Research Article

Stem Cell & Regenerative Medicine

SB cell® Therapy Ameliorates IL-1 RA-Deficiency-Associated Psoriasis

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ABSTRACT

In this study, we find a subject with a deficiency of IL-1 receptor antagonist (DIRA) – like syndrome who also has symptoms similar to that of psoriasis. Within 24 hours of intravenous infusion with autologous SB cells®, the subject's IL-1 RA expression is shown to increase drastically, during which the subject experiences relief from his psoriasis-like symptoms.

The subject was diagnosed with psoriasis as a young adult; however, no treatment has offered relief for the psoriatic plaques located along his forearms and back. Currently his diagnosis is classified as psoriasis and his management of the disease is minimal. Throughout the course of SB cell® treatments, we used ELISA to monitor a panel of cytokines, it was here we noticed the subject's deficient IL-1RA expression without treatment, his expression levels were near 0 pg/ml. 24 Hours after treatment, measurements of his expression levels were increased several thousand fold.

This case presents the possibility that SB cells can act as a treatment for psoriasis due to IL-1 RA deficiency.

Keywords

SB cell, small stem cell, psoriasis, IL- 1 RA.

Abbreviations

BLSC: Blastomere-Like Stem Cell, RT-PCR: Reverse Transcription polymerase chain reaction, DIRA: Deficiency of IL-1 Receptor Antagonist, Tc: Cytotoxic T-cells, ELISA: Enzyme-linked Immunosorbent Assay, Th: Helper T- cells, HSC: Hematopoietic stem cells, Treg: Regulatory T cells, IL-1: Interleukin 1, SB: StemBios Cell®, IL-1 a: Interleukin 1 alpha, VSEL: Very small embryonic-like stem cell, IL-1 b: Interleukin 1 beta, IL-1 RA: Interleukin 1 Receptor Antagonist, Lgr5: Leucine-Rich Repeat Containing G Protein-Coupled Receptor 5, MSC: Mesenchymal Stem cell; OOR: Out of range, PPAR-γ: Peroxisome proliferator-activated receptor gamma.

Introduction

Psoriasis is an autoimmune disease that affects the skin as it is typically characterized by red, itchy, scaly, patches on the dermis and can vary in severity from small and localized to whole body coverage. The exact cause of psoriasis is not fully understood but is thought to be due to an immune system response where overactive T-cells attack healthy skin cells. Ordinarily, T cells are lymphocytes that play a central role in cell-mediated immunity and can be divided into helper T cells (Th cells), cytotoxic T cells (Tc cells), and T regulatory cells (Tregs). The activation of Th and Tc cells in an immune response are balanced by the regulatory abilities of Tregs. An imbalance of T cell regulation is the cause of many types of inflammatory diseases such as rheumatoid arthritis, lupus, and a myriad of others [1]. Further upstream of T cells in the immune response pathway is Interleukin-1, a family of "pro-

inflammatory" cytokines. IL-1 is required for T cell proliferation. IL-1 signaling is inhibited by secretion of IL-1 receptor antagonists (IL-1 RA) as they competitively bind to the same receptor- the interleukin-1 receptor. IL-1 RA is highly involved with the regulation of inflammation, inhibiting the activities of interleukin 1-alpha (IL-1a) and interleukin 1-beta (IL-1B). As such, it is produced commercially to treat autoimmune diseases such as rheumatoid arthritis in the form of the drug, Anakinra. Recently, a newly discovered autoinflammatory disease related to the aberrant expression of IL-1 RA has emerged. Named, deficiency of IL-1 RA (DIRA), this autoinflammatory disease occurs because of mutations in the IL1RN gene that encodes IL1-RA. DIRA typically only affects infants and young children, however, the symptoms of this disease are extremely deleterious and often fatal, with those affected suffering from severe skin and bone inflammation [1]. Mutations of the IL-1RA gene are also associated with a risk of schizophrenia and bi-polar disease [2].

Mesenchymal Stem Cells (MSCs) have been indicated to demonstrate immunomodulation in in-vivo testing. They have shown to inhibit the proliferation and function of the major immune cell populations including T cells, B cells, and natural killer cells [3]. These properties make MSCs a great candidate for immunomodulatory therapies for those with autoimmune diseases. However, current practices to obtain MSCs are invasive and can cause trauma to the patient as they are most often harvested from the bone marrow or adipose tissue. Thus, stem cells that can be acquired through less deleterious methods are appealing. We present in this paper an alternative to MSCs that may be obtained from peripheral blood that demonstrates potential for autoimmune therapy.

StemBios (SB) cells® are somatic stem cells, small in diameter, around 3 to 7 microns, that are found in peripheral blood, bone marrow, cord blood, muscles, and adipocytes. SB cells® are CD133-, CD34-, and CD66e- while Lgr5+ and CD349+. Together this proves that SB cells® are a type of novel small stem cell as they are not VSELs, HSCs, or BLSC. They are multipotent and possess the ability to differentiate into the three germ layers: ectoderm, endoderm, and mesoderm. In vitro culture experiments demonstrate that suspension of SB cells® in specifically conditioned medium will increase their cell size and cause them to attach to the plate or dish, in a manner similar to MSCs [4]. We have seen evidence that medicinal signaling cells (MSCs) are capable of exhibiting significant immunomodulatory effects, for example, suppression of T-cells in vitro and in vivo [2]. Thus, we theorize that SB cells® should have immunomodulatory effects as well.

In this case study we report an adult male who has acquired a DIRA-like syndrome as a young adult but whose symptoms manifests phenotypically as psoriatic plaques along his forearm and back. The subject expresses very low levels of IL-1 RA which is alleviated temporarily by intravenous infusion of SB cells®, causing an increase in his IL-1 RA expression more than a thousand-fold. This increase of IL-1 RA coincides with the reduction of the subject's psoriatic inflammation, which was outwardly noticeable as well as testified by the patient who noted less itching, pain, and general irritation in the affected regions. As such, not only is this paper an examination of this unique case but also an exploration of SB cell® therapy for future applications.

Methods

Isolation and calculation of SB cells®

Peripheral blood was collected in EDTA tubes and incubated at 4°C for 48 hours, after which the blood was separated into two distinct layers. The SB cells® were collected from the top layer as described in our previous PLOS one paper [5]. SB cell® counts were obtained by flow cytometry, and were stained with Lgr5-PE.

Luminex assay for gene expression

Serum samples were sent out to City of Hope Clinical Immunobiology Correlative Studies Lab.

RT PCR for gene expression

RNA from the patient was extracted using Qiagen RNA extraction kit (catalog number: 74134). Reverse transcription was performed using the reverse transcription kit from qScript™ (catalog number: 101414-102), and real-time PCR was performed using SYBR green mix from BioRad (catalog number: 170-8882) according to the manufacturer's instructions. Fold calculations were done according to DoubleΔ CT Analysis. The primer information is shown below:

PPAR-γ (F: 5'-GCTGTGCAGGAGATCACAGA-3', R: 5'-GGGCTCCATAAAGTCACCAA-3')

IL- 1 RA (F: 5'-ATCAGTACCTCACGGCTGCT-3', R: 5'-TGGGTATCTCAGGCATCTCC-3')

Cyclophilin (F: 5'-AGGGTGGTGACTTTACACGCCATA-3', R: 5'-CAAAGACCACATGCTTGCCATCCA-3')

Case Presentation

The subject, an adult male aged 57, initially diagnosed with psoriasis was recommended to us as a patient for SB cell® therapy as a subject for our IRB (number SB-IN-4333) in September of 2015. His symptoms included inflamed skin congruent to psoriatic lesions along his forearms and back. The subject reported previously attempting UV therapy and platelet rich plasma therapy prior to entering our IRB trial. However, he reported no long-term benefits from either. At the time of admittance, the patient's lesions were most severe at his mid to lower back, and presented themselves as overlapping, circular, red patches with a layer of white flaky skin on top. His forearms, less severely affected, presented a few red patches accompanied by minor flakiness. The patient testified that the affected areas were itchy and cracking due to the dryness. This caused the subject discomfort and pain. Additionally, the subject reported his medical condition exacerbated due to his frequent business travels, which put more stress on him physically and mentally.

Our patient underwent multiple treatments with autologous SB

cells®. Cells were extracted from the patient's peripheral blood and reintroduced intravenously. Prior to blood draw, the patient ingested a fucoidan-containing stem cell-mobilization agent facilitating the enrichment of peripheral blood with SB cells® [6]. Each treatment occurred over the span of three days with follow-up blood draws up until 1 week. The patient received a total of four treatments. The first two treatments occurred consecutively in the month of February 2016. The third and fourth treatments were also administered consecutively in April 2016. In the first treatment, the patient received a count of 236 million SB cells®. In the second, he received 610 million. Lastly, in the third and fourth treatments, he received 12 million and 100 million SB cells®, respectively. After the first treatment, the redness and white, flaky skin abated from the affected forearm regions. During this time, the patient also reported less itchiness in all the affected regions. After the first two treatments, the mid-to-lower back did not show any signs of improvement; however, in the months following the third and fourth treatments, the patient exhibited significant improvement in the mid-to-lower back region (Figure 1). Half a year after his fourth treatment, the patient reported a reduction in steroid cream application from once a day to twice a week.

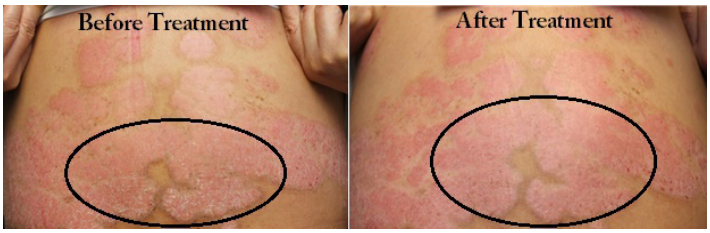

Figure 1: Mid-back region of patient before and one week after third treatment. Pictures taken 1 week apart showing a decrease in flakiness after the third treatment.

The subject's serum was sent out for City of Hope Clinical Immunobiology Correlative Studies Lab. After obtaining the results for his cytokine panel, of which included IL-1 RA, we discovered that his expression of IL-1 RA was significantly below the normal range 120 pg/ml to 250 pg/ml reaching levels below the lower threshold for ELISA detection typically around 30 pg/ml. The patient's blood samples were collected at time-points 48 hours before infusion, 24 hours after infusion, and 1 week after infusion. From these samples, we detected a significant increase in IL-1 RA expression 24 hours after SB cell® infusion for the first three treatments.

The first round of treatment increased IL-1 RA expression from below detectable values (<OOR) to 1156 pg/ml, the second round increased expression from <OOR to 2517 pg/ml, and the third round increased expression from <OOR to 3764 pg/ml. At the one week time point for each of the three treatments, the expression of IL-1 RA returned to levels similar to before the treatments. It should be noted, that the patient's IL-1 RA levels at the time point before each of the four treatments have increased from <OOR to 98.7 pg/ml, a value that although is still below the normal range does register on the ELISA assay. Additionally, the patient's blood was tested 4 months after his last treatment and his IL-1 RA levels registered at 89 pg/ml (Figure 2).

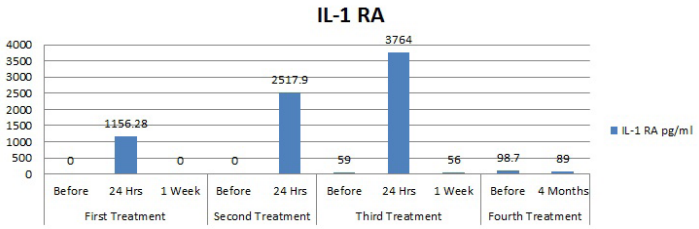

Figure 2: IL-1 RA ELISA results from patient's blood samples at time-points relative to treatment. Expression of IL-1 RA increased significantly 24 hours after SB cell® infusion but returns to native levels a week after. <OOR = out of (below) detectable range.

To confirm the Luminex data, we ran real time PCR on our subject's first treatment samples and noted a similar increase pattern in IL-1 RA expression (Figure 3).

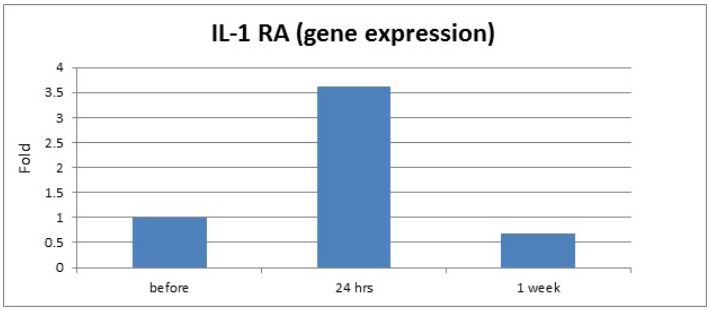

Figure 3: IL-1 RA gene expression. Expression of IL-1 RA as shown as fold changes from the patient's first treatment.

In addition, peroxisome proliferator-activated receptor gamma (PPAR-γ) has been shown to regulate IL-1 RA gene expression [7]. Here, we demonstrate PPAR-γ expression also significantly increases 24 hours after SB cell® treatment (Figure 4).

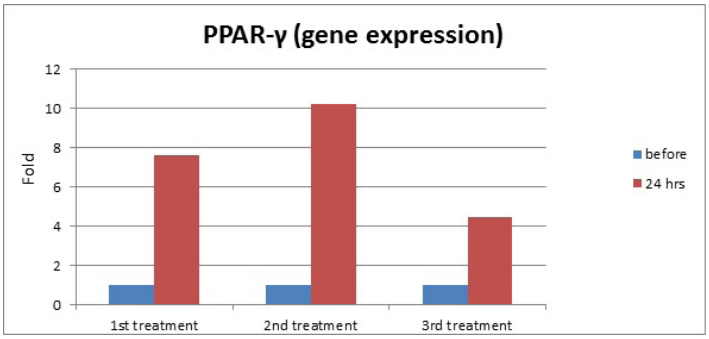

Figure 4: PPAR-γ gene expression. Before and 24 Hours after treatment expression of PPAR-γ as shown as fold changes across multiple treatments.

In correspondence to the IL-1 RA expression levels, the patient reported relief from his psoriatic symptoms when IL-1 RA was more highly expressed. The patient's relief included less flaking skin, less itchiness, and decrease in the severity of the lesions in size and appearance.

Discussion

Deficiency of IL-1 RA has previously only been classified in infants and toddlers [1]. However, this case presents the possibility of the disorder occurring later, perhaps by spontaneous mutation of the IL1RN gene or some other pathway. The consequences of the subject's abnormally low expression of IL-1 RA are not as severe as a traditional DIRA case the disease normally presents itself as life-threatening bone and skin inflammation [1]. This might be due to the delayed onset of the disease. There seems to be a correlation between the subject's psoriasis and his low IL-1 RA expression levels, as well as a correlation between the modulation of IL-1 RA expression and SB cell® therapy. This is evidenced by the increase in IL-1 RA expression after autologous SB cell® infusion. An explanation proposed for the regression of the subject's psoriasis involves the manifestation of IL-1 RA deficiency as a pattern of inflammation similar to psoriasis. If this were the case, the reduction of psoriatic lesions is caused by the increase in the subject's expression of IL-1 RA. To the best of our knowledge, there have been no other recorded cases of adult-onset DIRA thus symptoms of such are unknown. We have found no other deleterious symptoms that could be contributed to this abnormality. We hypothesize, due to the correlation, and lack of any other diagnosable indications, that the patient's deficiency of IL-1 RA is the cause of his psoriasis. The mechanism behind SB cell® therapy and the increase of IL-1 RA is still under investigation, there needs to be further research done. However, we propose that PPAR-γ plays an intermediatory role as we observed a significant increase in expression of this gene 24 hours after SB cell® injection. Furthermore, previous studies have indicated PPAR-γ to be involved in modulating inflammation, specifically by inhibiting secretion of IL-1 [8]. Perhaps there is a relationship between PPAR-γ and IL-1 RA as well.

Currently there are several treatments for psoriasis including topical corticosteroids, UV therapy, and immunomodulatory drugs such as Humira or Enbrel. However these treatments only treat the symptoms of psoriasis and there are many side effects associated with them, for instance skin thinning, melanoma, and nausea.

Previous studies have reported success with treating auto-inflammatory diseases such as psoriasis and arthritis with allogeneic stem cell transplants [3]. However, these treatments risk rejection by the host body and, until recently, stem cell extraction was an invasive process and could cause trauma to the patient as they were mainly harvested from the bone marrow or adipose tissue. Current methods of peripheral stem cell extraction and application have proven to be effective forms of treatment in some cases [4]. Our findings suggest that SB cells® may be useful for future applications.

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Competing Interests

The authors have received funding from a commercial source, TriMax SBT LLC. One or more of the authors are employed by a commercial company, StemBios Technologies, Inc. Both of these affiliations do not alter the authors' adherence to all the Stem Cell & Regenerative Medicine policies on sharing data and materials.

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