Immunomodulatory Properties of Adipose-Derived Stem Cells Cultured in Autologous Serum

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Introduction

Human adipose tissue is known to be an attractive and readily available source of mesenchymal stem cells (MSC). Most of the protocols, currently used for *in vitro* expansion of MSC, include fetal bovine serum (FBS) supplementation. MSC cultured in such a way for clinical applications rise the concerns about immunogenicity of FBS proteins (1). A possible solution to this problem is the use of autologous serum (AS) instead of FBS.

The immunomodulatory capacities of MSC were initially reported in T-cell proliferation assays where the ability of MSC to suppress T-cell proliferation can be readily determined (2). Such soluble factors as members of the transforming growth factor superfamily (transforming growth factor beta), hepatic growth factors, prostaglandin E2 and interleukin 10 secreted by MSC have all been reported to suppress T-cell-mediated antigen response *in vitro* (3). MSC inhibition of T-cell proliferation could also be due to depletion of tryptophan (4). Inducible nitric oxide synthase and heme oxygenase-1 expressed by MSC have also been implicated for their immunosuppressive properties (5,6). These properties of MSC might realize a novel therapeutic strategy to modulate immune responses in a variety of immune-mediated diseases.

Aim

The aim of this study was to investigate whether adipose-derived stem cells (ADSC), cultivated in the medium containing AS, maintain characteristics and immunomodulatory properties of MSC.

Cell lines

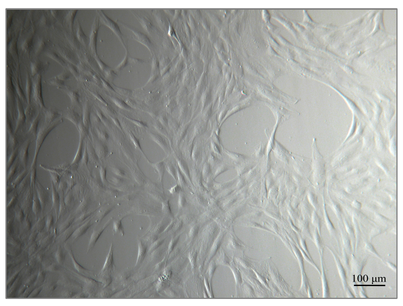

Figure 1. ADSC (*Invitrogen*) expanded in MesenPRO RS™ Medium at passage 3.

- ADSC isolated from human lipoaspirate tissue (StemPro® Human Adipose-Derived Stem Cell Kit; Invitrogen)
- Expanded in MesenPRO RSTM Medium containing 2% FBS (Invitrogen)
- Cell doubling time 36±4 h (7)
- Positive for expression of cell surface antigens CD29, CD44, CD73, CD90, CD105, CD166 (7)
- Negative for expression of cell surface antigens CD14, CD31, CD45, Lin1 (7)
- Can differentiate into adipocytes, osteoblasts and chondroblasts (7)

Figure 2. Morphology of ADSC expanded in the medium supplemented with 10% AS. (**A**) ADSC on the next day after isolation. (**B**) ADSC 2 days after isolation. (**C**) Monolayer of ADSC 8 days after isolation.

- ADSC isolated from human adipose tissue from abdominal cavity
- Cultivated in the medium (DMEM/F-12, 2 mM L-glutamine, 20 ng/ml bFGF, 100 u/ml : 100 µg/ml penicillin – streptomycin) supplemented with 10% AS for first 10 days and 5% AS afterwards
- Cell doubling time 40±4 h

Immunocytochemistry

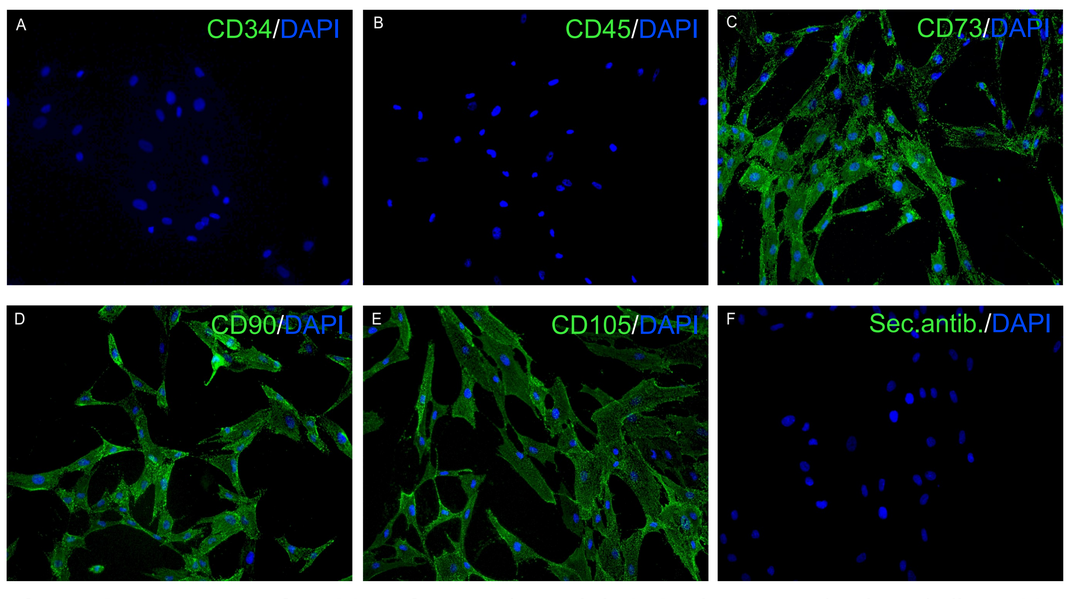

Figure 3. Expression of ADSC surface markers. (**A**) CD34 (mouse MAbs (1mg/ml) 1:100; *Abcam*) (**B**) CD45 (mouse MAbs (0,1mg/ml) 1:100, *Millipore*) (**C**) CD73 (mouse MAbs (0,5mg/ml) 1:100; *Invitrogen*) (**D**) CD90 (mouse MAbs (0,1mg/ml) 1:100; *Millipore*) (**E**) CD105 (mouse MAbs (0,1mg/ml) 1:100; *Millipore*) (**F**) negative control, secondary antibody only. Magnification 200x.

Differentiation

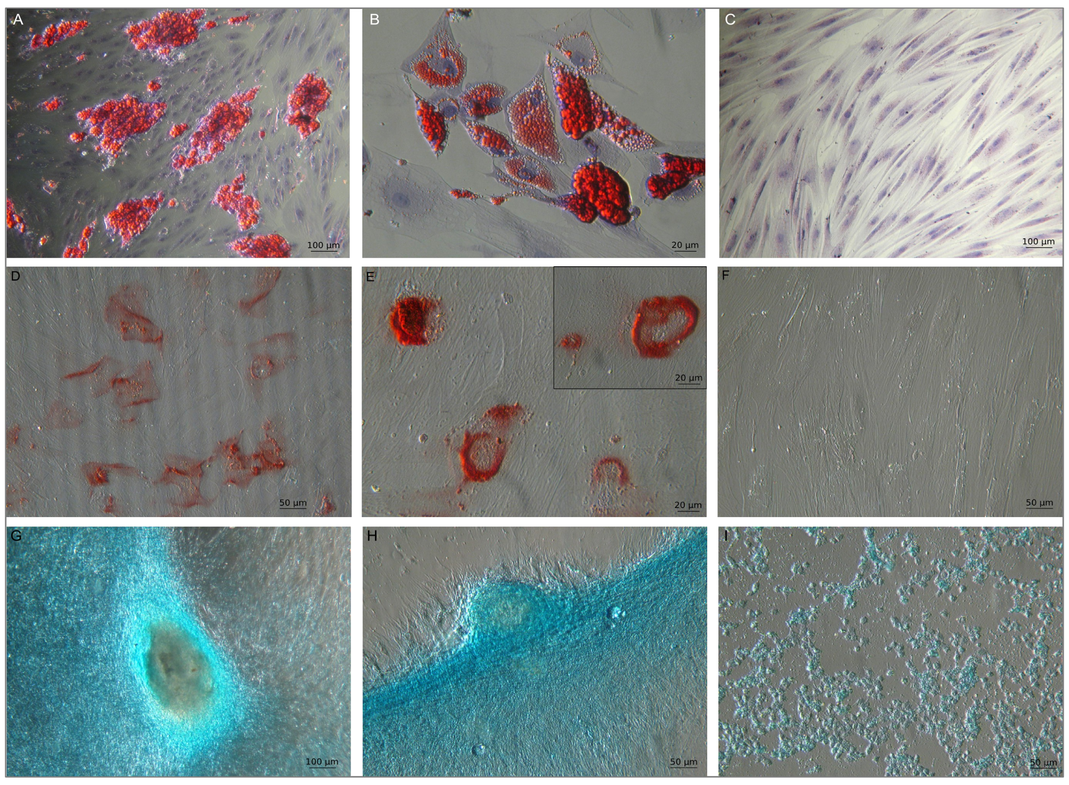

Figure 4. Differentiation of ADSC.
(**A**-**C**) ADSC differentiated towards adipogenic lineage. (**A**, **B**) Detection of lipids with Oil red O in the differentiated cells. (**C**) ADSC in a control medium. The cells counterstained with hematoxylin.
(**D**-**F**) ADSC differentiated towards osteogenic lineage. (**D**, **E**) Detection of calcified extracellular matrix with Alizarin Red S in the differentiated cells. (**F**) ADSC in a control medium.
(**G**-**I**) ADSC differentiated towards chondrogenic lineage. (**G**, **H**) Detection of sulfated proteoglycans with Alcian Blue in the differentiated cells. Initial and newly formed cell aggregates are shown. (**I**) ADSC in a control medium.

Blast transformation reaction

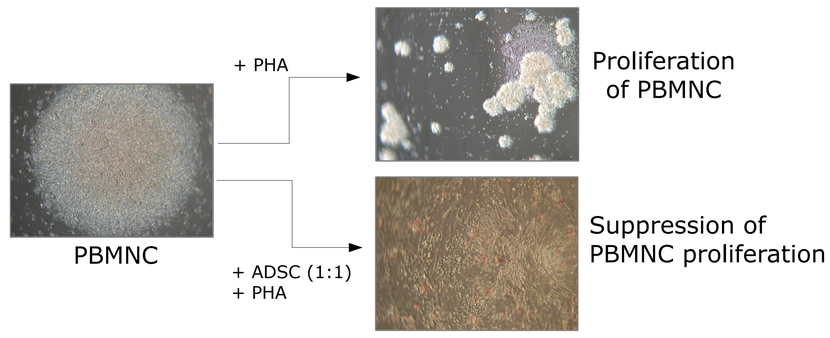

Figure 5. The effect of phytohemagglutinin (PHA) and ADSC on the proliferation of peripheral blood mononuclear cells (PBMNC).

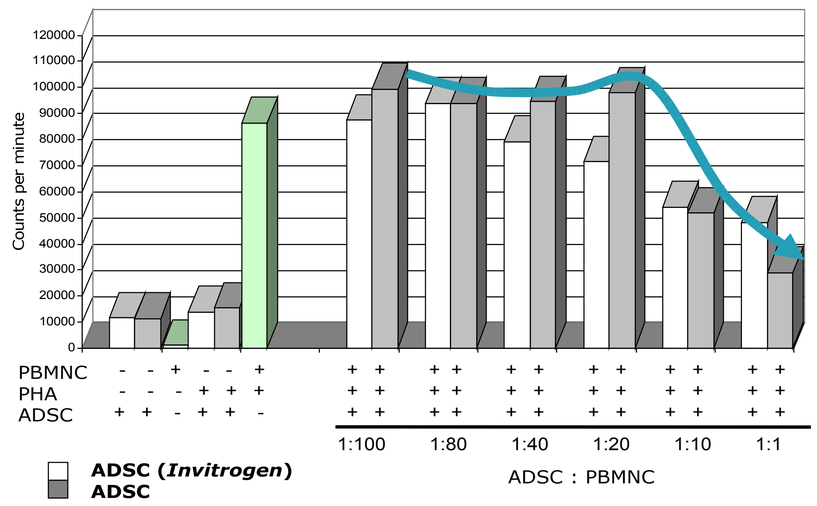

Figure 6. The suppressive effect of ADSC on the proliferation of PHA-stimulated PBMNC compared to control ADSC (*Invitrogen*). PBMNC alone served as a negative control and PHA stimulated PBMNC were used as a positive control. Data represents the average of three independent experiment sets.

Conclusions

- ADSC can be effectively cultured and expanded in the presence of AS. Such culture conditions do not influence the expression of characteristic MSC markers and ability to differentiate into adipocytes, osteoblasts and chondroblasts.
- ADSC suppress the proliferation of PBMNC in a dose dependent manner reaching the highest at ratio 1:1 (ADSC:PBMNC).
- At ratio 1:1 (ADSC:PBMNC) ADSC suppression of PBMNC proliferation is 1,7 times higher when compared to the control ADSC (*Invitrogen*).

References

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Acknowledgments

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