BACKGROUND

The growth and differentiation of haemopoietic cells in the bone marrow (BM) is regulated by complex interactions between the cells and their surrounding microenvironment alternatively called haemopoiesis supporting stroma. Anatomically, the BM microenvironment is a tridimensional structure consisting of cells, vessels, extracellular matrix (ECM) proteins such as adhesive glycoproteins and structural proteoglycans, extracellular fibres such as collagen and elastin and cytokines such as growth factors and inhibitors of haemopoiesis. In this tridimensional structure and especially, in highly organized compartments known as “niches”, complex cell-cell, cell-ECM, cell-cytokine interactions regulate the fate of haemopoietic stem cells in terms of self-renewal, differentiation, proliferation or apoptosis. We currently know that the majority of the haemopoiesis supporting stromal cells derive from a distinct progenitor, different of the haemopoietic stem cell, the mesenchymal stem cell (MSC). This cell displays a high degree of “plasticity” it terms of the potential to generate cells of varying lineages such as chondrocytes, osteoblasts, adipocytes and muscle cells. This cell is a novel therapeutic tool in degenerative medicine. And while the MSCs display immunosuppressive properties, periphery-derived, inappropriately activated immune cells such as monocytes and lymphocytes may settle in the BM microenvironment and may damage the growth and survival of haemopoietic cells in the niches through cell-to-cell contact interaction or through production of inhibitory cytokines. Such intricate interactions between the immune and haemopoietic system may result in marrow failure affecting one or more haemopoietic lineages and may even accelerate the process of clonal disorders of haemopoiesis. On the basis of this knowledge, current therapeutic approaches for clonal haemopoietic diseases target elements of the BM microenvironment rather than the clonal cells per se. Overall, the haemopoietic and the immune systems are mutually related.

RESEARCH ACTIVITIES

The Haemopoiesis Research Laboratory of the Medical School of Crete is linked to the Haematology Department of the University Hospital of Heraklion. The researchers and students work under the direct supervision of Dr Helen Papadaki who shares the time between the research and clinical arms of Haematology and also delivers a regular series of core lectures in Basic Immunology, Haematology and Pathophysiology. The Translation Research performed in Dr Papadaki’s Laboratory is focused on the biologic characteristics of the haemopoietic stem cells and MSCs and the role of the BM microenvironment in immune-mediated and clonal BM failure syndromes using highly sophisticated culture assays, molecular techniques, flow cytometry, cytogenetics, Fluorescence In Situ Hybridization (FISH) and immunohistochemistry.

1. Biology of Mesenchymal Stem Cells

From bench to bedside BM: MSCs are being considered as potential therapeutic agents in various inflammatory autoimmune diseases for their tissue-repair and anti-inflammatory tissue-protective properties. In this context, during the last two years we have investigated the reserves and function, the molecular and proteomic profile and the differentiation potential of BM MSCs in patients with rheumatoid arthritis using flow cytometry, apoptosis and proliferation studies, colony assays for cell differentiation towards the osteogenic, chondrogenic and adipogenic lineages and molecular and cytochemical techniques for cell identification. In col-
laboration with Reference Laboratories we have performed a proteomic and transcriptome analysis for identification of differences between patients and normal subjects.

Immune-mediated BM failure syndromes: MSCs normally display immune-suppressive properties. In immune-mediated BM failure syndromes such as hypoplastic Myelodysplastic Syndromes and hypoplastic neutropenías, MSCs may have a role in the pathophysiologic process of disease development by displaying defective immune-regulatory properties, by producing abnormal cytokines or even by participating in the abnormal clone. T-lymphocyte proliferation assays in the presence of MSCs from healthy subjects and patients with BM failure, evaluation of cytokines levels in MSC culture supernatants, study of the mean telomere length in normal and disease states, and comparative cytogenetic evaluation of haemopoietic and MSCs in patients with Myelodysplastic syndromes are projects under investigation.

2. Biology of Haemopoietic Stem Cells

Mechanisms regulating the survival/apoptotic characteristics of BM haemopoietic cells in early and late stages of differentiation in normal and disease states are under investigation with particular interest in the role of TNF/TNF-Receptor family members such as FasL/Fas and CD40L/CD40. In this context, we have defined the pathophysiologic basis of a previously “orphan” disease, the chronic idiopathic neutropenia by demonstrating increased Fas mediat-ed, TNF-induced apoptosis of the neutrophil early progenitor cells in the BM. We have also proposed as a new mechanism in the pathophysiology of anaemia of chronic inflammation, also known as anaemia of chronic disease, the TNF--mediated increased apoptosis of the BM ery-throid progenitor/precursor cells through downregulation of erythropoietin receptor. Translating these data into the clinic we have showed the beneficial effect of the in vivo anti-TNF- therapy in treating anaemia in patients with chronic inflammation and we have also investigated the effect of this treatment in patients with Myelodysplastic syndromes and increased levels of TNF- in the BM. The effect of the mutual interactions between TNF/TNF-Receptor family members in the reserves of BM stem cells in patients with autoimmune disor-ders undergoing stem cell transplantation is also an interesting field of investigation.

3. Interactions between Haemopoietic Stem Cells-BM microenvironment, Chronic Idiopathic Neutropenia: The pathophysiology of this disease remains our main research interest. An inflammatory BM milieu has been shown to mainly contribute to the pathophysiology of the disease. Activated T-lymphocytes with myelosuppressive properties and pro-apoptotic mediators such as IFNγ, TNF-, FasL, and TGFβ,1 result in accelerated apoptosis of the granulocytic progenitor cells. The decreased levels of the anti-inflammatory cytokine IL-
10 disturb further the balance between the survival and pro-apoptotic mediators. The role of viruses, the implication of specific TLR expression, investigation for polymorphisms in inflammatory cytokine genes and analysis of the CDR region of the TCR in association with specific HLA background, are projects under investigation.

**Translational Research in Myelodysplastic syndromes:** Myelodysplastic Syndromes comprise a heterogeneous group of haematological malignancies characterized by the paradox of BM hyperplasia with blood cytopenias due to abnormal, clonal proliferation of a pluripotent haemopoietic stem cell. Recent evidence suggests that an abnormal BM microenvironment may be involved in the pathogenesis of the disease through inflammatory cytokine production, abnormal angiogenesis and increased level of apoptosis. The effect of the treatment with anticytokine and immune-modulating agents by combining clinical data and research data from the in vitro BM studies is of particular interest in our laboratory.

**REPRESENTATIVE PUBLICATIONS**


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