Developing advanced therapeutics through the study of naturally occurring immune-mediated ocular disease in domestic animals

Brian C. Gilger, DVM, MS, DACVO, DABT*

Department of Clinical Sciences, North Carolina State University College of Veterinary Medicine, Raleigh, NC

*Corresponding author: Dr. Gilger (bgilger@ncsu.edu)
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ABSTRACT
This review, which is part of the “Currents in One Health” series, describes the importance of the study of immune-mediated ocular disease in the development of innovative therapeutics, such as cell and gene therapy for the eye. Recent examples of cell and gene therapy studies from the author’s laboratory are reviewed to emphasize the importance of One Health initiatives in developing innovative therapies for ocular diseases. Spontaneous immune-mediated corneal disease is common in horses, cats, dogs, and humans. Autologous bone marrow-derived mesenchymal stem cells (BM-MSCs) injected subconjunctivally resulted in the resolution of naturally occurring immune-mediated keratitis (IMMK) without adverse effects. These results support that autologous subconjunctival BM-MSC therapy may be a viable treatment alternative for IMMK. Furthermore, the use of subconjunctival MSCs may be an effective method to treat ocular surface immune-mediated diseases in humans and other species, including herpetic stromal keratitis and immunologic dry eye disease. Furthermore, the use of adeno-associated viral (AAV) vectors to deliver the immunosuppressive transgene cDNA of equine interleukin 10 (eqIL-10) or human leukocyte antigen G injected intravitreally was shown to be safe and inhibit the development of uveitis in the experimental autoimmune uveitis rat model. Efficacy and safety studies of ocular gene therapy in models will pave the way for clinical trials in animals with naturally occurring immune-mediated diseases, such as a therapeutic clinical trial for AAV-eqIL-10 in horses with equine recurrent uveitis.

Immune-mediated Keratitis
Equine IMMK is a nonulcerative, noninfectious keratitis that causes corneal opacity and vision loss in horses (Figure 1). The opacity develops secondary to corneal vascularization, cellular infiltration, and fibrosis; and calcification of the epithelial, stromal, and endothelial layers of the cornea. Histopathologically, the disease exists as a predominately lymphocytic-plasmacytic infiltration of the corneal tissue that supports the presence of a dysregulation of the local immune environment. Immunologic studies demonstrated that some horses with IMMK show autoantibodies against equine maspin, a corneal protein that inhibits corneal neovascularization, suggesting an autoimmune etiology. Equine IMMK is similar to the herpetic stromal keratitis in humans...
and cats, and chronic superficial keratitis in dogs, all of which have predominantly immune-mediated pathogenesis and similar clinical features. These cells have been shown to target sites of inflammation and to secrete cytokines that can reduce fibrosis and modulate the immune response. In human studies, MSCs regulate suppression of T-cell proliferation, inhibition of dendritic cell maturation, and decreased production of inflammatory cytokines. Autologous bone marrow-derived (BM) MSCs have demonstrated encouraging outcomes for the treatment of inflammatory and traumatic musculoskeletal injuries, including tendonitis and intra-articular disease such as meniscal injuries and osteoarthritis in horses. Equine MSCs demonstrate immunomodulatory effects through inhibition of lymphocyte proliferation and induction of interleukin (IL)-10. In rabbit ocular models, MSCs have been shown to accelerate corneal wound healing, decrease oxidative stress, and suppress proinflammatory cytokine profiles resulting in decreased opacification and neovascularization of an injured corneal surface. Collectively, these findings suggest that autologous MSCs may be an effective therapy for the treatment of ocular surface immune-mediated diseases such as IMMK in horses and stromal keratitis in humans.

In a pilot study, 4 horses with equine IMMK received a subconjunctival injection of autologous BM-MSCs. In these horses, 1 mL of MSCs (15 million cells/mL) were injected under the dorsal bulbar conjunctiva subconjunctivally. Injections were repeated approximately every 2 weeks for a total of 3 to 4 injections. Three of 4 horses treated with autologous subconjunctival BM-MSC therapy for IMMK demonstrated a complete resolution of their corneal disease without recurrence, with no adverse effects noted. These results support that autologous subconjunctival BM-MSC therapy may be a viable treatment alternative for horses that have not shown improvement with traditional therapeutic options for IMMK. Furthermore, the use of subconjunctival MSCs may be an effective method to treat ocular surface immune-mediated diseases in humans and other species, including herpetic stromal keratitis and immunologic dry eye disease. Further study of MSCs or genetically modified MSCs for ocular disease is warranted; targeted diseases include keratitis, uveitis, glaucoma, diabetic retinopathy, and age-related macular degeneration.

**Uveitis**

Horses spontaneously develop severe immunologic uveitis known as equine recurrent uveitis (ERU), which is frequently recurrent and chronic (Figure 2). Equine recurrent uveitis is a spontaneous, painful, noninfectious uveitis (NIU), and is the most common cause of blindness in horses. It is characterized by episodes of active ocular inflammation alternating with varying intervals of clinical
The accumulated effects of recurrent “bouts” or “flares” of inflammation lead to progressively destructive pathologic changes, including irreversible scarring, ocular cloudiness, cataract formation, and vision loss. The immunopathology of ERU has been studied extensively and has demonstrated that T cells are the predominant mononuclear inflammatory cells infiltrating ocular tissues in horses with naturally occurring chronic uveitis, with a significant number of CD4+ cells.22,25–27

The study of this common, spontaneous uveitis in horses has helped understand the pathogenesis of uveitis in humans, especially the identification of autoantigens and how the recurrence of uveitis develops immunologically.25,26–30 Several potential autoantigens have been identified in horses that could play a role in the development of autoimmune uveitis. T cells isolated from the eyes of horses with ERU proliferate in response to 2 common autoantigens in rodents: retinal S-antigen and interphotoreceptor binding protein (IRBP).31 In addition, several additional potential autoantigens were identified by analyzing antibodies in the sera of horses with ERU that reacted with retinal proteins. These include recoverin, cellular retinaldehyde-binding protein (CRALBP), and malate dehydrogenase.31 Although all these potential autoantigens are capable of inducing experimental uveitis in rodent models, only CRALBP and IRBP consistently produce uveitis in outbred horses.31 In addition, studies of horses with ERU have also helped elucidate how Leptospira infections induce immunologic uveitis, specifically autoimmune uveitis.32

Field studies of horses in the 1950s after an outbreak of acute leptospirosis caused by Leptospira interrogans serogroup Pomona demonstrated that 1 of the 6 horses (17%) developed intraocular inflammation during acute leptospiral disease and all horses developed ERU 18 to 24 months after the initial infection.33 Subsequent studies demonstrated cross-reactivity between equine ocular tissues and Leptospira antigens,34 and horses with uveitis associated with L. interrogans infections had high levels of immunoglobulin (Ig) A and IgG in their intraocular fluids that reacted to 2 Leptospira lipoproteins, LruA and LruB.32 These antibodies were also subsequently discovered in the serum of human patients with leptospiral uveitis.35 Furthermore, studies of spontaneous ERU have helped elucidate the immunopathogenesis of recurrent uveitis. Epitope spreading is defined as the diversification of epitope specificity from the initial focused, dominant, epitope-specific immune response, directed against a self or foreign protein to cryptic epitopes on that protein (intramolecular spreading) or other proteins (intramolecular spreading).36 The shifts in immunoreactivity, or epitope spreading, have been documented in ERU, and are likely to be responsible for the relapsing nature of many types of human uveitis.36

Equine recurrent uveitis, as a model of spontaneous immune-mediated uveitis, has also led to the study of promising therapeutics. Conventional treatment of uveitis, whether in humans or horses, is nonspecific, and includes the frequent use of topical and systemic corticosteroids and other topical or oral immunosuppressive agents, none of which are effective in preventing uveitis relapses.22,24,36,37 These therapies are limited by poor treatment compliance and long-term adverse effects, such as corneal degeneration, glaucoma, cataract, ocular hypertension, and infection, all of which may contribute to the development of blindness.24,38 Sustained-release ocular implants have shown promise in the treatment of ERU.38 Triamcinolone injections into the suprachoroidal space are currently under development for the treatment of human uveitis39 and have been evaluated in horses with ERU.40 However, improved therapies are needed that have fewer side effects and rely less on owner (or patient) compliance. Therefore, further study of ERU and its treatments will likely translate well to improving the understanding and treatment of human autoimmune uveitis.

Localized ocular gene transfer, or gene therapy, based on AAV vectors of an immunosuppressive or anti-inflammatory peptide may establish a long-term immunosuppressive effect that would serve as a novel and safe therapeutic strategy for immune-mediated diseases in the eye, such as uveitis.41 Adeno-associated virus transduces many different cell types and establishes long-term transgene production for years after a single injection.42 One approach to uveitis therapy is to target the autoantigenic T cells and inflammatory cytokines that orchestrate and exacerbate the uveitis disease process.43 One such cytokine is IL-10.

An immunomodulatory cytokine, IL-10 has potent anti-inflammatory and immunomodulating properties that play critical roles in limiting the immune response and preventing autoimmune disorders.44,45 Interleukin 10 is produced by immune cells and inhibits macrophages, natural killers, T helper cells type 1, and dendritic cell function (Figure 3).46 Interleukin 10 also inhibits the production of proinflammatory cytokines and costimulatory molecules, and interferes with general antigen-presenting cell function.44,46 Several studies47–49 have evaluated the anti-inflammatory effects and efficacy of IL-10 both
as a systemic and localized treatment. Interleukin 10 plays an essential role in uveitis by protecting the eye from chronic and relapsing inflammation, thus suggesting that ocular supplementation of endogenous IL-10 may be promising therapeutic for ERU and other NIU. Recently, AAV-equine (eq)IL-10 was demonstrated to be safe and effective in inhibiting uveitis in a well-established model of NIU—the experimental autoimmune uveitis (EAU) rat model. In these experiments, it was shown that both low and high doses of AAV-eqIL-10 suppressed the development of ocular inflammation significantly in rats with EAU. In addition, AAV-eqIL-10 treatment led to the reduction of aqueous humor inflammatory cell counts and histopathologic scores. Vector-derived eqIL-10 cDNA was detected in relevant ocular tissues, such as the iris/ciliary body and retina. Further studies are being planned to ensure safety and tolerability in horses. With regulatory approval, a clinical efficacy trial may then be conducted in horses.

Another promising gene transfer approach using AAV to treat immune-mediated disease is the use of therapeutic transgene human leukocyte antigen G (HLA-G). Human leukocyte antigen G is expressed by ocular tissues across species and contributes to the immunosuppressive microenvironment and immune privilege of the eye. Human leukocyte antigen G binding results in direct immune cell (natural killer cell, neutrophil, T cell, B cell, and dendritic cell) inhibition, reduced proliferation, and prevention of cytokine and chemokine release (Figure 4). The therapeutic use of AAV-HLA-G for ocular disease may be advantageous over other gene therapy approaches because of HLA-G’s wide and diverse immunomodulatory functions, which can target the multiple immunologic and inflammatory cascades active in uveitis. To evaluate the efficacy and safety of AAV-HLA-G for the treatment of NIU, AAV-mediated expression of the HLA-G-1 and -5 transgenes was evaluated in the rat EAU model. A single intravitreal injection of AAV–HLA-G-1/-5 decreased clinical and histopathologic inflammatory and cellular infiltration scores significantly compared to untreated EAU eyes. In other ocular disease models, AAV-mediated gene transfer of AAV-HLA-G has been shown to decrease corneal inflammation, vascularization, and fibrosis after chemical injury, prevent corneal transplant rejection, and inhibit the development of experimental ocular graft-vs-host disease—all immunologic ocular diseases. Thus, AAV gene therapy would not only treat ocular immune-mediated diseases via a single injection, but also may provide a novel comprehensive therapy for patients to reduce inflammation and prevent complications associated with NIU that lead to blindness. These studies of gene transfer of immunosuppressive transgenes demonstrate the value of the one-health approach in developing advanced therapies for naturally occurring immune-mediated diseases, such as uveitis in horses.

Conclusion

This review of studies that investigate the use of autologous MSCs or gene transfer using AAV vectors to target naturally occurring immune-mediated ocular diseases in animals epitomizes the one-health concept. The one-health approach is defined as the “collaborative efforts of multiple disciplines working locally, nationally, and globally, to attain optimal health for people, animals and our environment” (https://www.cdc.gov/onehealth/). The use of autologous MSCs in clinical patients or horses with IMM demonstrate the feasibility, safety, and efficacy of the MSCs delivered subconjunctivally in animals with naturally occurring disease. These results suggest that similar approaches could be considered for other species with similar diseases, such as stromal keratitis in humans and cats, and chronic superficial keratitis in dogs.

Gene transfer of therapeutic proteins to treat naturally occurring ocular diseases is becoming more feasible based on the results of research studies in models of uveitis and other ocular diseases. These efficacy and safety studies are paving the way for clinical trials in animals with naturally occurring immune-mediated diseases, such as a therapeutic clinical trial for AAV–eqIL-10 in horses with ERU. This AAV–IL-10 clinical trial will be the first trial of AAV gene therapy for the treatment of uveitis in any species. With the demonstration of safety and efficacy, these clinical trial results will translate well to other species, such as dogs, cats, and humans. The development of these innovative therapeutics uses multiple disciplines and will benefit people and animals globally—all supporting the one-health concept.

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Figure 4—Human leukocyte antigen-G (HLA-G) mechanism of action on various cell types. DC = Dendritic cell. IL = Interleukin. INF = Interferon. MHC = Major histocompatibility complex. NK = Natural killer. sHLA = Soluble human leukocyte antigen. TGF = Transforming growth factor. TH = T helper. TNF = Tumor necrosis factor. Treg = T regulatory cell.
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References