The use of mesenchymal stem cells in treatment of Immune Mediated Hemolytic Anemia

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Introduction

Immune mediated hemolytic anemia (IMHA) is among the most common autoimmune diseases of dogs, resulting in a 32% mortality rate despite therapy.[1] IMHA results from a failure of the immune system to recognize erythrocytes as self, resulting in their destruction. This occurs either through intravascular lysis mediated by the innate immune system, complement cascade, and membrane attack complex, or through the extravascular opsonization and phagocytosis by mononuclear cells in the spleen and liver.[2] The end result is a life threatening anemia, icterus, and coagulopathy.[3] This paper outlines the pathogenesis behind IMHA and explores the potential benefits of stem cell therapy.

Immunopathology of IMHA

Identifying and understanding the role each cell type plays and the cytokines present in the IMHA patient can help veterinarians direct therapy during this autoimmune crisis. The underlying pathology of IMHA is a failure of immune tolerance; the immune system's ability to recognize erythrocytes as "self". Through the activation of the reticuloendothelial system, self-antigen is presented to activate B cells, which then begin producing anti-self antibodies. This process is mediated by activator T cells and their effector cytokines. Increased concentrations of Interleukin-2 (IL-2), Interferon -g (IFN-g), and Tissue Necrosis Factor alpha (TNFα) suggest that the immune response may be driven by Th1 cells,[4] resulting in activation of mononuclear phagocyte system.[5] Th1 cells shift their environment towards a pro-inflammatory state through the release of TNFα and Interferon gamma (IFN-g).[6] Current therapeutic goals are aimed at combating the pro-inflammatory cytokine state, down regulating the antibody producing B cells, and promoting regulatory T cells (T_{reg}).[7,8] In humans with IMHA, increased number of Th17 cells in circulation and an increased serum concentration of Interleukin-17 (IL-17) have been observed and correlated with disease activity [7,8].[9,10] IL-17 when artificially blockaded in IMHA positive mice, resulted in a reduction in incidence of IMHA and a decrease in severity, suggesting a possible Th17 mediated IMHA [7].[9] Th17 elevations have been researched in dogs with IMHA. Cuq *et. al.* demonstrated a significant (P=0.0158) increase of IL-17 in IMHA dogs when compared to a healthy population.[11] The cross-species studies of Th17 cells in autoimmune disease suggests a potential therapeutic target for cases of IMHA.

Complement activation is an important component of the innate immune system responsible for intravascular hemolysis in cases of IMHA. Typically, the complement is activated through a series of proteolytic reactions that activate subsequent molecules, resulting in a cascade-like amplification and positive feedback that can result in severe and uncontrolled cell lysis. The protein C3b, created during the cascade, is deposited on cell surfaces and is responsible for triggering the subsequent erythrolysis. Cell

surface binding of C3b causes activation of the reticuloendothelial complex and results in the removal of the cell from circulation, and destruction in the spleen or liver. Additionally, binding of C3b on a cell surface activates the terminal pathway for the complement cascade, ending in formation of the membrane attack complex. The membrane attack complex is the primary affecter for intravascular hemolysis.[12]

Immunomodulatory Properties of Stem Cells

While the full catalogue of immunomodulatory effects exhibited by mesenchymal stem cells (MSCs) has not been discovered, recent studies have described their ability to affect T and B lymphocytes, macrophages, and complement.[13–15] Cell therapy differs from traditional pharmacologic therapy by nature of the bioactive properties of the cells. MSCs have been shown to respond to triggers such as IFN-g and TNF-α resulting in the secretion of anti-inflammatory compounds such as IL-1 receptor antagonist protein (IRAP), hepatocyte growth factor (HGF), prostaglandin E_2 (PGE2), and transforming growth factor-beta (TGF-β).[16,17] It has been shown that the immunomodulatory effects of MSCs includes blocking proliferation of activated lymphocytes, and the ability to shift a patient from a TH1 (inflammatory) phenotype to a TH2 (non-inflammatory) phenotype.[18] The induction of a Th2 phenotype in response to autoimmune inflammation has been shown to improve therapeutic outcomes in humans with multiple sclerosis.[19] MSCs also promote the generation of regulatory T cells, one of the key components in peripheral immune tolerance.[18] In a comparative, prospective study, Kol et al. demonstrated that MSCs inhibit Th17 polarization of canine cells.[20] MSCs have also been shown to drive a patient away from the Th17 phenotype towards a Th2 phenotype in murine models.[21] Inhibition of Th17 polarization reduces the secretion of the pro-inflammatory cytokine IL-17.

Modulation of the adaptive immune system is just one component of management in cases of IMHA. The innate immune system utilizes the complement cascade to affect intravascular lysis of red blood cells.[2] In healthy individuals, the complement cascade is down-regulated by the glycoprotein, Factor H, resulting in the protection of normal cells from damage. MSCs have demonstrated the ability to produce Factor H and prevent complement mediated erythrolysis.

These strong immunomodulatory capabilities are currently being harnessed to treat a number of veterinary immune-mediated diseases such as feline gingival stomatitis[23], dry eye[24], and inflammatory bowel disease[25]. Blocking of the pro-inflammatory pathways mediated by IL-1 and TNFα has been used for years in the treatment of inflammatory bowel disease.[26]

Stem Cell Therapy

The use of MSCs to treat IMHA focuses on their immunomodulatory effects to both the adaptive and innate immune system. Adipose-derived mesenchymal stem cells block proliferation of activated lymphocytes, decreasing the production of potential alloreactive antibodies. They reduce systemic inflammation caused by Th1 cells by shifting the cell population to a Th2 phenotype. Finally, they release Factor H, causing inhibition of complement mediated red blood cell destruction. MSCs have been shown

in studies to be a safe treatment that acts on the 3 primary pathologic mechanisms of IMHA.

Therapeutic MSCs derived from adipose tissue are harvested with the stromal vascular fraction (SVF). The SVF contains a heterogenous population of cells consisting of high concentrations of ad-MSCs, pericytes, and endothelial and mesenchymal progenitor cells. It is harvested and processed in a way to produce consistent and quantifiable concentrations of cells for therapeutic use.

The current VetStem Biopharma recommendation for treatment of immune mediated diseases is two administrations of 5 mLs of freshly isolated Stromal Vascular Fraction (containing concentrated MSCs) or cryo-banked cells. The cells are administered intravenously, approximately 1 month apart, dosed on a cells/kg body weight basis. Retreatment is recommended if clinical signs return, or if cell therapy is being used aggressively, retreatment with an additional dose every 90 days. For animals that do not respond adequately to the first round of therapy, not showing improvement after 90 days of therapy, consider repeat doses with a higher cell count.

Conclusion

IMHA is a hypersensitivity reaction that results in destruction of red blood cells. Anti-erythrocyte antibodies bind to the cell surface resulting in both intravascular hemolysis, mediated by complement; and extravascular hemolysis, governed by the reticuloendothelial system. Intravascular hemolysis occurs when complement proteins bind together and ultimately form the membrane attack complex. Factor H, a molecule secreted by MSCs, inhibits the assembly of complement proteins and accelerates their decay. This has been shown to protect red blood cells from complement mediated lysis.[15] Activated lymphocytes producing self-reactive antibodies are blocked through the immunomodulatory effects of MSCs. Cells that are not bound to alloantibodies are not phagocytized by macrophages in the liver or spleen. MSCs also promote Treg cell population, helping reestablish peripheral immune tolerance.

Taking all of this into account, there is potential for stem cell therapy to ameliorate both intravascular erythrolysis and autoantibody production in cases of Immune Mediated Hemolytic Anemia. While we are still early in the collection and generation of clinical data, the proven immunomodulatory effects of cell therapy appear to affect pathways linked to IMHA disease. Given the evidence that cell therapy has shown an improved outcome in treatment of other immune mediated disease, adding cell therapy to an IMHA protocol may be beneficial.

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