination of the Wavelength for Suppression

All molecules absorb light at specific wavelengths and have a characteristic action spectrum.\textsuperscript{10} Therefore, to begin identifying the action spectrum of molecules that mediate the effect of light on the ocular immune response, we used Wratten filters, photographic filters that pass light based on color. The experiment in Figure 2 shows that when Wratten filters for red, blue, and green light are used, only green light supports the development of suppressed CHS.

Because the green filter lets light pass at multiple wavelengths, we purchased narrow band filters, fitted the apparatus with them, and repeated the experiment in Figure 2. Filters from 400–460 nm and 530–750 nm were uniformly negative, i.e., no suppressed CHS was seen (data not shown). The most interesting points were 510 and 500 nm, as shown in Figure 3. Light at 510 and 500 nm support suppression, demonstrating that light near these wavelengths was responsible for the induction of TNP-ACAID. These wavelengths are two of the principle wavelengths in green light.

Role of the Retina

The experiment in Figure 3 demonstrates that the wavelengths responsible for suppression are near 500–510 nm. These wavelengths are very near the optimal absorption of several photopigments in the eye. Ocular pigments in the human eye absorb maximally at 496 nm (rod rhodopsin), 419 nm (blue cones), 531 nm (green cones), and 558 nm (red cones).\textsuperscript{10} Rodent
pigments are very similar, and evidence exists for another pigment very near the absorption spectrum of rhodopsin. Because these pigments are located in the photoreceptors of the mammalian retina, we took advantage of the availability of C3H/HeJ rd/rd mice. These animals have severe degeneration of photoreceptors by 4 wk of age, and a total degeneration of rods by 26 wk. In several experiments we determined that 4–6-wk-old C3H/HEJ mice and 26–30-wk-old C3H/HEJ mice displayed the same light/dark effect as Balb/c mice (data not shown). Therefore, we did the experiment in Figure 4 with 8–10-wk-old C3H/HeJ mice. These data show that the same wavelength requirement existed for C3H/HEJ rd/rd and Balb/c mice.

**Extension of Light/Dark to Protein Antigens**

The photoreactivity of TNP is a potential problem that’s encountered when the role of light in the suppression of CHS after anterior chamber injection of TNP-spl is being assessed. Therefore, we determined whether the effect of light could be extended to other antigen systems. We have been unable to induce suppressed DTH to protein antigens by injecting soluble protein into the anterior chamber. However, we have determined (data not shown) that antigen specific suppression can be induced by including a small number of antigen primed T cells in the anterior chamber inoculum with small amounts of the immunizing protein. As little as 1.5 µg of HEL can be included with $1 \times 10^5$ HEL primed T cells to induce potent suppression of DTH. Therefore, an experiment was done using HEL and HEL primed T cells in C3H/HeJ mice. The data in Figure 5 demonstrate that suppression of DTH to HEL was supported by light at 500 and 510 nm, as was suppression to TNP. The injection of $1 \times 10^5$ T cells plus 1.5 µg of HEL intravenously, intraperitoneally, or subcutaneously has no effect on immunity to HEL (data not shown).

**Role of the Pineal Gland**

Recent studies have suggested the pineal gland is a light-sensitive organ. Therefore, we considered the role of this organ in the light/dark effect in the eye. This organ produces important compounds such as melatonin and responds to circadian influences controlled by light. To test the role of the pineal gland, we purchased neonatally pinealectomized (pinlx) mice. These mice and sham pinealectomized controls were injected in the anterior chamber with TNP-spl, immunized 24 hr later, and tested for CHS 6 d later by ear challenge. After the experiment, the animals were examined surgically for successful pinealectomy. The results in Figure 6 show that pinealectomized and sham operated mice display suppressed CHS.

**The Effect of Light on Immunity**

Throughout the experiments in this report, we have been determining the effect of various wavelengths of light on ACAID. Our previous studies demonstrated that ACAID is initiated by an intraocular T cell reaction that is influenced by visible light. We already have shown that dark rearing animals and placing light-reared animals in the dark post immunization has no effect on intravenously induced tolerance of CHS responses. However, a question arose regarding the effect of the various wavelengths of light on systemic immunity. To address this question, we per-

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**Fig. 4. Determination of the wavelength for suppression in C3H/HeJ mice—role of the retina.** C3H/HeJ mice were AC-injected with TNP-T cells and immediately placed in boxes fitted with narrow band wavelength filters. Twenty-four hours later the mice were immunized with 0.2 ml of 10 mM TNBS SC, and 5 days later ear-challenged with TNCB. Ear thickness was measured 24 hr later. Values are expressed as units of CHS with 1 unit = $10^{-3}$ cm (+ denotes significant suppression vs. immune control).
formed the experiments in Figure 7. Mice were immunized for CHS (Fig 7A) or DTH (Fig 7B) and placed in the various lighting conditions. These results demonstrate that the wavelengths of light that influence ACAID have no effect on systemic immunity.

**Discussion**

The results presented in this report extend our previous observations concerning the effect of visible light on the ocular immune response. We show that suppressed CHS after anterior chamber injection of antigen is governed by green light in the 500-510 nm range. Although these wavelengths are near the absorption of rhodopsin, the effect of light apparently is not governed by detectable retinal pigments. This is because the same wavelengths supported suppression in C3H/HeJ rd/rd mice, which are devoid of photoreceptors. In addition, the pineal gland, a light-sensitive organ, does not appear to play a role because pinealectomized mice display suppressed CHS.

The results presented here show that the effect of light on ocular immune responses extends beyond the TNP-ACAID system. The suppression of DTH after anterior chamber injection of HEL and T cells also depends on light at 500-510 nm. HEL absorbs light in the UV range (280 nm) as do most other protein antigens. In addition, we also have extended our observations to the von Szily model of experimental retinal necrosis. The contralateral retinitis observed after the anterior chamber injection of HSV-1 depends on visible light. When animals are dark reared, retinal necrosis is not observed in the ipsilateral or contralateral eye. Whether this is governed by 500-510 nm light remains to be determined.

The effect of UV light on immunity is well studied, but little is known about the effect of visible light.
light on immune responses. To our knowledge, there is virtually no literature that documents an effect of visible light directly on in vivo immune responses. Studies have been performed that show an effect of visible light on cultured cells, such as Chinese hamster ovary cells. These effects have been attributed to the photochemical interaction between fluorescent light and media components such as riboflavin, tryptophan, tyrosine, and HEPES buffer. This interaction can lead to the formation of lipid peroxidases, superoxide anion, and H$_2$O$_2$, which inhibit or kill cultured cells. Some studies have attempted to document a direct interaction between visible light and DNA. It has been shown that visible light can cause single strand breaks in the DNA.

One recent study documented the effect of constant darkness on autoimmunity to type II collagen. These results were attributed to circadian effects and melatonin synthesis by the pineal gland. The results of our previous study suggest that circadian influences may not be directly involved in this system and the present study argues against a role for the pineal gland.

While the effects of visible light on the immune response have not been studied, the interactions between light and the eye have been studied extensively. The eye absorbs light through its system of visual pigments located in the retina. These pigments include the well known rod pigment rhodopsin and three cone pigments: blue, green, and red. The maximum absorption for these pigments in humans are: rhodopsin, 496 nm; blue cone, 419 nm; green cone, 531 nm;
pigments starts a complex series of chemical reactions and the initiation of the visual cycle. The initial isomerization of the photopigment is the only reaction in vision that involves light. The remainder of the reactions are non-light dependent and result in the production of many products and intermediates that may potentially affect immune reactions in the eye. Unfortunately, our experiments with rd/rd mice may rule out a role for the retina. However, the presence of photopigment in other ocular structures, such as the iris, has not been ruled out. Amphiibians have been shown to have rhodopsin localized in the iris. Also, although retinal pigments absorb maximally at the above wavelengths, they also have absorption at other visible wavelengths and in the UV range. Even the possibility that T cells included in the anterior chamber inoculum have 500–510 nm absorbing pigments has to be considered.

Adult rd/rd mice are devoid of photoreceptors. However, they do retain a normal ganglion cell layer, retinal pigment epithelium, and Müller cells. Although these layers are intact and may inhibit immune function (eg, Müller cells), they do not result in a light-induced neuronal signal. This is solely a property of the photoreceptors in the neurosensory retina. Our results seem to rule out the possibility that visual phototransduction through the neurosensory retina is responsible for the observed effect of green light because rd/rd mice have no photoreceptors and thus no phototransduction.

It is interesting that the effect of light on intraocular immune reactions is confined to such a narrow range. Further study will be required to evaluate the limited wavelength requirements. The molecular mediator (or mediators) for the light-induced effects on the immune response and the eye is not known, so further study on the wavelength of light responsible for the production of neurotransmitters (dopamine, serotonin, melatonin, histamine) and neuropeptides (vasointestinal peptide, substance P) may provide clues to the role of light in ocular immunoregulation.

Key words: light, anterior chamber associated immune deviation, delayed-type hypersensitivity, immunoregulation, T cell

References