Intraperitoneal human adipose-derived stromal vascular fraction suppresses synovial inflammation and induces an increase in splenic Tregs in a mouse CIA model

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INTRODUCTION: Rheumatoid arthritis (RA) is an autoimmune disorder characterized by chronic inflammation and progressive destruction of the joints due to the immune system's attack on synovial tissues. Cell-based therapies, particularly those using mesenchymal stromal cells (MSCs), have gained attention for their potential to modulate the immune response and reduce inflammation in RA[1, 2]. Adipose-derived stromal vascular fraction (SVF) cells, which contain a heterogeneous population of cells including MSCs, have been highlighted for their ease of isolation from subcutaneous adipose tissue without the need for cell culture, making them an attractive candidate for clinical application[3]. SVF have shown promise in various regenerative therapies[4, 5], however, their specific role and mechanisms of action in the context of RA remain underexplored. The aim of this study is to evaluate the anti-inflammatory effects and therapeutic potential of SVF cell administration in a mouse collagen-induced arthritis (CIA) model, a widely used experimental model of RA, and to elucidate the mechanisms underlying these effects.

METHODS: This study was approved by the Institutional Animal Care and Use Committee of our institution. SVF was isolated from subcutaneous human abdominal or breech fat. A CIA model was established in 6-week-old mice. Three weeks post-immunization, 2.0×10^6 SVF cells were administered intraperitoneally to the SVF group, while phosphate-buffered saline (PBS) was administered intraperitoneally to the control group. The arthritis score, which assesses the degree of swelling in the limbs, was measured every week. At 6 and 8 weeks post-immunization, mice were sacrificed; knee joints were harvested for histological and immunohistochemical analyses, spleens were collected for flow-cytometric quantification of $CD4^+Foxp3^+$ Tregs and $CD4^+ROR\gamma t^+$ Th17 cells to calculate the Treg/Th17 ratio, and blood was obtained for ELISA measurement of serum TNF-α, IL-6, TGF-β, and IL-10.

RESULTS: Differences in arthritis scores between the control and SVF group began to emerge from 6 weeks post-immunization, with a significant difference observed at 8 weeks (Figure 1(a)). Histological assessment using hematoxylin and eosin staining revealed an increased number of lining cell layers, proliferation of subsynovial tissue and infiltration of inflammatory cells in the synovium of the control group, whereas the SVF group showed a thinner lining cell layer and reduced proliferation of subsynovial tissue (Figure 1b). Safranin O-fast green staining showed significant staining loss, erosions and a decrease in chondrocyte density in the control group, whereas some cartilage was preserved in the SVF group (Figure 1c). In the spleen, the proportion of $Foxp3^+$ Tregs in the SVF group increased at 6 and 8 weeks , whereas Th17 cells remained unchanged or slightly decreased, resulting in a higher mean Treg/Th17 ratio (4.8 ± 2.4 at 8weeks). In contrast, in the control group, Treg frequencies remained stable while Th17 cells increased, leading to a lower mean Treg/Th17 ratio (1.8 ± 0.8 at 8weeks) (Figure 2). Serum cytokine analysis showed elevated TNF-α levels at 8weeks and IL-6 levels at 6weeks in the control group, whereas the SVF group significantly exhibited increased levels of TGF-β ($6{,}400 \pm 1{,}500$ pg/ml at 6weeks) and IL-10(15.0 ± 5.2 pg/ml at 8weeks) (Figure 3).

DISCUSSION: In this study, synovitis was significantly attenuated and cartilage destruction was reduced in the SVF group compared with control. SVF administration enhanced splenic Treg responses while suppressing Th17 expansion, leading to a favorable Treg/Th17 balance. This shift was accompanied by increased anti-inflammatory cytokines (TGF-β, IL-10) and reduced pro-inflammatory cytokines (TNF-α, IL-6), indicating that SVF exerts systemic immunomodulatory effects. These findings suggest that intraperitoneally administered SVF suppresses arthritis in a mouse CIA model primarily through immune modulation, which results in the suppression of synovial inflammation and protection of cartilage. The cellular heterogeneity of SVF, including stromal and immune regulatory components, may underlie its capacity to induce such immune rebalancing.

SIGNIFICANCE: This study demonstrates that intraperitoneal administration of human SVF alleviates arthritis in a mouse CIA model by restoring immune balance through Treg enhancement and cytokine modulation. These findings highlight the potential of SVF as an immunomodulatory cell therapy for rheumatoid arthritis.

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