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CTLA4-ATMSC Treatment Therapy of Steroid-refractory Pemphigus Foliaceus

Pemphigus is an autoimmune disease characteristic of Type II hypersensitivity. Typical signs of acantholysis are observed when desmosomal proteins are targeted by autoantibodies, as they mediate release of proteases from keratinocytes that destroy intercellular bridges. Free floating keratinocytes form bullae that rupture easily, leading to areas that are prone to secondary infections. In the case of the admitted Shih Tzu patient, extreme pruritus is seen as crusting and pustules are observed in specific areas all over the body, along with histopathological indications of acantholytic keratinocytes and high neutrophil concentrations. Pemphigus foliaceus is the definitive cause as bacterial and parasitic infections are ruled out through cytology smears; serum biochemistry also points to an autoimmune cause to the perceived issue. From a pathophysiological standpoint, lesions stemming from pemphigus typically occur around mucocutaneous junctions. Antibody depositions in such type II hypersensitivity cases usually show up as ribbonlike deposits when observed microscopically.

Since it is an autoimmune condition by nature, the most obvious course of action would be immunosuppressive therapy to combat the body's uncontrollable response against its self-antigens. Common medicine used include cyclosporine, corticosteroids, azathioprine, and more, along with immunosuppressive skin care management in the form of topical antimicrobial therapy and systemic treatments. Symptomatic treatment using keratolytic shampoo is also recommended until the disease is under control. However, despite being a manageable chronic disease, pemphigus treatment is volatile in the sense that clinical efficacy of combination therapy –even though has increased survival rate of treated patients – remains inconsistent from patient to patient. Certain combinations of such drugs (i.e. azathioprine and prednisolone) also contribute detrimental side effects. Immunosuppressive side effects included melena, anorexia, and lesions throughout the body. Due to the inefficiencies of such drug cocktails, researchers have turned to mesenchymal stem cells as a form of therapeutic tool. According to research, MSCs have immunosuppressive properties through cytokine/TLR ligant modulation. However, due to its poor immunogenicity, allogenic MSCs are preferred in clinical settings. Specifically, a combination of CTLA4 gene overexpression of AMSCs (previously tested on peripheral blood mononuclear cells against autoimmune thyroiditis and proved to be effective) as well as naïve ATMSCs was used over a period of 20 months.

Pemphigus foliaceus symptoms are fairly evident in the conspicuity of their gross appearances. Scales, crusts, pustules, epidermal collarettes, erosions, erythema, and more such obvious signs of skin disease damage readily indicate the presence of an autoimmune disease provided that other primary infection sources (i.e bacterial, parasitic) are ruled out. In more severe or chronic cases, lymphadenopathy, edema, depression, and fever may also be observed. As mentioned above, the diagnosis was all but confirmed through observations of neutrophils, fibrin debris, and acantholytic keratinocytes within the lesions, as well as mastocytic and neutrophilic dermal inflammation. When normal immunosuppressive drugs proved to be ineffective, therapy measures turned to ATMSC injection therapy. Almost immediately, positive results can be seen as skin lesions improved and the severity of the pruritus markedly decreased. To further increase the effectiveness of the treatment, a combination of CTLA4 overexpression and naïve ASMC injection was put on a specific schedule of alternating administration with occasional concurrent transplantation. To minimize side effect damage from the immunosuppressive drugs, a tapered continuous dose of prednisolone was also used along with the cessation of azathioprine usage. Clinical remission is thus achieved after 20 months, and with the cessation of ATMSC treatment along with a continuous low dose of prednisolone, the skin lesions were well controlled for 12 months with obvious results of systemic

improvements. Body weight returned to normal as improvements to leukocytosis, anemia, and the abnormally high liver enzyme elevation were also seen. Body condition score was also ameliorated after the treatment.

To further understand why the CTLA4-AMSC therapy treatment is as effective as it is, one must turn to the specific biochemical functions of mesenchymal stem cells. Their immunosuppressive properties allow inhibition of a variety of cell types involved with both adaptive and innate immune response. Among other things, target suppression of note by MSCs includes CD4+ and CD8+ lymphocytes as well as B cell differentiation and antibody production. MSCs are also thought to target NK cell activation and expansion along with having a modulating effect on T-cell proliferation and function. Upon reading more into other research papers, the specific functions of MSCs seem to expand further to include the inhibition of T-cell cytotoxicity and cytokine secretion, dendritic cell development, and antigen presenting obstruction; a phenomenon called division arrest anergy is seen as MSCs interfere with major aspects of the immune system. The main inhibitory mechanism of choice observed seems to be direct cell-to-cell interaction and soluble factor secretion. (MP 2012) Though there is strong evidence, the actual mechanism behind MSC regulation of immune response is still up for debate amidst controversies regarding the molecules involved. It is thought that MSC recruits regulatory T lymphocytes to lymphoid organs and grafts as well as other possible players such as Prostaglandin E2, TGF-Beta, and IL-6 and 10. (MP 2012)

The other piece of the puzzle in regards to the treatment rests in the mechanisms behind CTLA-4 activity and how it negatively regulates T-cell activation. It is know that as a protein co-receptor on the surface of T-cells, CTLA4 helps regulate immune activation and tolerance by acting as the counterpart to CD28, a positive modulatory signal of immune response. On the other hand, CTLA4 inhibits T-cell activation during strong immune responses. It is precisely this balance of stimulatory and inhibitory signals that give rise to a stable immune response in which avoidance of a severe inflammation response as well as prevention of autoimmune attacks are both achieved. Through experimental testing, it is seen that interference with the processes that govern the regulation of CTLA4 surface expression that alternative approaches to tools that can combat autoimmune responses can be achieved. One of the first demonstrations of CTLA4 function involved mice that had this specific gene deficiency, which resulted in an autoimmune phenotype of massive tissue infiltration, most likely due to unchecked immune homeostatic response and tolerance. The CTLA-4 gene, especially in humans, have also been tied to the implications of other autoimmune diseases should any sort of mutation occurs. Unlike traditional T-cell lymphocytes, it was discovered that CTLA-4 receptors are expressed constitutively on the surface of Treg cells. This high level of surface expression despite the receptor being intracellular in origin speaks to the importance of its function when it comes to T-cell regulation and autoimmune response. Currently there are studies underway that uses this premise in order to block such an expression. Naturally, this blockade would theoretically aid in tumor treatment, as a greater degree of antitumor T-cell response would be generated due to a lack of inhibitory signaling. (Schneider 2014) Conversely, the logical progression of this discovery would imply that the opposite would also hold true when using CTLA-4 as a therapeutic tool against overaggressive autoimmune responses, such as pemphigus foliaceus. Though the specific mechanism of how CTLA-4 affects T-cell regulation is still not definitively known, the promise shown through early clinical trials has warranted its use as an experimental therapeutic tool in both veterinary and human medicine.

In conclusion, in the specific case of the 10-year-old castrated male Shih Tzu that went into immunosuppressive therapy utilizing the fundamental principles behind CTLA4 expression in AMSCs, the results positively correlate to the confirmation of the findings. For 12 months after

the application of AMSCs that overexpress CTLA4 receptors to augment the T-cell inhibitory effects of the co-receptor protein, most of the symptoms had been alleviated and only requiring a continuous low dose of prednisolone in order to keep the lesions in check. The combination of the CTLA4-AMSCs as well as the naïve AMSCs was potent enough to curb the negative effects of the disease as well as avoid the possible exacerbated effects of immunosuppression. Unfortunately, the dog eventually passed away due to pulmonary complications, so it is not known whether the lasting effects of the treatment would have continued after the 12-month observation period. Even so, the clinical results speak positively to the possibility of non-drug therapy and may greatly improve prognosis of autoimmune diseases in the future as well as the survival rate of patients undergoing such treatments.

Misc. Sources besides CTLA4-AMSC Paper:

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