Regenerative and immunomodulatory potential of mesenchymal stem cells

Ph.D. Thesis

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1. INTRODUCTION

Tissue stem cells isolated from the bone marrow hold a great promise for the regenerative medicine. Hematopoietic stem cells (HSCs) are not only able to sustain hematopoiesis, but, under certain circumstances, can also contribute to the regeneration of other tissues both under *in vitro* and *in vivo* conditions. Likewise, aside from constantly renewing bone marrow stroma and providing a niche for HSCs, mesenchymal stem cells (MSCs) can also (trans)differentiate into non-mesenchymal cell types, as well as ameliorate diseases caused by cell degradation. The underlying mechanism is quite complex, but the remarkable immunological properties of MSCs seem to be a very important component. Mesenchymal stem cells evoke only minimal immune response when applied in either allogeneic or xenogeneic settings, and they also show a strong immunosuppressive effect.

In the first part of our work, we examined the effect of gal-1, an endogenous lectin with strong immunosuppressive and anti-inflammatory properties, on Cy/G-CSF-induced mobilization of hematopoietic stem- and progenitor cells.

Many attempts have been made to use stem cell therapy for the treatment of type I. diabetes, with controversial results. Thus, in the second part of our work, we developed our own, combined method of treatment, which could permanently cure diabetic mice, and we also attempted to reveal the underlying mechanism.

Sufficient hematopoiesis is only possible with the regulation of the stem cell niche, provided by mesenchymal stromal cells. Therefore, we studied two diseases (myelodysplastic syndrome – MDS, and myeloma multiplex – MM) which affect the hematopoietic system, in order to find out, whether the symptoms are caused by the defective hematopoietic clone alone, or an alteration of the stromal cells might also contribute to the onset of the disease?

2. MATERIALS AND METHODS

In our gal-1 experiments we used Cy/G-CSF-induced mobilization as a model. In order to assess the *in vivo* repopulating ability of the cells isolated from the blood of the treated animals, we transplanted them into lethally irradiated recipients. Number of hematopoietic progenitors in the blood and bone marrow samples was determined in a colony forming assay. Surface markers on the nucleated BM cells were measured with flow cytometry. Lymphocytes and granulocytes in the blood samples were counted after Giemsa staining.

Type I diabetes was induced by STZ treatment. Sublethally irradiated female C57Bl/6 recipients were transplanted with BMCs freshly isolated from male animals, and mesenchymal stem cells of syngeneic or allogeneic origin.

The samples used for the stem cell niche experiments were obtained from untreated MDS and MM patients, or healthy donors. Number of progenitors was determined in a semi-solid colony forming cell-assay. The stomal ability to support hematopoiesis was examined in a cobblestone area forming cell (CAFC) culture setting. For the MSC plasticity experiments, culture media supporting osteogeneic and adipogeneic differentiation were prepared according to the method of Pittenger's group.

3. RESULTS

Gal-1 strongly inhibited Cy/C-CSF-induced mobilization of the HSPCs, and decreased peripheral monocytosis and neutrophilia in a dose- and time-dependant manner. However, gal-1 treatment also caused an elevation in the number of hematopoietic progenitor cells in the bone marrow. The inhibitory effect of the lectin occured in the second half of the mobilization. Gal-1 also inhibited SDF-1-induced transendothelial migration of the BM cells, while having no effect on their chemotactic ability itself.

Rapid normalization of the blood sugar and serum insulin levels, and regeneration of the pancreatic islets could be achieved in diabetic mice by the co-transplantation of 10^6 BMCs and 10^5 MSCs. Neither MSC, nor BMC treatment was effective alone. Regeneration was not due to transdifferentiation of the transplanted cells, since no donor-derived β-cells were found in the recovered animals. It is more likely, that the transplanted cells induced endogeneous regeneration of the recipient's pancreas. We also proved, that after the MSC trasplantation no β-cell-specific, autoreactive T-cell clones can be detected in the pancreas of the treated animals.

The stromal cells of MDS patients show a decreased myelosupportive ability and plasticity in comparision to the MSCs of healthy donors. In MM patients however, no changes in the stroma could be observed.

4. CONCLUSIONS

Effect of gal-1 is based on the inhibition of transendothelial migration, with no effect on their viability and chemotactic ability. This might contribute to the strong anti-inflammatory effects of the protein under *in vivo* conditions.

Our findings suggest, that the co-transplanted stem cells cooperate in the treatment of STZ-induced diabetes: BMCs and MSCs induce the endogenous regeneration of the recipient's pancreas, while MSCs inhibit T-cell mediated immune response against newly formed β-cells, thus providing the possibility of total recovery. Thus, our therapy might be applied successfully in humans, either in diabetes, or other diseases with a cell degradation of autoimmune origin.

Finally, our conclusion is that MDS – unlike MM - is caused not only by the neoplastic transformation of a hematopoietic cell clone, but also an alteration of the stromal cells contributes to the disease. These alterations in the MSCs are consistent with those that are observed during the aging of stromal cells.

5. LIST OF PUBLICATIONS CONNECTED WITH THIS DISSERTATION

1. Judit **Kiss**, Aliz Kunstár, Roberta Fajka-Boja, Valéria Dudics, József Tóvári, Ádám Légrádi, Éva Monostori and Ferenc Uher (2007) *Function of Human Galectin-1: Inhibition of Hematopoietic Progenitor cell mobilization.* Experimental Hematology, 35: 305-313 (IF=3,15)

2. Veronika S. Urbán, Judit **Kiss**, János Kovács, Elen Gócza, Virág Vas, Éva Monostori, Ferenc Uher (2007) *Mesenchymal Stem Cells Cooperate with Bone Marrow Cells in Therapy of Diabetes.* Stem Cells, 26: 244-253. (IF=7,74)

3. Gergely Varga, Judit **Kiss**, Judit Várkonyi, Virág Vas, Péter Farkas, Katalin Pálóczi and Ferenc Uher (2007) *Inappropriate Notch Activity and Limited Mesenchymal Stem Cell Plasticity in the Bone Marrow of Patients with Myelodysplastic Syndromes.* Pathology Oncology Research, 13: 311-319. IF=1,27

6. FURTHER PUBLICATIONS

1. Zsuzsanna Kertész, Virág Vas, Judit **Kiss**, Veronika S. Urbán, Éva Pozsonyi, András Kozma, Katalin Pálóczi, Ferenc Uher. (2006) *In vitro expansion of long-term repopulating hematopoetic stem cells in the presence of immobilized Jagged-1 and early acting cytokines*. Cell biology International, 30: 401-405. (IF=1,36)

2. Kiss Judit, Urbán S. Veronika, Dudics Valéria, VasVirág, Uher Ferenc (2008) *A mesenchymális őssejtek és az immunrendszer – immunszuppresszió gyógyszerek nélkül?* Orvosi Hetilap, 149: 339-346.

3. Urbán S. Veronika, **Kiss** Judit, Vas Virág, Kovács János, Uher Ferenc. (2006) *A diabetes mellitus őssejtterápiája: eredmények, lehetőségek,és kérdőjelek.* Orvosi Hetilap, 147:791-797.

4. Beáta Hegyi, Bernadett Sági, János Kovács, Judit **Kiss**, Veronika S. Urbán, Gabriella Mészáros, Éva Monostori, Ferenc Uher (2010)
Identical, similar or different? learning about immunomodulatory function of mesenchymal stem cells isolated from various mouse tissues: bone marrow, spleen, thymus and aorta wall
International Immunology, in press (IF=3,18)