PREFACE

Stem Cells are primal undifferentiated cells that retain the ability to divide and differentiate into other cell types. In higher plants, this function is the defining property of the meristematic cells. Stem cells have the ability to act as a repair system for the body, because they can divide and differentiate, replenishing other cells as long as the host organism is alive. Medical researchers believe stem cell research has the potential to change the face of human disease by being used to repair specific tissues or to grow organs. This book presents related research in this field. Topics include, circulating platelets, hematopoietic progenitor cells, bone defects and fractures, adult neural stem cells, human embryonic stem cells, therapeutic use of cells, cognitive research, hemopoietic stem cells and tissue regeneration.

Chapter 1 - Megakaryocytopoiesis involves the commitment of hematopoietic stem cells, proliferation and differentiation of the megakaryocytic progenitors, and cell maturation including endoreduplication and cytoplasmic acquisition of structural and functional properties of platelets. Megakaryocytopoiesis is primarily controlled by thrombopoietin (TPO), which induces the expansion of early progenitors, as well as, the proliferation, endoreduplication and differentiation of megakaryocytes (MKs). Thrombopoietin avidly binds to the c-Mpl receptors on platelets and MKs, and the circulating levels of unbound TPO induce concentration-dependent proliferation and maturation of MK progenitors. Accordingly, decrease in platelet turnover rate results in increased concentration of unbound TPO, enabling the compensatory response of marrow MKs to increased demand for peripheral blood platelets. The process of megakaryocytopoiesis is also mediated by multiple pleiotropic hematopoietic growth factors which include the stem cell factor - c-kit ligand, interleukin-3 (IL-3), IL-6, IL-11, granulocyte-macrophage colony-stimulating factor (GM-CSF) and FGF, as well as chemokines such as SDF-1 with its receptor

CXCR-4. The stimulatory effect of the regulating factors is mediated by a concerted action of specific transcription factors such as GATA-1 and FOG, Runx1, Ets and Nuclear Factor-Erythroid-2, while C-myb plays a central negative regulatory role. The demise of senescent MKs involves a specialized form of apoptosis during the processes of proplatelet formation and platelet release. In this review, the regulation of megakaryocytopoiesis with the quantitative alterations in the megakaryocytic cells and platelet production, in both humans and animal models, is discussed.

Chapter 2 - Objective: We sought to develop a culture system in which the interactions between human hematopoietic progenitor cells (HPCs) and bone marrow stromal cells could be studied *in vitro* in the absence of exogenously added serum and/or hematopoietic cytokines.Methods: Human bone marrow (BM) hematopoietic and stromal cells were cultured *in vitro* in a serum-free, cytokine-free system. Flow cytometric and hematopoietic assays were employed to assess the maintenance of human HPCs as a function of time and culture conditions. Results: In the absence of serum, HPCs and stromal cells were observed to form clusters (microaggregates, or MAGs). These MAGs appear to promote interactions important for the maintenance and maturation of CD34+ as well as more primitive CD34+CD38- multilineage HPCs, possibly upon provision of hematopoietic cytokines by stromal cells and/or by cell-cell interactions between BM stroma and hematopoietic cells. Additionally, the behavior of HPCs in the MAGs mirrors that observed in more traditional (e.g., serum replete) cultures. Thus, predictable hematosuppressive effects were seen in response to treatment of MAG cultures with IFNα and azidothymidine. Reciprocally, the cytokines thrombopoietin and erythropoietin enhanced the survival of CD34+CD38- HPCs and erythroid cells, respectively. Conclusions: The MAG culture system provides an *in vitro* system in which human HPCs and their progeny can be evaluated in the absence of exogenously added serum and/or hematopoietic cytokines.

Chapter 3 - In the emerging age of cellular therapy, bone marrow stromal stem cells (BMSSC) represent a population of cells with enormous therapeutic potential. Whilst in their relative infancy, numerous studies suggest that BMSSC are capable of restoring cardiac function, neural regeneration, defects of articular cartilage, improving hematopoietic engraftment and most promisingly, the repair of large osseous defects. Segmental defects of bone, or non-union fractures represent a significant and intractable clinical problem, in which the volume of bone loss results in delayed healing or non-union. Currently there are several therapies employed to facilitate osseous repair, ranging from the use of autograft bone, allograft bone and an array of artificial bone substitutes. However, all these

approaches are often associated with various limitations making it difficult to predict outcome. Major problems include secondary site morbidity associated with autologous bone, availability and suitability of allogeneic bone, and the variable osteo-conductivity of different biomaterials. The ability to generate osteogenic progenitor cells derived from culture expanded BMSSC has provided, for the first time, the potential to overcome many of these limitations. When used in conjunction with existing osteo-conductive bone void fillers, BMMSC may not only facilitate the repair of large segemental defects following disease or trauma, but may also provide a cellular therapy for spinal fusion and craniofacial reconstructive surgery. The following review outlines the possible applications and highlights the potential limitations of BMSSC for developing novel tissue engineering approaches for orthopaedic applications.

Chapter 4 - Considerable efforts and means have been invested to find treatments for neurological diseases and injuries, yet there is still no cure for these ailments and new alternatives for therapy must be explored. Because they generate the main phenotypes of the central nervous system (CNS), neural stem cells (NSCs) hold the promise to cure a broad range of CNS diseases and injuries. With the confirmation that neurogenesis occurs in the adult brain, and the recent isolation and characterization *in vitro* of neural progenitor and stem cells from the adult CNS, new avenues for the treatment of neurological diseases and injuries are being considered. Cell therapeutic interventions may involve both *in vivo* stimulation and transplantation of neural progenitor and stem cells of the adult brain.

Chapter 5 - Embryonic stem cells (ESC) have become attractive for prospective cell therapy of degenerative diseases. Both mouse and human embryonic stem cell lines provide also a powerful biological system to study the early stages of mouse and human development. More specifically, the heart is the first functional organ to develop in the embryo. Cardiac congenital and genetic diseases are the most frequent inborn defects in the foetus. Mouse ES cells recapitulate the genetic programme of the first stages of cardiac cell differentiation. They can give rise to all cardiac phenotypes. They thus represent a precious cell model to better comprehend these diseases. The early stages of development of the primate embryo share many similarities with mouse embryogenesis. However, differences also exist including the temporal and spatial patterns of gene expression. Little is known about the cardiogenic potential of human ES cells. This overview discusses the true potential of cardiogenesis of both mouse and human ES cells and proposes future lines of research within the prospective of developmental biology and therapeutic use.

Chapter 6 - Thymus is the major site where hemopoietic stem cell progenitors develop into functionally mature T cells, which reconstitute the peripheral lymphocyte pool. Repopulation of the thymus by stem cells begins in the embryonic life and continues through adolescence. In the thymus, tolerance to self is established by elimination of autoreactive T lymphocytes expressing T cell receptors (TCR) with high affinity to self-peptides, the process known as negative selection, and by generation of regulatory T cells. Breakdown in tolerance toward self-antigens, together with complex genetic and environmental factors may lead to autoimmunity. In recent years, there have been advances in the understanding of molecular and cellular pathways controlling negative selection in the thymus and in the periphery, including the discovery of the genes mediating protection from autoimmune responses. In this review, we discuss the structure and function of the thymus as relates to the mechanisms of thymocyte development, selection and egress from the thymus, as well as the factors that mediate susceptibility to autoimmunity or the induction of tolerance. Unraveling these mechanisms in animal models may further our understanding of human autoimmune diseases, ultimately leading to the development of safer treatment regiments.

Chapter 7 - For centuries oxygen has been thought to be the key to all life. Without it, eukaryotic organisms including cells cannot survive. Oxidative stress, reactive oxygen and antioxidants have become household terms, yet it has only been recently that researchers have started to unravel the essential complex regulatory role oxygen plays in stem cell maintenance, proliferation, migration, and engraftment for tissue regeneration. Both adult and embryonic stem cells have now been shown to be housed in "niches" which possess 1-5% oxygen levels, considerably lower than 21% ambient oxygen. The term hypoxia is used to describe these lower oxygen levels but recent critics suggest this may be more accurately described as normoxia. Much evidence has come to light that cells possess several key factors that function as oxygen sensors and that oxygen status within target tissues may play a key role in stem cell engraftment and differentiation during tissue repair. One of the key factors involved is hypoxia inducible factor (HIF). This factor can both enhance cellular differentiation, as well as rapidly change local oxygen levels through stimulation of angiogenesis and reduction of cellular oxygen requirements, by switching the cell from aerobic to anaerobic respiration. Thus during tissue injury, hypoxic areas within the damaged region can induce expression of factors essential for the recruitment and engraftment of stem cells for regeneration. Further, stem cells when exposed to higher oxygen in the blood stream during mobilization, switch from anaerobic respiration in their hypoxic niche, to aerobic metabolism during proliferation. During this period of high energy production and cellular proliferation, reactive

oxygen is generated, which in concert with other factors alters gene expression towards terminal differentiation. Thus oxygen tension may be an essential component for the regeneration of tissue.