MTA Doctoral Thesis

Summary

BONE MARROW DERIVED STEM CELLS IN HEALTH AND DISEASE

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I. INTRODUCTION

This thesis summarizes my work of the last 15 years studying the role of bone marrow derived cells in the maintainance of a healthy environment in the body. My interest stemmed from the observation that circulating cells are able to enter the brain. The first experiments we performed confirmed the presence of blood derived microglia and astrocytes in the rat brain. Consequently, using transgenic and molecular biolgy techniques we have shown that cells from gender-mismatched transplanted bone marrow make their way into the brain and differentiate into all neural linages. These results were confirmed by independent goups before we also demonstrated the same phenomenon in humans. In addition to the nervous system, we have studied and showed that bone marrow cells from donor marrow contribute to the oral mucosa and salivary glands in humans. While circulating hematopoetic cells can contribute to tissue regeneration, we also started to examine if bone marrow derived - non hematopoetic - cells might participate in regulating the immune system. These studies lead to results showing that bone marrow stromal cells (also called mesenchymal stem cells) have the ability to recognize immune inbalance and are capable of correcting the problem. Based on our and other groups studies a variety of clinical trials are underway to test if we can exploit this feature of MSCs in patient therapy.

Our studies and results:

I. Contribution of circulating cells to tissue regeneration

A. CNS

B. Rodents

Cells that circulate in the bloodstream can gain access to the brain in rodents. In animals without brain injury these cells are evenly distributed and can become neural cells including microglia, macroglia, endothelial cells and neurons.

When a brain injury (stroke) occurs, the number of circulating cells entering the brain seems to increase. Many of these cells become vascular endothelial cells and form new vessels. The neovascularization facilitates healing, decreasing the necrotized volume and increasing the number of surviving cells.

C. Humans

In samples of postmortem human brains we determined the presence of donor derived cells in the CNS following gender mismatched bone marrow transplants. As in rodents, we found that cells from the donor bone marrow entered the brains and differentiated into neural cells there. We found the greatest numbers of these cells in the youngest patient (2 years old) who also survived the longest following BM transplantation. Although we can not do functional tests in postmortem human samples, based on morphology and multiple neuronal markers, the donor derived cells were undistinguishable from the host neurons. Another important observation is that statistical analysis of the donor derived cells suggest that they are clonal, i.e. a cell from the circulation seeds the brain and multiplies and differentiates there.

B. Epithelial tissues

1. Human Cheek Cells

Using a similar approach to the brain studies, we found that donor derived blood cells contribute to the population of cheek epithelium. Due to the unique patient population we were also able to establish the fact that in two patients the new cheek cells derived from hematological progenitors, since these patients did not receive BM, but isolated hematological progenitors from peripheral blood. We also examined the possibility that Y chromosome containing cheek cells might derive from an earlier pregnancy with a male fetus. DNA analysis conclusively showed that the Y chromosome in the cheek cells was from the donor vs the male offspring of the recipient.

B. Mouse Uterine Epithelium

Based on the human cheek cell data we used a genetic tool to see if hematopoetic progenitors can indeed seed epithelial tissues. Using genetic marking of hematopoetic progenitors we have demonstrated that these cells get into the endometrium and participate in replenishing lost cells during the cycle. In some mice 70% of the epithelium was of hematopoetic origin following a pregnancy, which is known to inclrease the uterine surface area by 20 fold.

Significance of the above findings: ***the demonstration that circulating cells cross the blood-brain suggests that these cells might be used as vehicles to introduce growth factors/differentiation factors or enzymes into the CNS. The enzymes might correct or slow the progression of genetic neurological diseases. The finding that GCSF/SCF treatment significantly enhances neovascularization indicates that this treatment may be beneficial in patients with stroke.***

II. Immunoregulatory effect of BMSCs

A. Septic conditions

We analyzed the role that BMSCs play in regulating immune function in sepsis. Our results demonstrated that iv injected BMSCs are entrapped in the lungs where they communicate with monocytes/macro-phages that surround them. In the septic environment, these monocytes/macrophages are proinflammatory and make TNF-a. When they come in contact with BMSCs they change character and becomy anti-inflammatory. This tunes down the immune attack on body organs and allows animals to survive the septic process.

The crosstalk between BMSCs and the monocytes/macrophages involves the production and release of PDG2 by the BMSCs and a resulting increase of the monocytes/macrophages IL-10 production. The final outcome is a decreased number of neutrophils in body organs and less oxidative damage. On the other hand, there are more circulating neutrophils, and consequently more efficient clearence of bacteria.

B. Th2 dominant (allergic) environment

Using a ragweed induced allergy mouse model we determined that BMSCs are able to "sense" the allergic environment (increased levels of IL-4 /IL-13) and respond by producing large amounts of TGF-β that (either alone or by recruiting regulatory T cells) will ultimately lead to a decrease of lung eosinophil infiltration, and the allergy-specific cytokine and Ig production.

C. Histamine rich environment

As a continuation of the above studies, since mast cells (MCs) play a significant role in humans in allergic settings, we examined how BMSCs interact with MCs and how they respond to histamine, which is the major MC mediator. We demonstrated that BMSCs have the ability to affect the biology of MCs by limiting their activation and migration. Our data additionally show that these effects are mediated through the EP4 receptor on MCs.

Significance of the above findings:

Our results in the sepsis model indicate that BMSCs might be a very effective therapeutic intervention in human sepsis – when no other treatment option is available. Live cells may be superior to drugs, because the cells respond to their environment while drugs can only play the role that they were designed for. Since a large number of people die of sepsis every year (over 250000 in the US alone), a new therapy is badly needed.

The second set of studies indicate that in steroid resistant asthmatic conditions the use of BMSCs should be considered, since they are able to mitigate the allergic response and to counter the effects of MC degranulation.

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