THE EFFECTS OF CUTANEOUS ULTRAVIOLET SUNLIGHT EXPOSURE ON T CELL-MEDIATED NEUROINFLAMMATION

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Abstract

Neuroinflammatory disease is becoming increasingly common in our society-- affecting individuals of all ages, causing cognitive deficits, motor decline, and even propagating anxiety and depression. As the field of neuroimmunology continues to develop, there is increasing evidence of the implication of T cells in inflammation of the central nervous system. At the same time, in the field of photobiology, advances are being made to detail the absorption of photons by chromophores in the human body-- a process rooted in quantum physics-- and the downstream effects that this has on homeostasis. Humans have evolved with a strong dependence on sunlight as it regulates our physiology through both the eye and skin, not only dictating circadian rhythms but also driving immune cell differentiation. Upon intertwining discoveries in each of these fields, I propose that moderate doses of cutaneous sunlight exposure may help ameliorate T cell-mediated neuroinflammation through mechanisms involving upregulated tolerogenic dendritic cells and a shift in the balance of regulatory and effector T cells, which are capable of crossing the blood brain barrier and dictating inflammatory responses.

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INTRODUCTION

Like most components of nature, T cell-mediated immunity comprises a delicate balance: the yin and yang of creating and healing inflammation, mediated by effector and regulatory immune cells. If T cell differentiation is tipped too far in favor of inflammation, pathology ensues; furthermore, if these effector cells begin attacking self, autoimmune conditions arise. Immune cell differentiation and proliferation are highly dependent on environmental cues, stemming from the extracellular microenvironment, systemic fluid components, and extending out to environmental exposure of surface tissues including the skin, lungs, eyes, gastrointestinal tract, and other mucus membranes.[1] Neuroinflammation is defined as an immune response within the brain or spinal cord, mediated by the production of cytokines, chemokines, and reactive oxygen species. The central nervous system (CNS) is equipped with a highly regulated neuroimmune system, though disease can lead to increased permeability of its protective barriers resulting in increased infiltration of peripheral immune mediators. While neuroinflammation is primarily associated with neurodegenerative disorders including Alzheimer's disease (AD), Parkinson's disease (PD), and multiple sclerosis (MS), it is also implicated in anxiety, cognitive dysfunction, and aging in a large percentage of the population.[2–4] While neuroinflammation may manifest differently in various age groups and according to external influences, the aforementioned conditions share common mechanisms of dysfunction.

As the number of cases of neuroinflammatory disease continues to increase, it is timely to consider how everyday inputs to cellular signaling affect the immune system, including sunlight. Humans have evolved with both direct and indirect dependence on sunlight, and there is an increasing amount of evidence that light exposure plays a critical role in maintaining homeostasis. The field of photobiology is increasingly expanding, and different wavelengths of light are being used to treat disease of all types. Ultraviolet (UV) light is currently used by major medical institutions to treat skin conditions including atopic dermatitis and psoriasis.[5] Jaundiced newborns are placed in filtered sunlight for treatment in hospitals.[6] LED lights are utilized for wound healing.[7] Timed light exposure through both the eye and skin is used to treat countless

conditions including cancer-related fatigue, depression, and even inflammatory bowel disease.[8–10] Transcranial photobiomodulation is used as behavioral and cognitive therapy in patients with dementia.[11] Infrared light and near-infrared light is used to increase ATP production in mitochondria, affecting the health of the body as a whole.[12]

Sunlight exposure is commonly discouraged by the medical community due to associations with skin cancer, however there is an increasing amount of evidence supporting its therapeutic effects, including those beyond benefits provided by vitamin D production.[13] While excess exposure to ultraviolet light from the sun can have deleterious effects on human health, there are undeniable benefits to sunlight exposure in moderate amounts and under appropriate conditions, taking into account factors such as skin type, diet, hydration, and level of systemic inflammation (indicated by markers such as C-reactive protein).[14]

Advances in literature demonstrate light's ability to regulate the immune system through the eye by dictating circadian rhythms and through the skin by commanding T cell development in the lymph nodes. There is substantial evidence of visible light modifying circadian rhythms via the suprachiasmatic nucleus in the hypothalamus, affecting overall body function through transcription of CLOCK genes in nearly every body cell.[15–17] On the other hand, the connection between skin exposure to ultraviolet light and systemic immunomodulation is less understood. The mechanism of sunlight's effect on dendritic cells in the epidermis has been documented[18] and there is evidence of general translocation of these cells systemically,[19] though there is not significant documentation of the extended mechanism through which sunlight modulates T cell-mediated neuroinflammation specifically.

Through this thesis, I aim to describe how moderate levels of ultraviolet sunlight exposure can help ameliorate neuroinflammation caused by T cell-mediated mechanisms. This will be accomplished through first detailing the physiology of T cell-mediated immunity, the involvement of T cells in neuroinflammation, the mechanisms of chromophore excitation in the

skin and their connection to the systemic immune system, and finally how modification of peripheral immune cell differentiation can affect brain inflammation.

CHAPTER 1: T CELL-MEDIATED IMMUNOLOGY

1.1 T Cell Differentiation

T lymphocytes originate from stem cells in the bone marrow and develop in the thymus, where they undergo positive and negative selection. During this process, the precursor cells are considered double negative, as they possess neither surface marker CD4 nor CD8. The genes for both CD4 and CD8 surface markers are then turned on.[20] The double positive cells undergo positive and negative selection in which immature T cells that bind to major histocompatibility complex (MHC) complexes with moderate affinity are positively selected for, while those that bind too strongly and will likely become self-reactive are negatively selected for, preventing autoimmunity. Selected T cells continue on to become single positive, maintaining either CD4 or CD8 surface markers.[21] Mature naïve CD4+ cells exist as T helper type 0 (Th0) and later differentiate into Th1, Th2, Th17, follicular helper T cells (Tfh), or regulatory T cells (Treg), while CD8+ cells develop into cytotoxic T cells. The mature naïve T cells then migrate to the paracortex of peripheral lymph nodes, where they await activation.[22] CD4+ T cells recognize antigenic peptides presented on MHC class II molecules, while CD8+ T cells recognize antigenic peptides presented on MHC class I molecules. MHC class II is expressed by professional antigen presenting cells (APCs), including dendritic cells, B cells, and macrophages, while MHC class I is expressed on all nucleated cells.[23]

Specialized phagocytic white blood cells called dendritic cells (DCs) exist in the skin, lungs, and mucous membranes and serve as the interface between the environment and adaptive immune system. When immature DCs become activated they undergo maturation, take up nearby molecules in their environment, and travel through the lymphatic system to the nearest draining

lymph node. There, they encounter immature CD4+ T cells and induce their differentiation into helper T cells. To accomplish this, DCs present the antigenic peptides they have gathered via MHC class II. For this reason, DCs are classified as APCs. The MHC II is recognized by the T cell receptor (TCR) on one of the immature T cells. In addition to the TCR/MHC II interaction, a second signal is necessary for T cell activation and is provided by interaction between costimulatory molecules on the DC and T cell. Based upon this interaction, the DC then releases specific cytokines that prime T cells for unique differentiation into Th1, Th2, Th17, or Treg cells. Naïve CD4+ differentiation is dependent upon cytokines and transcription factors secreted into their microenvironment. It is through this mechanism that T cell differentiation is modulated by the external environment, mediated through DCs.[23,24] The cytokines and transcription factors required for differentiation into each of the primary T helper cells are detailed below, in addition to a brief overview of their functions. See Figure 1 for a visual representation of naïve CD4+ T cell.

Th1 cells are activated by interleukin 12 (IL-12) and interferon gamma (IFNγ). IL-12 activates transcription factor STAT1, which induces the master transcription factor T-bet, inducing a Th1 phenotype. Th1 cells are inhibited by IL-10, an anti-inflammatory cytokine.[25] Th1 cells secrete IFNγ, which is chemotactic for blood monocytes and tissue macrophages and activates cytotoxic T cells and B cells. IFNγ classically activates macrophages to become M1, which are powerful phagocytes that destroy tissue, creating inflammation. A single Th1 cell can elicit an immune response by classically activating thousands of macrophages.[2627] Due to their pro-inflammatory effects, Th1 cells are key players in autoimmune disease. This is further detailed below.

Th2 cells are activated by IL-2 and IL-4, which induce the master transcription factor GATA3. The primary function of Th2 cells is fighting helminths, protozoa, and other parasites. They are chemotactic for both eosinophils and macrophages and secrete cytokines including IL-4, IL-5, IL-10, and IL-13, which alternatively activate macrophages. These M2 macrophages wall off pathogens and promote healing from the damage caused by M1 macrophages, as detailed below.

Th2 cells also induce mucus production and facilitate B cell class-switching to IgE, further eliciting immune response.[25,26,28]

Tfh cells are activated by IL-6 and IL-21 via induction of transcription factor BCL6. Both DCs and B cells play a role in activating Tfh cell differentiation and migration. They are primarily involved in activating B cell maturation and regulating antibody class-switching from IgM to IgG, IgA, or IgE. Tfh cells are also critical to the induction of high-affinity plasma cells and memory B cells.[26,29,30]

Th17 cells are activated by cytokines IL-6, IL-21, IL-23, IL-1β, and transforming growth factor beta (TGF-β), which turn on transcription factors STAT3 and RORγt. The primary role of Th17 cells is defending against extracellular bacteria. Like Th1 cells, they also play a critical role in inflammatory and autoimmune disease due to the effector cytokines they secrete, primarily IL-17. Th17 are pro-inflammatory and chemotactic for classically activated M1 macrophages.[26,31]

Treg cells can develop either in the thymus during negative selection (tTreg cells) or from mature naïve CD4+ T cells in the periphery (pTreg cells). Differentiation of pTreg cells is induced by IL-2 and TGF-β, which activate the transcription factor FoxP3. Differentiated Treg cells express both surface markers CD4 and CD25. These CD4+CD25+FoxP3+ Treg cells maintain the delicate balance between pro-inflammatory and anti-inflammatory responses, suppressing proliferation and cytokine production of both Th and CD8+ T cells. This is carried out through their secretion of IL-10 and TGF-β, two anti-inflammatory cytokines that suppress activation of Th1 and Th17 cells and induce M2 macrophage polarization.[32] This role makes them instrumental in prevention of inflammatory pathologies and autoimmune disease.[33,34] FoxP3+ Treg cells are crucial for survival, as evidenced by autoimmune lethality in mice modified with the deletion of *FoxP3*.[34,35] Treg cells make up approximately 6% of CD4+T cells.[1] While Treg cells exist in fewer numbers than Th cells, they are much more potent as one Treg cell can suppress approximately 1,000 Th cells.[27]

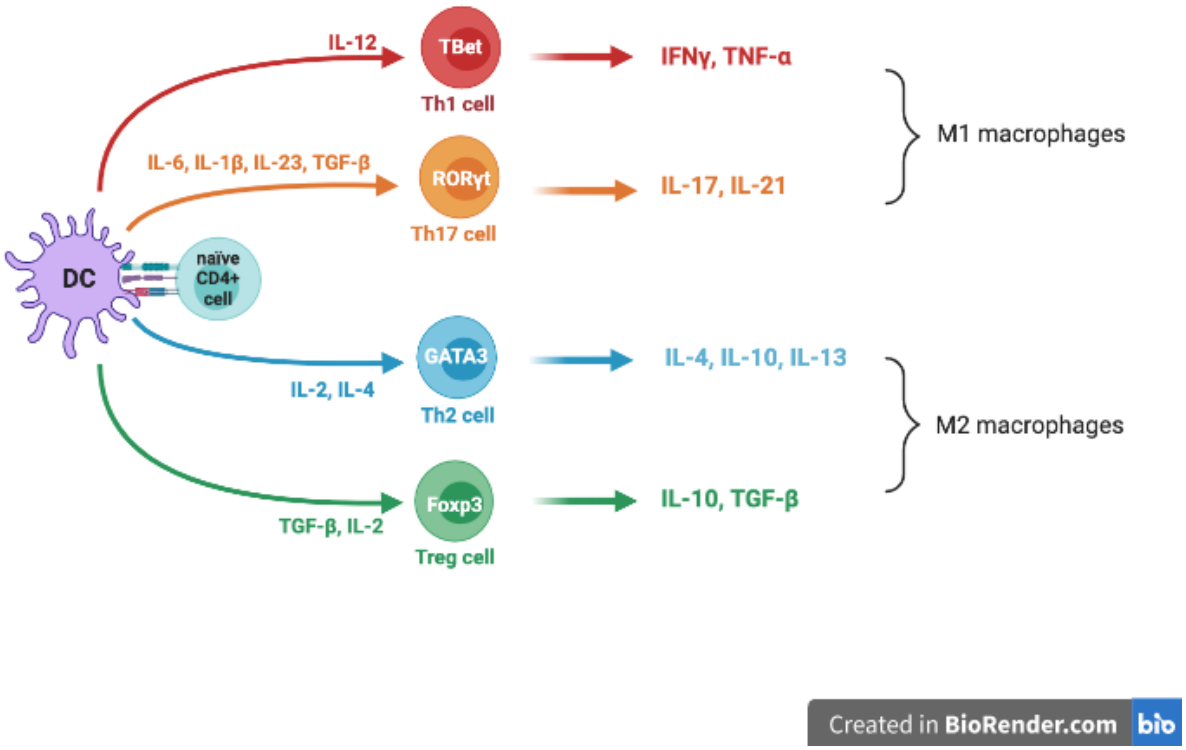

Figure 1: Differentiation of naïve CD4+ T cells is determined by cytokines released by the activated dendritic cell which binds to it. The various cytokines that specialized T cells then secrete can classically or alternatively activate macrophages.

Macrophages

The word macrophage is Greek for "big eater", and these cells are named for their phagocytic function in engulfing debris and devouring anything deemed as foreign. Macrophages are a type of plastic phagocytic leukocyte that can be activated through one of two methods: classical activation leading to M1 macrophages, or alternative activation leading to M2 macrophages. The population balance of M1 and M2 macrophages is concerted, and imbalance can lead to pathogenesis.[37] M1 macrophages express transcription factors including NF-kB and STAT1, which lead to production and secretion of proinflammatory cytokines including high levels of IL-12 and IL-23 and low levels of IL-10, in addition to reactive oxygen species (ROS).[25] They

create inflammation and destruction which can be a beneficial response to infection but can have pathological effects when dysregulated, as further described below. The M1 phenotype is stimulated by IFNγ, tumor necrosis factor alpha (TNFα), or toll-like receptor (TLR) ligands. Active M1 macrophages secrete proinflammatory cytokines including TNFα, IL-1, and IL-6, inducing local edema and triggering endothelial barrier leakiness.[37]

Conversely, M2 macrophages are anti-inflammatory and are involved in healing by cleaning up debris and walling off damage. They produce ornithine via the arginase pathway, which contributes to the restorative effects of M2, in addition to high amounts of the anti-inflammatory cytokines IL-10 and TGF-β.[25,38,39] Macrophages are alternatively activated by IL-4 and IL-13, primarily secreted by Th2 cells. Polarization of macrophages is ultimately influenced by T cell types: a large amount of Th1 cells correlates with a high number of M1, while a relatively large amount of Th2 and Treg cells correlates with a high number of M2. Overabundance of M1 cells can lead to chronic inflammation and autoimmunity.[40]

Tolerogenic vs. Immunogenic Dendritic Cells and Their Role in T Cell Proliferation

As stated above, dendritic cells exist in the tissues bordering the external environment and serve as mediators between innate and adaptive immune responses. As DCs secrete cytokines that activate Th differentiation, it is important to note that they may also exert tolerogenic effects in addition to their immunogenic properties. Polarization of DCs is dependent on environmental cues. Interestingly, increased levels of autoimmunity have been correlated with decreased DC count, supporting a tolerogenic role for DCs rather than solely immune response.[41] Tolerogenic DCs prevent inflammatory and autoimmune response via peripheral tolerance both by inhibiting T helper cells directly and by activating FoxP3+ regulatory T cells. They express low levels of costimulatory molecules and high levels of surface molecules involved in T-cell inhibition, such as programmed cell death ligand 1 (PDL1) and CD95L. Tolerogenic DCs also synthesize Indoleamine 2, 3-dioxygenase (IDO), a molecule which depletes levels of intracellular tryptophan leading to inhibition of proinflammatory T cell proliferation and induction of autophagy and apoptosis.[41,42] Tolerogenic DCs promote T cell anergy through production of low

levels of costimulatory molecules, inhibiting T cells from receiving the secondary signals that they need to differentiate. By eliciting development of Tregs, tolerogenic DCs indirectly upregulate secretion of IL-10 and TGF-β, resulting in both resolution of existing inflammation and inhibition of further pro-inflammatory responses. As such, tolerogenic DCs play a vital role in prevention and amelioration of T cell-mediated inflammation and autoimmune disease.[43,44] As will be described in subsequent sections, it is this switch to tolerogenic DCs that enables ultraviolet light to inflict immune modulation.

CHAPTER 2: NEUROINFLAMMATION

2.1 Overview

The CNS comprises the brain and spinal cord. Its microvasculature is surrounded by the blood brain barrier (BBB), a highly selective barricade created by specialized endothelial cells. The BBB serves as the buffer between the CNS and periphery to preserve homeostasis within the microenvironment of the neurons. The BBB differs from other endothelial cell layers in the body in that it has tight junctions and lacks fenestrations, preventing the majority of blood-borne molecules from being transported across.[45] The BBB has a very high concentration of mitochondria to enable its selectivity. Interestingly, mitochondrial dysfunction can lead to opening of the BBB, resulting in an increase of cerebral disease and neuronal degradation.[46] I will further detail the key components of neuroinflammation and their relationship to peripheral immunity below.

Neuroinflammation is fundamentally involved not only in neurodegenerative disease including AD, PD, and MS, but also in the aging process, anxiety and depression, and in response to stress.[3,4,47] Thus, neuroinflammation is constituted by varying degrees of severity. Though the pathology of each of these states and diseases has unique components, they share underlying mechanisms of T cell-mediated neuroimmune dysfunction. The neuroimmune system is primarily mediated by microglia and the cytokines, chemokines, and second messengers that they release. Resulting edema can impair cognitive function, incite deficits in learning and

memory, and engender progression of neurodegenerative disease. While chronic neuroinflammation may be triggered by traumatic brain injury or infection, it can also be related to diet and lifestyle, including consumption of a diet high in carbohydrates.[48] As further described below, the neuroimmune system is receptive to that of the periphery, and environmental exposures including sunlight can influence this.

2.2 Microglia

Microglia, the resident macrophages of the CNS, play a critical role in neuroinflammation and are the key intermediaries between T cells and neuronal fate. Under normal conditions, CNS infection or injury leads to acute M1-like activation of microglia, which have phagocytic and antimicrobial effects through the secretion of inflammatory cytokines just like their peripheral counterparts. This is typically followed by M2-like activation of microglia, which reduce inflammation and alleviate damage caused by M1-like microglia, cultivating a neuroprotective environment.[49] However, when M1-like microglia undergo prolonged activation, they chronically secrete inflammatory cytokines including TNFα, IL-6, and IL-1β, ROS including hydrogen peroxide and superoxide anion, and the excitatory neurotransmitter glutamate.[32,50] Each of these mediators have neurotoxic effects when large quantities persist in the brain. Furthermore, TNFα and IL-1β upregulate the expression of surface molecules on endothelial cells of the BBB, increasing its permeability to lymphocytes.[32] This further enables activated CD4+ T cells and DCs from the periphery to enter into the CNS. Figure 2 provides a visual representation of microglial polarization and its effects on neuronal fate.

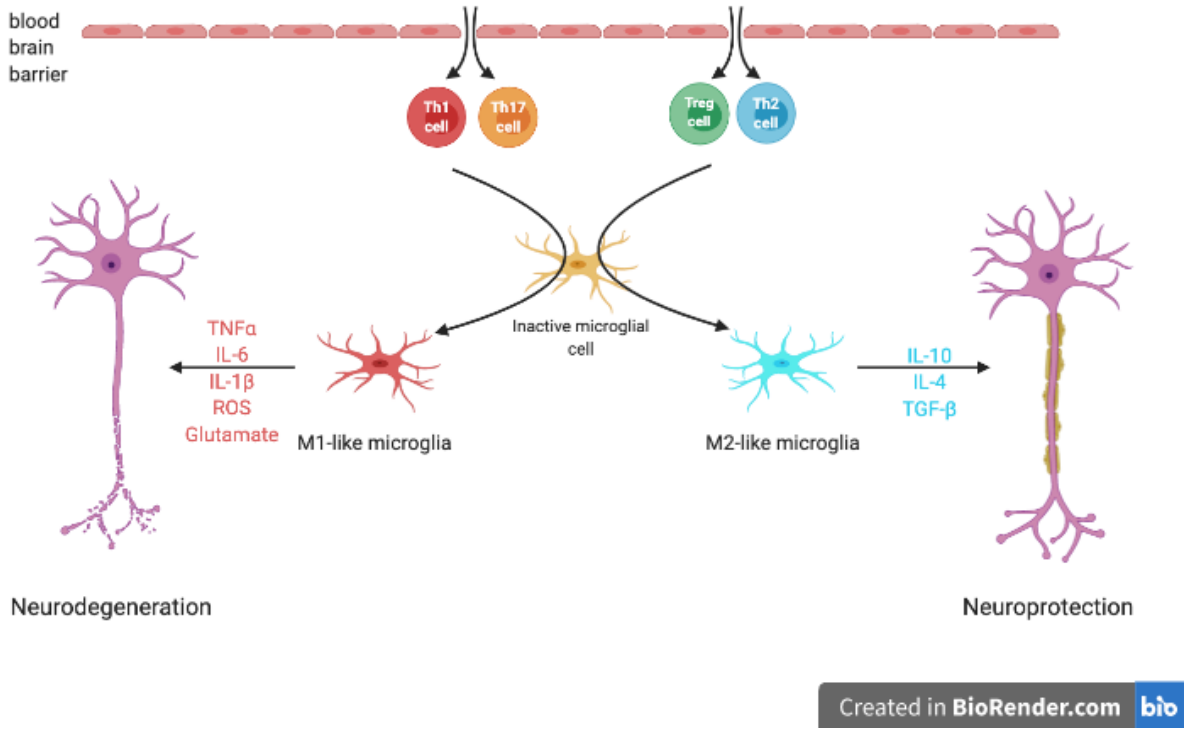

Figure 2: Polarization of M1/M2-like microglia and their effect on neuron health.

2.3 T Cell Involvement in Neurodegenerative Disease

Just as with peripheral immunity, microglial polarization is dependent upon activation by T cells. Once Th17 cells infiltrate the CNS, they can be re-stimulated by cerebral APCs, increasing their secretion of IL-6, IL-1β, and TGF-β. Secretion of these cytokines leads to M1-like activation of microglia, thus stimulating the inflammatory cascade detailed above.[51]

Though the full function of Tregs in the CNS has yet to be elucidated, there is evidence suggesting their neuroprotective role in maintaining immune tolerance and mitigating overactivation of M1-like microglia. Within the CNS, Treg cells have been demonstrated to promote microglial polarization toward the M2-like phenotype, providing anti-inflammatory effects.[32] Additionally, cerebral Treg secretion of IL-10 and TGF-β may serve to downregulate Th1 and Th17 cell activation as it does in the periphery.[52]

Alzheimer's Disease

AD is the most common neurodegenerative disease. It is marked by the aggregation of the naturally occurring protein amyloid beta (Aβ) in the brain. Abnormal levels of Aβ form plaques which block electrical and chemical signals from being transmitted between neurons. This dysfunction results in loss of memory and cognitive ability. In AD, effector T cells have been discovered to infiltrate the brain and aggregate in close proximity to Aβ plaques. Moreover, M1-like microglial secretion of IL-1β and TNFα contribute to increased deposition of cerebral Aβ plaques. IFNγ produced by Th1 cells increases M1-like microglial activation, Aβ plaque deposition, and cognitive impairment.[53] Thus, increased filtration of T cells into the CNS has been shown to accelerate pathogenesis of AD. Interestingly, adoptive transference of Treg cells into a mouse model of AD has been demonstrated to significantly reduce the amount of Aβ deposits and improve cognitive function.[51,54]

Parkinson's Disease

Similar to AD, PD also involves the aggregation of misfolded proteins in the CNS. PD is characterized by loss of dopaminergic neurons in the substantia nigra pars compacta, resulting in symptoms of altered motor function. There is emerging evidence of T cell involvement in PD, indicated in part by altered frequency and ratios of CD4+ T cells in blood samples of PD patients.[51,55] It has also been demonstrated that Tregs serve a neuroprotective role in PD, with injected Treg cells slowing progression of neurodegeneration in mice.[55] This is again similar to the role of Tregs in AD.

Multiple Sclerosis

MS is a primarily T cell-mediated inflammatory autoimmune disease. It is characterized by chronic and progressive degradation of the axonal myelin sheath in neurons of the brain and spinal cord. The main culprits of neuronal destruction and neuroinflammation in MS and the complementary animal model of experimental autoimmune encephalomyelitis (EAE) are Th1

and Th17 cells, which become self-reactive for myelin. These self-reactive T cells originate in peripheral lymph nodes and then migrate to the CNS. Their overproduction of proinflammatory cytokines including IL-1β, granulocyte-macrophage colony-stimulating factor (GM-CSF) and TNFα results in increased permeability of the BBB.[51] This increased permeability allows greater amounts of Th1 and Th17 cells to enter into the CNS where they release inflammatory cytokines, activate microglial cells, and exacerbate demyelination and neuronal damage.[2] Just as with PD and AD, several separate studies have been executed which display neuroprotection in EAE mice injected with FoxP3+ Treg cells, as well as disease progression in those depleted of Tregs.[56] FoxP3+ Treg cells have been demonstrated to protect mice against EAE, while depletion of the cells has been demonstrated to exacerbate severity of the disease.[43,56,57]

2.4 BBB Disruption in Neuroinflammatory Disease

Each of the above immunopathologies involves increased permeability of the BBB which enables more immune cells to enter the CNS, exacerbating neuroinflammation. This process indicates increased sensitivity to systemic inflammation in neurodegenerative disease. It is important to note that BBB permeability is not limited to effector T cells, but also enables migration of Tregs and Th2 cells to the CNS, where they have the ability to decrease inflammatory response and create a neuroprotective microenvironment.[51] Blood samples from patients with varying levels of neuroinflammatory conditions show an imbalanced ratio of peripheral effector to regulatory T cells, mirroring the imbalance of pro- and anti-inflammatory cells in the brain and spinal cord.[58] This not only demonstrates a shortage of Treg cells, but also shows that T cells are able to infiltrate the BBB in large numbers and in ratios representative of those in the periphery. As aforementioned, multiple experiments in which murine models of AD, PD, and MS were injected with Tregs demonstrated decreased neuroinflammation and disease progression.[51,55–57] This leads to the conclusion that upregulating peripheral Tregs may help ameliorate neuroinflammation through their migration across the BBB.

To summarize, the role that infiltrating CD4+ T cells play in neuroinflammation is dependent on peripheral activation which determines their functionality.[32] This can result from a shift in the

balance of upstream tolerogenic and immunogenic DCs, dictated by microenvironmental factors of all DCs that can migrate to peripheral lymph nodes. Interestingly, DCs have also been shown to migrate to the CNS from the periphery, demonstrating a mature phenotype upon migration and thereby activating T cell differentiation and proliferation within the CNS itself.[59]

CHAPTER 3: ULTRAVIOLET LIGHT

3.1 UV Absorption: A Quantum Process

Electromagnetic radiation from the sun has shaped life on Earth by providing photoenergy, which is converted to chemical energy that drives molecular organization and therefore organismal complexity. The electromagnetic spectrum of sunlight that reaches Earth's surface includes UVB (280-320 nm), UVA (320-400 nm), visible light (400-700 nm), and infrared light (700-1000 nm). UVC radiation (<280 nm) is absorbed by ozone in the atmosphere and rarely reaches Earth's surface. Electromagnetic radiation exists in packets or quanta termed photons. Organic molecules with functional groups capable of absorbing light, called chromophores, convert energy from photons into usable chemical energy. This interaction is founded in the quantum mechanics of electron excitation. Basic molecular structure involves energy stored in electron bonding orbitals (wave functions) between atoms. When a photon is absorbed by a chromophore, it promotes an electron to a higher energy orbital. For example, electrons can transition from $n \rightarrow \pi^*$ or $\pi \rightarrow \pi^*$, moving from a bonding to an antibonding orbital. This demonstrates that photons contain sufficient energy to break or make covalent bonds, having profound effects on molecular structure and therefore function. The chemical structure of each chromophore dictates which wavelengths of light it can absorb. Molecules with conjugated C-C or C-O double bonds including aromatic amino acids (tryptophan, tyrosine, and histidine) are capable of absorbing light with wavelengths in the ultraviolet region.[60] Other compounds with benzene rings including purines, pyrimidines, melatonin, urocanic acid, NADH, and porphyrins are also highly photobiologically relevant.[61–64] While the landscape of light's effects on the human body is rapidly expanding, we have barely scratched the surface in terms of the profound effects that it has on our physiology.

3.2 UV Light and Skin

The skin is the major front-line defense against the external environment, serving as a barrier and communicating signals with the immune system in a bidirectional manner to regulate homeostasis. Various chromophores in the skin, including DNA, urocanic acid, aromatic amino acids, melanin, and others, facilitate the transduction of electromagnetic energy into chemical and neural signals.[61,62] Locally stimulated components of the epidermis can have wide downstream effects, transferring information from light to systemic modulation.[13]

UV light that reaches Earth's surface is composed of approximately 95% UVA and 5% UVB. While the longer wavelengths of UVA can penetrate deeper into the dermis, UVB light is mostly absorbed by the epidermis.[65] The epidermis contains Langerhans cells (LCs), which are resident DCs. There are multiple mechanisms through which UV affects LC differentiation and migration, as described below.

UVB is perhaps best known for its role in vitamin D production in the skin. Vitamin D is a steroid hormone with profound systemic endocrine and immune effects, exemplified by the fact that the majority of body cells express vitamin D receptors.[66–68] Keratinocytes in the epidermis synthesize vitamin D from its precursor, a form of cholesterol called 7-dehydrocholesterol. Upon stimulation by UVB light of wavelength 280-320 nm, 7-dehydrocholesterol isomerizes to cholecalciferol D3, which is transported to the liver and then kidney where it is converted to a usable form.[69]

Urocanic Acid as a Chromophore

One of the primary chromophores in the skin that is involved in immunoregulation is urocanic acid (UCA). *Trans*-UCA is synthesized from histidine in the epidermis and undergoes isomerization to *cis*-UCA upon exposure to UV radiation. This isomerization takes place with greatest quantum efficiency when excited by wavelengths 300-320 nm.[70] The role of *cis*-UCA in mediating UVR suppression was initially demonstrated in contact hypersensivity and has been

evidenced to directly reduce immunogenic effects of LCs in the skin.[71] The mechanism through which *cis*-UCA induces immunosuppression has yet to be fully elucidated, though it has been shown to be mediated through binding to serotonin receptors.[72] *Cis*-UCA has been demonstrated to upregulate production of IL-10 and inhibit production of IFN-γ by T cells, demonstrating immunosuppressive effects.[73] Additionally, a study using cultures from MS patients showed significantly increased numbers of FoxP3+ Treg cells in the presence of *cis*-UCA.[68] This argument was further supported by evidence of similar immunosuppressive effects in topical administration of *cis*-UCA to mice.[74] Modulation of APCs by *cis*-UCA affects their downstream activity in the draining lymph nodes and therefore mediates their tolerogenic and immunosuppressive function.[75]

Receptor Activator of NF-kappaB (RANK)

It has been demonstrated that keratinocytes in the skin contain receptor activator of NF-kappaB (RANK) ligand (RANKL). RANK is a key modulator of thermoregulation, liver function, mammary gland function, bone metabolism, and CNS as well as gut immunity. LCs, a subset of DCs and components of the epidermis, contain RANK. They are capable of migrating from the epidermis to the peripheral lymph nodes, acting as one of the primary communicators between external stimuli and internal reaction. It has been demonstrated that RANKL expression by keratinocytes increases upon skin exposure to UV light, binding to RANK on LCs and stimulating their production of IL-10 and tolerogenic activation of Treg cells.[76] In addition to cases of disease, RANK activation has been shown to maintain Treg population in healthy individuals.[75] Through this mechanism, RANK-RANKL interaction leads to immunosuppression implicated in the healing of atopic dermatitis, a type IV hypersensitivity.[77] It is through this same mechanism that exposure of skin to UV light can have systemic immune effects, as further described in Chapter 4.

Once activated, Treg cells have been shown to migrate not only to inflammatory sites in the skin, but to areas of inflammation throughout the body.

CHAPTER 4: CONNECTING CUTANEOUS UV EXPOSURE AND DECREASED BRAIN INFLAMMATION

There is significant documentation of the correlation between geographical location and prevalence of neurodegenerative disease including MS, with lower numbers of cases occurring at latitudes closer to the equator (where UV light exposure is the highest).[78] While vitamin D is the most feasible measure of UV light exposure, there is also significant evidence of immunomodulation through mechanisms independent of vitamin D, which are more difficult to measure.[61] These alternative effects must be considered in studies correlating vitamin D levels and immune function. A decrease in T cell-mediated pathology may be related to the mechanisms outlined in this thesis, or a to a number of other effects that sunlight has on the immune system independent of vitamin D.

UVB light exposure has been demonstrated to activate LCs as tolerogenic through chromophores and receptors including UCA and RANK, thus upregulating differentiation and proliferation of Treg cells and downregulating that of Th1 cells.[18,71,76,79] Additionally, keratinocytes themselves have been documented to secrete IL-10 upon exposure to UV light, resulting in immunosuppression.[18] As described in previous sections, both Treg/Th2 and Th1/Th17 cells have the ability to migrate from peripheral lymph nodes to the CNS. This, combined with evidence of increased ability of DCs and Tregs to migrate systemically and cross the BBB in cases of neuroinflammatory disease, supports the hypothesis that UV radiation on the skin has the potential to downregulate neuroinflammation through a cascade involving T cell-mediated mechanisms. Mimicry of sunlight in therapeutic treatments, whether or not that is the stated goal, demonstrates the properties of ultraviolet and visible light to modulate the immune system.

UV Effects Mirrored in Graphene Quantum Dots

Graphene quantum dots (GQDs) are an emerging class of photoluminescent nanoparticles made of carbon. They have diameters less than 10 nm and disperse well in water. Their small size even enables them to traverse the BBB. As GQDs exert low levels of cytotoxic activity, they have become candidates for biological application.[80] GQDs emit light in the UV range and have been demonstrated to have similar immunological effects as skin exposure to UVR, but on an organ-specific basis, as they are injected into the body intravenously. Their effects have been documented in MS, inflammatory bowel disease, and hepatitis, amongst others.[9,81–83] While much of the current literature on GQDs does not describe UVR as the direct cause of immunosuppression and amelioration of inflammatory disease, the mechanisms appear to be consistent with this and this is an area that I believe warrants further exploration.

In another study, GQDs were injected into EAE rats. The GQDs used had photoluminescence between 330-675 nm, wavelengths which fall both in the ultraviolet and visible ranges of the electromagnetic spectrum. The GQD-treated rats displayed significantly decreased levels of Th1 in extracted lymph node and CNS tissue, along with decreased levels of inflammatory cytokines including IFNγ, IL-1, and GM-CSF. The GQD-treated rats had not only confirmed delayed onset of disease, but also significant alleviation of axonal damage in the CNS.[82] In another study involving the murine model of AD, transgenic mice treated with GQDs with peak absorption at 320 nm displayed significant inhibition of Aβ plaque aggregation in the brain.[81] The immunological effects shown in these studies mirrors the proposed mechanism of sunlight modification of the neuroimmune system via excitation of chromophores in the skin, further illustrating the beneficial role of UV light on the immune system.

CONCLUSION

Based upon a constellation of seemingly unrelated studies, I propose that skin exposure to a moderate amount of ultraviolet sunlight can alleviate neuroinflammation through T cell-mediated mechanisms. This is accomplished through multiple points along the extensive path between the skin and brain. The energy contained in photons from the sun excites electrons in chromophores

of the skin, converting photoenergy into chemical signals. This triggers modification of DCs in the epidermis, inducing a phenotype for peripheral tolerance.[79] The DCs then migrate from the skin to nearby peripheral lymph nodes. There, they encounter mature naïve CD4+ T cells and induce their differentiation into Treg cells rather than effector cells. As one Treg cell can modulate approximately 1,000 Th cells, significant change in the inflammatory balance can be made with the production of even a small number of Treg cells.[27] These Treg cells, along with tolerogenic DCs, are capable of migrating across the BBB to areas of CNS inflammation. There, they are able to alter the components of the cerebrospinal fluid to contain less IL-6, IL-1β, TNFα, glutamate, and ROS, and more IL-10, IL-4 and TGF-β, shifting neuronal fate from neurodegeneration to neuroprotection.[25,32,39]

While nanotechnology is evolving with the ability to inject light-emitting quantum dots into the body to ameliorate disease, similar healing effects may be available through appropriate amounts of sunlight exposure to the skin. As research in this area expands, sunlight exposure may be a key piece in the puzzle of reducing neuroinflammation. This would provide an accessible and low-risk therapeutic intervention to those affected by neurodegeneration, anxiety, and other neuroinflammatory conditions.

In short, current data supports the cohesion between the puzzle pieces that I have connected to demonstrate that sunlight can increase anti-inflammatory cells and cytokines, decrease proinflammatory cells and cytokines, and activate the brain's natural healing functions to ameliorate neuroinflammation.

References

1. Thornton AM. *Regulatory T Cells: History and Perspective*. Vol 5.; 2010. doi:10.1016/B978-0-08-046884-6.00605-9
2. DiSabato, D., Quan, N., Godbout J. Neuroinflammation: The Devil is in the Details. *J Neurochem*. 2016;139(2):136-153. doi:10.1016/j.jtcvs.2018.03.025
3. Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: When the immune system subjugates the brain. *Nat Rev Neurosci*. 2008;9(1):46-56. doi:10.1038/nrn2297
4. Won E, Kim YK. Neuroinflammation‐associated alterations of the brain as potential neural biomarkers in anxiety disorders. *Int J Mol Sci*. 2020;21(18):1-19. doi:10.3390/ijms21186546
5. Nimbalkar M, Yawalkar M, Mahajan N, Dhoble SJ. Potential of Luminescent Materials In Phototherapy. *Photodiagnosis Photodyn Ther*. 2020;(October):102082. doi:10.1016/j.pdpdt.2020.102082
6. Slusher TM, Olusanya BO, Vreman HJ, et al. Treatment of neonatal jaundice with filtered sunlight in Nigerian neonates : study protocol of a non-inferiority , randomized controlled trial. Published online 2013:1-10.
7. Em M, Chaves A, Piancastelli CC. Effects of low-power light therapy on wound healing : :616-623.
8. Starreveld DEJ, Daniels LA, Valdimarsdottir HB, et al. Light therapy as a treatment of cancer- related fatigue in (non-) Hodgkin lymphoma survivors (SPARKLE trial): study protocol of a multicenter randomized controlled trial. Published online 2018:1-11.
9. Lee BC, Lee JY, Kim J, et al. Graphene quantum dots as anti-inflammatory therapy for colitis. *Sci Adv*. 2020;6(18). doi:10.1126/sciadv.aaz2630
10. Maiese K. Moving to the Rhythm with Clock (Circadian) Genes, Autophagy, mTOR, and SIRT1 in Degenerative Disease and Cancer. *Curr Neurovasc Res*. 2017;176(10):139-148. doi:10.2174/1567202614666170718092010.Moving
11. Chao LL. Effects of Home Photobiomodulation Treatments on Cognitive and Behavioral

Function, Cerebral Perfusion, and Resting-State Functional Connectivity in Patients with Dementia: A Pilot Trial. *Photobiomodulation, Photomedicine, Laser Surg*. 2019;37(3):133-141. doi:10.1089/photob.2018.4555

12. Li S, Mason C, Melnick A. Mechanisms and Mitochondrial Redox Signaling in Photobiomodulation. *Curr Opin Genet Dev*. 2016;36(1):100-106. doi:10.1016/j.gde.2016.03.011
13. Vieyra-Garcia PA, Wolf P. From early immunomodulatory triggers to immunosuppressive outcome: Therapeutic implications of the complex interplay between the wavebands of sunlight and the skin. *Front Med*. 2018;5(SEP). doi:10.3389/fmed.2018.00232
14. Pepys MB, Hirschfield GM. C-reactive protein: A critical update. *J Clin Invest*. 2003;111(12):1805-1812. doi:10.1172/JCI200318921
15. Musiek ES, Holtzman DM. Mechanisms linking circadian clocks, sleep, and neurodegeneration. *Science (80-)*. 2016;354(6315):1004-1008. doi:10.1126/science.aah4968
16. Ding G, Gong Y, Eckel-Mahan KL, Sun Z. Central circadian clock regulates energy metabolism. In: *Advances in Experimental Medicine and Biology*. Vol 1090. Springer New York LLC; 2018:79-103. doi:10.1007/978-981-13-1286-1_5
17. Herzog ED, Hermanstyne T, Smyllie NJ, Hastings MH. Regulating the Suprachiasmatic Nucleus (SCN) Circadian Clockwork: Interplay between Cell-Autonomous and Circuit-Level Mechanisms. *Cold Spring Harb Perspect Biol*. 2017;9(1):a027706. doi:10.1101/cshperspect.a027706
18. Schmitt DA, Ullrich SE. Exposure to Ultraviolet Radiation Causes Dendritic Cells/Macrophages to Secrete Immune-Suppressive IL-12p40 Homodimers. *J Immunol*. 2000;165(6):3162-3167. doi:10.4049/jimmunol.165.6.3162
19. Lipscomb MF, Masten BJ. Dendritic cells: Immune regulators in health and disease. *Physiol Rev*. 2002;82(1):97-130. doi:10.1152/physrev.00023.2001
20. Janeway CA Jr, Travers P WM. *Generation of Lymphocytes in Bone Marrow and Thymus*. 5th ed. Garland Science; 2001.
21. Kaiko GE, Horvat JC, Beagley KW, Hansbro PM. Immunological decision-making: How

does the immune system decide to mount a helper T-cell response? *Immunology*. 2008;123(3):326-338. doi:10.1111/j.1365-2567.2007.02719.x

22. Pekalski ML, García AR, Ferreira RC, et al. Neonatal and adult recent thymic emigrants produce IL-8 and express complement receptors CR1 and CR2. *JCI insight*. 2017;2(16). doi:10.1172/jci.insight.93739
23. Luckheeram RV, Zhou R, Verma AD, Xia B. CD4 +T cells: Differentiation and functions. *Clin Dev Immunol*. 2012;2012. doi:10.1155/2012/925135
24. Tai Y, Wang Q, Korner H, Zhang L, Wei W. Molecular mechanisms of T cells activation by dendritic cells in autoimmune diseases. *Front Pharmacol*. 2018;9(JUN):1-10. doi:10.3389/fphar.2018.00642
25. Couper KN, Blount DG, Riley EM. IL-10: The Master Regulator of Immunity to Infection. *J Immunol*. 2008;180(9):5771-5777. doi:10.4049/jimmunol.180.9.5771
26. Zhu X, Zhu J. CD4 T Helper Cell Subsets and Related Human Immunological Disorders. *Int J Mol Sci*. 2020;21(21):8011. doi:10.3390/ijms21218011
27. Cohen Z. PSIO431 Introduction to the Immune System. *PSIO431 Introd to Immune Syst*.
28. Chuang Y, Knickel BK, Leonard JN, et al. Regulation of the IL-10-driven macrophage phenotype under incoherent stimuli. *Innate Immunol*. 2017;22(8):647-657. doi:10.1177/1753425916668243.Regulation
29. Liu Y, Bouteiller O De, Fugier-vivier I. Mechanisms of selection and differentiation in germinal centers. Published online 1997:256-262.
30. Crotty S. T follicular helper cell differentiation, function, and roles in disease. 2015;41(4):529-542. doi:10.1016/j.immuni.2014.10.004.T
31. Lee SY, Lee SH, Yang EJ, et al. Metformin ameliorates inflammatory bowel disease by suppression of the stat3 signaling pathway and regulation of the between Th17/Treg Balance. *PLoS One*. 2015;10(9):1-12. doi:10.1371/journal.pone.0135858
32. González H, Elgueta D, Montoya A, Pacheco R. Neuroimmune regulation of microglial activity involved in neuroinflammation and neurodegenerative diseases. *J Neuroimmunol*. 2014;274(1-2):1-13. doi:10.1016/j.jneuroim.2014.07.012
33. Kelsey C. Martin Mhatre V. Ho J-AL. tTregs, pTregs, and iTregs: Similarities and

Differences. 2012;23(1):1-7. doi:10.1111/imr.12160.tTregs

34. Lin X, Chen M, Liu Y, et al. Advances in distinguishing natural from induced Foxp3+ regulatory T cells. *Int J Clin Exp Pathol*. 2013;6(2):116-123.
35. Liu Y, Zheng P. FoxP3: A life beyond regulatory T cells. *Int J Clin Exp Pathol*. 2009;2(3):205-210.
36. Marson A, Kretschmer K, Frampton GM, et al. Foxp3 occupancy and regulation of key target genes during T-cell stimulation. *Nature*. 2007;445(7130):931-935. doi:10.1038/nature05478
37. Wang N, Liang H, Zen K. Molecular mechanisms that influence the macrophage M1-M2 polarization balance. *Front Immunol*. 2014;5(NOV):1-9. doi:10.3389/fimmu.2014.00614
38. Gordon S, Martinez FO. Alternative activation of macrophages: Mechanism and functions. *Immunity*. 2010;32(5):593-604. doi:10.1016/j.immuni.2010.05.007
39. Gong D, Shi W, Yi S ju, Chen H, Groffen J, Heisterkamp N. TGFβ signaling plays a critical role in promoting alternative macrophage activation. *BMC Immunol*. 2012;13. doi:10.1186/1471-2172-13-31
40. Funes SC, Rios M, Escobar-Vera J, Kalergis AM. Implications of macrophage polarization in autoimmunity. *Immunology*. 2018;154(2):186-195. doi:10.1111/imm.12910
41. Audiger C, Rahman MJ, Yun TJ, Tarbell K V., Lesage S. The Importance of Dendritic Cells in Maintaining Immune Tolerance. *J Immunol*. 2017;198(6):2223-2231. doi:10.4049/jimmunol.1601629
42. Heidari F, Ramezani A, Erfani N, Razmkhah M. Indoleamine 2, 3-Dioxygenase: A Professional Immunomodulator and Its Potential Functions in Immune Related Diseases. *Int Rev Immunol*. Published online October 29, 2020:1-18. doi:10.1080/08830185.2020.1836176
43. Xie Z, Zhang H, Wu X, Zhu J, Ma D, Jin T. Role of the Immunogenic and Tolerogenic Subsets of Dendritic Cells in Multiple Sclerosis. 2015;2015.
44. Morelli AE, Thomson AW. Tolerogenic dendritic cells and the quest for transplant tolerance. 2007;7(AUGUST):610-621. doi:10.1038/nri2132
45. Sharma K, Kalakoti P, Nanda A, Sun H. *Blood-Brain Barrier Disruption During*

Neuroinflammation. Elsevier Inc.; 2018. doi:10.1016/b978-0-12-811709-5.00030-2

46. Doll DN, Hu H, Sun J, Lewis SE, Simpkins JW, Ren X. Mitochondrial Crisis in Cerebrovascular Endothelial Cells Opens the Blood-Brain Barrier. *Stroke*. 2015;46(6):1681-1689. doi:10.1161/STROKEAHA.115.009099
47. Prajeeth CK, Huehn J, Stangel M. Regulation of neuroinflammatory properties of glial cells by T cell effector molecules. *Neural Regen Res*. 2018;13(2):234-236. doi:10.4103/1673-5374.226385
48. Gomes JAS, Silva JF, Marçal AP, et al. High-refined carbohydrate diet consumption induces neuroinflammation and anxiety-like behavior in mice. *J Nutr Biochem*. 2020;77:108317. doi:10.1016/j.jnutbio.2019.108317
49. Helmut K, Hanisch UK, Noda M, Verkhratsky A. Physiology of microglia. *Physiol Rev*. 2011;91(2):461-553. doi:10.1152/physrev.00011.2010
50. Poulose SM, Miller MG, Scott T, Shukitt-Hale B. Nutritional factors affecting adult neurogenesis and cognitive function. *Adv Nutr*. 2017;8(6):804-811. doi:10.3945/an.117.016261
51. González H, Pacheco R. T-cell-mediated regulation of neuroinflammation involved in neurodegenerative diseases. *J Neuroinflammation*. 2014;11(1):1-11. doi:10.1186/s12974-014-0201-8
52. Luokun Xie, Gourav Roy Choudhury, Ali Winters, Shao-Hua Yang and KJ. Cerebral regulatory T cells restrain microglia/macrophage-mediated inflammatory responses via IL-10. *Eur J Immunol*. 2015;23(1):1-7. doi:10.1002/eji.201444823.Cerebral
53. Browne TC, McQuillan K, McManus RM, O'Reilly J-A, Mills KHG, Lynch MA. IFN-γ Production by Amyloid β–Specific Th1 Cells Promotes Microglial Activation and Increases Plaque Burden in a Mouse Model of Alzheimer's Disease. *J Immunol*. Published online 2013. doi:10.4049/jimmunol.1200947
54. Yang H, Yang H, Xie Z, Wei L, Bi J. Systemic Transplantation of Human Umbilical Cord Derived Mesenchymal Stem Cells-Educated T Regulatory Cells Improved the Impaired Cognition in AβPPswe/PS1dE9 Transgenic Mice. *PLoS One*. 2013;8(7):1-10. doi:10.1371/journal.pone.0069129

55. Bas J, Calopa M, Mestre M, et al. Lymphocyte populations in Parkinson's disease and in rat models of parkinsonism. *J Neuroimmunol*. 2001;113(1):146-152. doi:10.1016/S0165-5728(00)00422-7

56. Legroux L, Arbour N. Multiple Sclerosis and T Lymphocytes: An Entangled Story. *J Neuroimmune Pharmacol*. 2015;10(4):528-546. doi:10.1007/s11481-015-9614-0

57. Reddy J, Illes Z, Zhang X, et al. Myelin proteolipid protein-specific CD4+CD25+ regulatory cells mediate genetic resistance to experimental autoimmune encephalomyelitis. *Proc Natl Acad Sci U S A*. 2004;101(43):15434-15439. doi:10.1073/pnas.0404444101

58. Fiszer U, Mix E, Fredrikson S, Kostulas V, Olsson T, Link H. γδ+ T cells are increased in patients with Parkinson's disease. *J Neurol Sci*. 1994;121(1):39-45. doi:10.1016/0022-510X(94)90154-6

59. Alla L. Zozulya, Emily Reinke, Dana C. Baiu, Jozsef Karman, Matyas Sandor and ZF. Dendritic Cell Transmigration through Brain Microvessel Endothelium Is Regulated by MIP-1α Chemokine and Matrix Metalloproteinases1. *J Immunol*. 2007;178(1):520-529. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3624763/pdf/nihms412728.pdf

60. Rapf RJ, Vaida V. Sunlight as an energetic driver in the synthesis of molecules necessary for life. *Phys Chem Chem Phys*. 2016;18(30):20067-20084. doi:10.1039/c6cp00980h

61. Slominski AT, Zmijewski MA, Plonka PM, Szaflarski JP, Paus R. How UV Light Touches the Brain and Endocrine System Through Skin, and Why. *Endocrinology*. 2018;159(5):1992-2007. doi:10.1210/en.2017-03230

62. Young AR. Chromophores in human skin. *Phys Med Biol*. 1997;42(5):789-802. doi:10.1088/0031-9155/42/5/004

63. Lappin MB, Simon JC. Urocanic acid and cutaneous antigen presentation. *J Photochem Photobiol B Biol*. 1998;44(2):112-116. doi:10.1016/S1011-1344(98)00129-8

64. Roberts JE. Light and immunomodulation. *Ann N Y Acad Sci*. 2000;917:435-445. doi:10.1111/j.1749-6632.2000.tb05408.x

65. D'Orazio J, Jarrett S, Amaro-Ortiz A, Scott T. UV radiation and the skin. *Int J Mol Sci*. 2013;14(6):12222-12248. doi:10.3390/ijms140612222

66. Mabley JG, Szabo C. Inflammatory disease and sunlight: The vitamin D-poly (ADP-ribose) polymerase connection. *Fut Rheumatol*. 2008;3(2):169-181. doi:10.2217/17460816.3.2.169
67. Pike JW, Meyer MB. The vitamin D receptor: New paradigms for the regulation of gene expression by 1,25-dihydroxyvitamin D3. *Endocrinol Metab Clin North Am*. 2010;39(2):255-269. doi:10.1016/j.ecl.2010.02.007
68. Correale J, Farez MF. Modulation of multiple sclerosis by sunlight exposure: Role of cis-urocanic acid. *J Neuroimmunol*. Published online 2013. doi:10.1016/j.jneuroim.2013.05.014
69. Weiterbildung CME, Fortbildung Z. Vitamin D and SKin. 2008;(August):737-744. doi:10.1007/s00105-008-1499-x
70. Ryan WL, Levy DH. Electronic spectroscopy and photoisomerization of trans-Urocanic acid in a supersonic jet. *J Am Chem Soc*. 2001;123(5):961-966. doi:10.1021/ja003261h
71. Morison WL, Bucana C, Kripke ML. Systemic suppression of contact hypersensitivity by UVB radiation is unrelated to the UVB-induced alterations in the morphology and number of Langerhans cells. Published online 1984.
72. Walterscheid JP, Nghiem DX, Kazimi N, et al. Cis-uronic acid, a sunlight-induced immunosuppressive factor, activates immune suppression via the 5HT2A receptor. *Proc Natl Acad Sci U S A*. 2006;103(46):17420-17425. doi:10.1073/pnas.0603119103
73. Holáň V, Kuffová L, Zajícová A, et al. Urocanic acid enhances IL-10 production in activated CD4+ T cells. *J Immunol*. 1998;161(7):3237-32341.
74. Noonan FP, De Fabo EC. Immunosuppression by ultraviolet B radiation: initiation by urocanic acid. *Immunol Today*. 1992;13(7):250-254. doi:10.1016/0167-5699(92)90005-R
75. Bernard JJ, Gallo RL, Krutmann J. Photoimmunology: how ultraviolet radiation affects the immune system. *Nat Rev Immunol*. 2019;19(11):688-701. doi:10.1038/s41577-019-0185-9
76. Loser K, Mehling A, Loeser S, et al. Epidermal RANKL controls regulatory T-cell numbers via activation of dendritic cells. *Nat Med*. 2006;12(12):1372-1379. doi:10.1038/nm1518

77. Ono T, Hayashi M, Sasaki F, Nakashima T. RANKL biology: Bone metabolism, the immune system, and beyond. *Inflamm Regen*. 2020;40(1):1-16. doi:10.1186/s41232-019-0111-3

78. Ghareghani M, Reiter RJ, Zibara K, Farhadi N. Latitude, Vitamin D, Melatonin, and Gut Microbiota Act in Concert to Initiate Multiple Sclerosis: A New Mechanistic Pathway. *Front Immunol*. 2018;9:2484. doi:10.3389/fimmu.2018.02484

79. SIMON J, TIGELAAR R, BERGSTRESSER P, EDELBAUM D, CRUZ P. Ultraviolet B radiation converts langerhans cells from immunogenic to tolerogenic antigen-presenting cells : induction of specific clonal anergy in CD4. *J Immunol*. 1991;146(2):485-491.

80. Shen J, Zhu Y, Yang X, Li C. Graphene quantum dots: Emergent nanolights for bioimaging, sensors, catalysis and photovoltaic devices. *Chem Commun*. 2012;48(31):3686-3699. doi:10.1039/c2cc00110a

81. Xiao S, Zhou D, Luan P, et al. Graphene quantum dots conjugated neuroprotective peptide improve learning and memory capability. *Biomaterials*. 2016;106:98-110. doi:10.1016/j.biomaterials.2016.08.021

82. Tosic J, Stanojevic Z, Vidicevic S, et al. Graphene quantum dots inhibit T cell-mediated neuroinflammation in rats. *Neuropharmacology*. 2019;146:95-108. doi:10.1016/j.neuropharm.2018.11.030

83. Volarevic V, Paunovic V, Markovic Z, et al. Large graphene quantum dots alleviate immune-mediated liver damage. *ACS Nano*. 2014;8(12):12098-12109. doi:10.1021/nn502466z