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Applications of the Role of α-MSH in Ocular Immune Privilege

Andrew W. Taylor* and **Darren Lee**

Abstract

There is an important role for α-MSH and the melanocortin receptors in ocular immunity, development and health. This chapter will cover what is known about how α-MSH is part of the mechanisms of ocular immune privilege, about the expression of melanocortin receptors and the implications of these findings on the role of α-MSH in ocular physiology and its potential use to treat ocular pathologies.

Ocular Immune Privilege

It is greatly appreciated that the ocular microenvironment has an unique relationship with the immune system, in that immunity is highly regulated within the eye. It is considered to be an evolutionary adaptation to prevent excessive inflammatory responses that are often accompanied with collateral damage caused by vascular leakage, cell death and fibroses, which can permanently impair vision and cause blindness.[1] This immunoregulation is characterized by immunity failing to mount a response that would reject an allograft placed into the ocular microenvironment even if the immune response is primed.[2] Moreover, there is an induction of regulatory immunity to the foreign antigens.[3] This feature of ocular immunity defines immune privilege. The mechanisms of ocular immune privilege actively suppress the activation of effector T-cells, suppresses proin-flammatory activation of macrophages and neutrophils and alters the presentation of antigen by antigen presenting cells to suppress inflammation and to promote regulatory T-cell activation.[4–7] The mediators of this immunosuppression are membrane bound proteins and soluble immunomodulating factors produced by parenchymal cells, neurons and immune cells within the ocular microenvironment.

The soluble immunomodulating factors within the ocular microenvironment are found primarily in aqueous humor, the fluid filling the anterior chamber of the eye. Aqueous humor suppresses inflammation mediated by macrophages stimulated with bacterial products.[8] In addition, aqueous humor treated macrophages and dendritic cells cannot function as antigen presenting cells that promote proinflammatory activity by T-cells.[9] Moreover, aqueous humor treatment of macrophages induces anti-inflammatory cytokine production and the presentation of antigen in a manner that promotes regulatory T-cell activation.[10,11] These findings suggest that resident ocular macrophages and dendritic cells are inhibited from mediating inflammation while they are still able to clear pathogens and damaged cells. When effector T-cells are treated with aqueous humor, the T-cells can no longer mediate hypersensitivity responses.[12] Aqueous humor changes the T-cell cytokine profile from interferon-gamma (IFN-γ), to transforming growth factor-beta (TGF-β).[11,13] This change is associated with the T-cells changing their functionality from inflammatory to regulatory. The very ability of the ocular microenvironment to resist the activation of effector T-cells can be seen when effector T-cells are placed into the anterior chamber along

*Corresponding Author: Andrew W. Taylor—Schepens Eye Research Institute, 20 Staniford Street, Boston, Massachusetts 02114 USA. andrew.taylor@schepens.harvard.edu.

with their specific antigen and antigen presenting cells.[12] Whereas, if this adoptive transfer of effector T-cells, antigen and antigen presenting cells were injected into the skin they would mediate a vigorous hypersensitivity response which does not occur when injected into the anterior chamber of the eye. Also, treatment of the effector T-cells with aqueous humor before injecting them with antigen and antigen presenting cells into the skin suppresses the expected inflammatory response.[12] Moreover, such aqueous humor treated T-cells function as regulatory T-cells and suppresses immunity.[13] Therefore, in aqueous humor there are constitutively expressed immunomodulating soluble factors that suppress the activation of inflammatory immunity while turning the immune response onto itself, further promoting immune privilege.

α-MSH in the Eye

Aqueous humor subjected to HPLC size separation revealed two fractions of immunomodulating activity.[14] The first fraction was found to be centered on 25 kDa associated with activated transforming growth factor-beta2 (TGF-β2). The second immunomodulating fraction was found to be the peptides contained in a fraction that was 2 kDa or less in molecular weight. It was in this low molecular weight fraction that the immunomodulating neuropeptides of aqueous humor were found. The first described immunomodulating neuropeptide in the eye was alpha-melanocyte stimulating hormone (α-MSH).[15] This thirteen amino acid long neuropeptide that is derived from sequential endoproteolytic cleavage and posttranslational modifications of pro-opiomelanocortin hormone (POMC) was originally described for its melanin-inducing activity in frogs.[16] In mammals it has become more evident that α-MSH has a more fundamental role in homeostasis of metabolism and immunity.[17–19]

The immunomodulating role of α-MSH is demonstrated by the suppression of endotoxin and proinflammatory cytokine (such as IL-1β and TNF-α) induced systemic inflammatory responses of animals injected with α-MSH.[20–27] At the cellular level, it is clear that α-MSH binds its melanocortin receptors on cells of innate immunity (macrophages, dendritic cells and neutrophils). The ability of these cells to generate reactive oxygen intermediates, nitric oxide, produce pro-inflammatory cytokines and to migrate are profoundly suppressed by α-MSH.[28–33] Endotoxin, IL-1β and TNF-α activated intracellular signaling pathways are blocked by α-MSH preventing the translocation of NF-kB from the cytoplasm to the nucleus.[34–41] This suppression is early in the signaling cascade and involves IRAK-M binding to IRAK-1.[42] In addition, α-MSH promotes production of anti-inflammatory cytokines like IL-10, its own production and up-regulates melanocortin receptor expression.[30,43–47] This action of α-MSH is not only anti-inflammatory, but it promotes a self-perpetuating anti-inflammatory autocrine loop.

In aqueous humor, α-MSH is constitutively present at a concentration of approximately 10^{-11} M.[15] Aqueous humor depleted of α-MSH can no longer modulate T-cell activity. The function that α-MSH plays in aqueous humor is to suppress IFN-γ production by effector T-cells. Also, it is through α-MSH that aqueous humor mediates a change in an antigen-specific T-cell response from proinflammatory to regulatory.[13,48] In this process, TGF-β2, also found in aqueous humor, helps enhance this activity of α-MSH induction of regulatory activity in T-cells.[48] The aqueous humor/α-MSH-induced regulatory T-cells express the expected regulatory T-cell marker of CD25 and are only $CD4^+$ T-cells. These regulatory T-cells produce TGF-β, but not IL-4 or IL-10. It is possible to generate these T-cells in vitro by treating antigen-stimulated $CD4^+$ T-cells with only α-MSH at its 10^{-11} M physiological concentration.[13,49,50] In adoptive transfer experiments it has been shown that these α-MSH-treated T-cells require restimulation with their specific antigen to activate their suppressive functionality, but they suppress the activation of neighboring T-cells that can be responding to other antigens.

The potential for α-MSH to induce regulatory T-cells in vivo was demonstrated by finding that melanocortin 5 receptor (MC5r) knocked-out mice naturally recovering from autoimmune uveitis lack the presence of retinal autoantigen-specific regulatory $CD25^+$ $CD4^+$ T-cells in their spleens, unlike wild type mice.[51,52] These results suggest that the natural recovery from autoimmune disease is not dependent on these regulatory T-cells, but that the induction of the regulatory T-cells through MC5r is a by-product of the recovery. A possible role for these regulatory T-cells is to prevent the expression of memory immunity to the autoantigens. When adoptively transferred, the regulatory T-cells from postrecovered wild type mice into post recovered MC5r mice prevent a rapid and severe uveitic response to a second immunization with retinal autoantigen.[51]

Melanocortin Receptors and the Eye

There are four melanocortin receptors for α-MSH that are differentially expressed throughout the body. In the eye, the melanocortin receptors MC3r, MC4r and MC5r are expressed in the inner neural retina layers with MC3r and MC4r localized to retinal ganglion cells, MC5r in the neural outer plexiform layers and the retinal pigment epithelial cells express MC1r and MC5r.[40,53] The expression of the melanocortin receptors in the eye are evolutionarily conserved and there is evidence thatα-MSH, through its receptors, play an important role in retinal and neuronal development.[54–56] Neurite outgrowth from embryonic retinal explants can be stimulated with α-MSH treatment and there has been found a spatial and temporal expression of α-MSH in chick embryonic eye development with the retinal pigment epithelial cells as the source of α-MSH.[53,54] These indicate that with our previously described immunomodulating activity, α-MSH is also a neurotropic factor that may be necessary for development and survival of the retina.

Support of the dual role of α-MSH in the immune privileged eye is seen when we induce autoimmune disease in mice with MC5r knocked-out and when α-MSH is used as ocular therapy, which we will discuss in the next section.[51,57] As previously stated, the induction, tempo and resolution of autoimmune uveitis in the MC5r knock-out mice is no different from the wild type mouse. Also, while there is an induction of regulatory T-cells in the spleens of the wild type mice and not in the MC5r knock-out mice they are not necessary for the resolution of uveitis. One of the most striking differences is seen when comparing the histology of the post-uveitis retinas of the wild type and MC5r knock-out mice. The post-uveitic wild type mouse retina displays retinal folds and some granulomas formations, but is mostly intact with discernible retinal layers. In contrast, the retinas of post-EAU MC5r knock-out mice are severely damaged with losses in photoreceptors and disorganization of the retinal layers.[51,57] When a gene therapy approach was used to deliver α-MSH into the uveitic eyes of wild type mice and MC5r knock-out mice, only the uveitis of the wild type mouse was suppressed. The MC5r knock-out mice received no immunosuppressive benefit as observed in the wild type mice, but histological examination of the post-uveitis retina revealed less damage and some preservation of the retinal layers.[57] Such a benefit from α-MSH treatment may be due to it working through the other expressed melanocortin receptors in the eye. Such findings indicate the importance of the melanocortin system in the development, health, survival and immune privilege of the retina.

Application of α-MSH in Ocular Therapy

The immunomodulating activity and possible natural role in immune homeostasis of α-MSH has long suggested that it may be an effective and safe immunosuppressive therapy. There are multiple publications demonstrating the application of α-MSH either through peptide (cytokine-like) or gene therapy to suppress animal models of septic shock, contact hypersensitivity, allograft survival and multiple sclerosis.[23,24,27,31,35,43–45,57–66] Because of

the intimate role of α-MSH in ocular immune privilege, the potential of delivering α-MSH into an inflamed or wounded eye to reimpose ocular immunosuppression has been an intriguing possibility. Several rodent models of ocular pathology have been tested and treated with α-MSH to see if it is possible to prevent, suppress and restore ocular immune privilege. Experimental autoimmune uveitis (EAU) and endotoxin induced uveitis (EIU) are rodent models for endogenous uveitis in humans, which in the USA 2 million persons are afflicted. Persistent and reoccurring uveitis leads to visual impairment and possible blindness. The current treatments are limited to steroids, which because of serious side effects limits their use. The potential of using an immunomodulating neuropeptide like α-MSH that has the additional possibility of (re)imposing immune privilege or immune tolerance is an exciting possibility of a new therapeutic approach.

The mouse model of EAU is an antigen specific, $CD4^+$ T-cell dependent, uveitis, in which mice are immunized to break tolerance and activate effector T-cells specific to the retinal antigens such as interphotoreceptor retinoid-binding protein (IRBP) and retinal soluble antigen (SAg).[67] The resulting inflammation of the retina is easily monitored and scored. The uveitis, unlike in many humans, is limited in that it lasts from 30 to 90 days depending on the strain of mouse when it spontaneously resolves. Analyzing how mouse uveitis spontaneously resolves led to finding the presence of MC5r-dependent regulatory T-cells in the spleens of EAU-recovered mice, which we discussed in the last two sections.[51,52] The methods of delivering α-MSH into mice with EAU have included systemic injections of α-MSH peptide and conjunctival injection of an α-MSH expression plasmid. If α-MSH is systemically injected when the uveitis is at its maximum, the treatment accelerates the recovery.[68] When α-MSH is injected at the start of uveitis it can delay the onset of the disease and when an α-MSH encoded plasmid is injected into the conjunctiva of mouse eyes at the onset of inflammation it suppresses the severity and hastens the resolution of the autoimmune disease.[57] The mechanism of immunosuppression mediated by these α-MSH therapies are not completely understood, but it is likely a combination of α-MSH antagonizing proinflammatory cytokines and chemokines and its induction of regulatory and immunosuppressive activity in immune cells. Whether these α-MSH therapies alone are sufficient to reestablish immune privilege is to be seen.

The EIU model of uveitis in rats is induced by the injection of bacterial endotoxin, lipopolysaccharide, which results in ocular inflammation within hours of the injection and is fully resolved by 48 hours.[69] This model of uveitis involves a stepwise change in the expression of intraocular inflammatory cytokines and chemokines.[69] These are followed by a breakdown in the blood ocular barrier and infiltration of immune cells. Around 24 hours, intraocular immunosuppressive molecules begin to be re-expressed, inflammation subsides and immune privilege is restored. It is viewed that in EIU, immune privilege is able to naturally reassert itself in response to the induction of innate immune mediated intraocular inflammation. A systemic injection of α-MSH peptide effectively inhibits the ocular inflammation and suppresses infiltration of cells into the ocular microenvironment.[63,64] The mechanism by which α-MSH can suppress EIU is through suppression of cyclooxygenase 2 production by macrophages within the ocular microenvironment.[64] The effect of α-MSH treatment is the suppression of the early events of EIU; therefore, preventing the subsequent infiltration of immune cells and production of proinflammatory cytokines and chemokines in the eye.[63] A similar anti-inflammatory efficacy was seen when α-MSH was systemically injected or topically applied to the eye of rabbits with surgical trauma to the cornea.[70]

The potential benefits of α-MSH therapy are not limited to only ocular inflammatory disease. There is a potential of α-MSH therapy to be effective in treating other ocular pathologies. Intravitreal injection of α-MSH analogs retard photoreceptor loss in RCS rat, which is a model of retinal dystrophy.[71] This may be working through the melanocortin

receptors inducing some undefined neurotropic activity. It has also been demonstrated that topical application of α-MSH is effective in lowering the intraocular pressure in normal tension rabbits for up to six hours by stimulating PGE2 and prostacyclin levels in iris and ciliary body cells.[72] This finding suggests a potential for α-MSH therapy to be an effective antiglaucoma drug.

There is also the potential of using α-MSH in more unconventional therapies that involve manipulating immune cells ex vivo to generate antigen-specific regulatory T-cells. It is possible in a mouse model to generate ocular autoantigen specific T-cells that when restimulated and treated with α-MSH they become antigen-specific regulatory T-cells capable of suppressing EAU.[48] The only requirement of antigen specificity for these regulatory T-cells is that they are specific for any retinal autoantigen, not necessarily the target autoantigen.[13,48,50] Adoptive transfer of similarly generated α-MSH regulatory T-cells specific to retinal autoantigens into mice with neonatal retinal allografts suppressed graft ejection and promoted retinal tissue development.[65] This activity of the α-MSH generated regulatory T-cells tells us that these regulatory T-cells need to see their antigen to activate their immunosuppressive activity in vivo and the regulatory T-cells can create a supportive environment that promotes tissue survival and development. Therefore, a potential exists to take advantage of this feature of α-MSH generated regulatory T-cells and use the regulatory T-cells to suppress inflammation by generating α-MSH regulatory T-cells to any antigen expressed within the target tissue. It may even be possible to suppress autoimmunity by using an antigen that is purposely introduced into the tissue to activate the regulatory T-cells. This would be a benefit of using the α-MSH generated regulatory T-cells in autoimmune diseases where it is unclear as to what is the target antigen.

Conclusion

The role of α-MSH in mediating the expression of ocular immune privilege has further added to our understanding of α-MSH in the maintenance of immune homeostasis. The mechanisms of α-MSH modulation of immunity have suggested new therapeutic approaches to inflammatory and autoimmune diseases. Whether it is necessary to find ways that single out the immunomodulating activity of α-MSH from α-MSH's role in skin pigmentation and metabolic homeostasis is to be determined. The characterization of α-MSH in ocular immunity defines its importance and its place in the evolutionary adaptation of the ocular microenvironment to highly regulate immunity for the purpose of preserving vision.

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