CASE REPORT

Purified umbilical cord derived mesenchymal stem cell treatment in a case of systemic lupus erythematosus

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Abstract

Introduction: Systemic lupus erythematosus (SLE) is a multiple organ system autoimmune disorder for which there is no known cure.

Methods: We report a case of a young adult lady with SLE and Sjogren’s with diagnostic and clinical resolution following purified umbilical cord derived mesenchymal stem cell (MSC) and globulin component protein macrophage activating factor (GcMAF) therapy in a combined multidisciplinary integrative medicine protocol.

Results: Our patient had complete reversal of all clinical and laboratory markers.

Conclusion: We recommend a prospective randomized double blind study to assess the sustained efficacy of MSC and GcMAF in the treatment of autoimmune connective tissue diseases such as systemic lupus erythematosus.

Keywords: Lupus, Systemic lupus erythematosus, Mesenchymal stem cells, Sjogren’s, Systemic sclerosis, Autoimmune diseases, Connective tissue diseases, gcMAF

Introduction

Systemic lupus erythematosus (SLE) is an inflammatory mediated autoimmune disorder which forms a part of a spectrum of connective tissue disorders which can often attack any or many organ systems involving joints, skin, kidneys, lungs, brain, etc. Genetics and environmental factors can influence the incidence of disease. Connective tissue diseases (CTD) can present in several members of a family or underlying propensity can be unmasked by environmental factors such as drugs, infections, and solar radiation. Sjogren’s syndrome (SS) is a CTD characterized by xerostomia, xerophthalmia, and parotomegaly in combination with CTD spectrum pathology. The pathophysiology is autoimmune inflammatory destruction of tissue compartments with deposition of lymphocytic immune complexes which trigger and enhance inflammatory destruction in multiple organs. CTD destructive process is cyclical with immune dysfunction leading to inflammatory destruction. The proteinaceous components provide for further triggering of the inflammatory cascade. SS is characterized by infiltrates predominantly in the lacrimal and salivary glands, whereas SLE tends to be a more diffuse organ system disease process. Therapeutic options for CTD have been less than satisfactory; anti-inflammatories and immunosuppressants have been the primary focus since no cure is known at this time. Long-term immunosuppressants and anti-inflammatories carry significant morbidity, estimated mortality is 6.8% while the most frequent predictors are: active SLE, thromboses, and infections, respectively [1].

Mesenchymal stem cells are pluripotent cell lines that are able to differentiate into specialized cell types after mitosis. These pluripotent stem cells are progenitor cells able to differentiate into specialized cell types such as: cartilage, bone, and connective tissue, etc. The International Society for Cellular Therapy (ISCT) defines the minimal criteria for assessing multipotent MSC: (1) plastic adherent, (2) expression of CD105, CD73, and CD90 but not CD45, CD34, CD14, CD11b, CD79 alpha, CD19, or HLA-DR, (3) must differentiate to osteoblasts,

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adipocytes, and chondrocytes in vitro. MSC have also been demonstrated to affect T and B lymphocytes, natural killer, and antigen presenting cells [2]. Mesenchymal stem cells have anti-inflammatory properties but is hypo-immunogenic since MSC's have low major histocompatibility complex (MHC) class I expression and lack MHC II or co-stimulators: CD40, CD40L, CD80, CD86 [3]. MSC's therefore, do not require HLA testing making mesenchymal stem cells relatively safe for administration. Several sites have been used to harvest MSC’s including, bone marrow, brain, and adipose tissue. Cras et al. demonstrated in their study the potential of MSC in the treatment of autoimmune connective tissue diseases such as systemic lupus erythematosus and scleroderma. In that study, the authors examined several published studies which used MSC from various sources and demonstrated efficacy [4]. A more recent evaluation completed in 2014 demonstrated that umbilical cord derived MSC resulted in satisfactory clinical response but the response was time limited to approximately 6 months [5]. MSC therapy has the advantages of modulating the inflammatory cascade while stabilizing the progenitor cell lines thereby reducing the inflammatory and autoimmune dysfunction.

GcMAF is a serum glycoprotein which can be converted by beta-galactosidase of B cells and sialidase of T cells to a potent macrophage activating factor (MAF) [6]. Macrophage activating factor is a protein with an N-acetylgalactosamine sugar moiety attached. Macrophages are also activated by superoxides in vivo, the generation of which include inflammation and cellular stress. Somali et al. [7] demonstrated the interaction of exercise and alcohol on antioxidant enzymes in brain regions of the rat in 1996. Macrophages operate as a common final pathway for immunological and inflammatory waste management. Macrophages are the white blood cell component that consume the proteinaceous byproducts and help to tag other byproducts for immune destruction. It seems reasonable to posit that in diseases where chronic immunologic triggering of the inflammatory cascade are predominant, more efficient clearing of the debris should result in less activation of the inflammation. GcMAF appears to have antineoplastic, antioxidant, and anti-angiogenic effects. Inui et al. [8] report three cancer patients treated via multimodality integrative immunotherapy with GcMAF with promising results. The same group in Japan also reported successful treatment of a patient with multiple sclerosis using GcMAF in 2016 [9].

**Methods**

A 26 year old lady diagnosed with primary SLE and secondary SS via 1997 American College of Rheumatology (ACR) guidelines at the age of 13 years old. She has several family members with CTD diagnoses. She has cycled through various anti-inflammatory and immunosuppressants over the years with limited efficacy. Thirteen years after diagnosis, she presented for mesenchymal stem cell therapy at IntelliHealth Plus Clinic, Bangkok, Thailand. At the time of diagnosis the following laboratory results were obtained: positive ds-DNA, positive ANA (speckled 1:1280, nucleolar 1:80), positive RF. Using ACR criteria, the following were met: malar rash, photosensitivity, nonerosive arthritis, pericarditis, mild hemolytic anemia with reticulocytosis, immunologic disorder (positive ds-DNA), and positive ANA. Medications before treatment include: plaquenil, methocarbamol, klonopin, imuran, trazodone, prozac, etodolac, synthroid, prilosec. Allergies: PCN.

A multidisciplined integrative medicine treatment protocol was designed using 100 million MSC delivered intravenously and 0.25 mL (440 ng/mL) GcMAF intramuscularly weekly in combination with vitamin D3 5000 IU/600 mg calcium supplementation daily. The MSC were delivered in four injections of 25 million each over 3 weeks. The treatment protocol was tolerated well without complications and patient returned to the USA to follow up with her primary physicians and rheumatologist. She reported improvement in multijoint pain and stiffness at approximately 3 months and began tapering off medications. All medications were discontinued by month 8 without complication; JM reported complete resolution of all symptoms. The following laboratory results were obtained: centromere Ab, histone Ab, ds-DNA Ab, JO Ab, RNA Ab, Smith Ab, SCL Ab, SSA Ab, SSB Ab all negative. JM is approximately 1 year status post treatment, off all medications, and continues to remain symptom free. Repeated laboratory analyses are negative.

Our umbilical cord derived purified mesenchymal stem cells are obtained from Stem Cell 21 Co., Ltd., Thailand which uses a five step process for 1st pass culturing to ensure maximal numbers of young, metabolically active cells. The pooled donated MSC are obtained from live, healthy, babies from the umbilical cord remnant and processed to exclude infectious or immunologic contamination. The MSC are processed to ensure (1) donated cells are examined for uniform cell morphology. (2) Cell viability is examined using fluorescence tagging, Muse analyzer, and Guava Easycyte flow cytometer to ensure healthy, active, viable cells above 80%. (3) Apoptosis, autophagy, and necrosis are assessed using Annexin V assay. (4) Cell cycle is a highly ordered process that results in transmission of genetic information from one generation to the next. Assessment follows the process through the phases of mitosis: G1-S-G2-Cytokinesis. (5) Ensure high surface markers: surface expression of CD105, CD73, CD90 and absence of CD45, CD34,
CD14/CD11b, CD79 alpha, CD19, and HLA-DR. Cell lines must differentiate to osteoblasts, adipocytes, and chondroblasts in vitro as indicated by ISCT guidelines.

**Results**
Comparing the laboratory and clinical data before and after treatment reveals a complete reversal of the immunological and inflammatory markers for this patient. Additionally, the laboratory results have been consistent since treatment (see chart #1 and chart #2). Unfortunately, SLEDAI and BILAG evaluations for SLE and ESSDAI evaluations for SS were not done because these studies are most appropriate for clinical research studies for which she was not enrolled at the time of diagnosis. Our clinical measurements use the ACR criteria and laboratory measurements include what we have for pre and post treatment immunological markers.

| Before treatment | After treatment |
|------------------|-----------------|
| ANA+             | –               |
| RF+              | –               |
| dsDNA+           | –               |
| Smith antibody+  | –               |

| Before treatment | After treatment |
|------------------|-----------------|
| Malar rash present | Absent          |
| Pericarditis present | Absent         |
| Photosensitivity present | Absent       |
| Arthritis present | Absent          |

**Conclusion**
Systemic lupus erythematosus is an inflammatory mediated autoimmune disease which often severely limits the patient’s activities of daily living (ADL) through fatigue, arthritis, pain, and multiple organ impairment. Curative therapies for SLE have been elusive to date making chronic immunosuppressive therapy, with it’s associated long term side effects problematic. To our knowledge, this is the first SLE case to show clinical and laboratory remission after therapy using mesenchymal stem cells and GcMAF. The combination of MSC with GcMAF has three main advantages: (1) mild immunosuppression, (2) robust anti-inflammatory effects, and (3) repair of damaged cell lines. Mesenchymal stem cell therapy with GcMAF is well tolerated with minimal potential complications. Our patient had no complications or side effects of therapy, clinical and diagnostic improvement has been sustained for approximately 1 year. Whether combined therapy offers an advantage over MSC therapy alone and if the therapy should be repeated over time remains to be answered by additional research. Research studies would need to evaluate for possible macrophage activation syndrome as a potential complication of GcMAF therapy. Additional research should help shed light on whether MSC and GcMAF therapy will show a consistent pattern of disease reversal over time.

**Abbreviations**
- SLE: systemic lupus erythematosus; SS: Sjogrens syndrome; CTD: connective tissue disease; MSC: mesenchymal stem cells; MHC: major histocompatibility complex; GcMAF: globulin component protein macrophage activating factor; ACR: American College of Rheumatology; DNA: deoxyribonucleic acid; ds-DNA: double stranded deoxyribonucleic acid; ANA: antinuclear antibodies; RF: rheumatoid factor; ADL: activities of daily living; PCN: penicillin; ESSDAI: Sjogren’s syndrome disease activity index; SLEDAI: SLE disease activity index; BILAG: British Isles Lupus Activity Group.

**Authors’ contributions**
All authors were involved in concept and design while Dr. Phillips wrote the manuscript. All authors read and approved the final manuscript.

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**Competing interests**
Drs. Pornpatcharin and Htut are employees of IntelliHealth Plus Clinic. Stem Cell 21 Co Ltd. paid the publication fees for this manuscript. Authors have no other financial interest to declare.

**Availability of data and materials**
Laboratory and clinical data are kept at IntelliHealth Plus Clinic, Bangkok, Thailand.

**Consent for publication**
Patient has given written consent to publish data relating to her case for educational purposes.

**Ethics approval and consent to participate**
Ethics approval is n/a as this is a case report. Informed consent to treatment was obtained from the patient.

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