Stem Cell Biology and Regenerative Medicine

David S. Allan
Dirk Strunk *Editors*

Regenerative Therapy Using Blood-Derived Stem Cells

Humana Press

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Series Editor
Kursad Turksen, Ph.D.
kturksen@ohri.ca

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David S. Allan • Dirk Strunk
Editors

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Editors
David S. Allan
Ottawa Hospital Research Institute
University of Ottawa
ON, Canada
daallan@ohri.ca

Dirk Strunk
Center for Medical Research (ZMF)
Medical University of Graz
Graz, Austria
dirk.strunk@medunigraz.at

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Preface

Blood has long been viewed as a conduit for therapy, stemming from the ancient days of phlebotomy to remove evil humors to the development of successful blood transfusions to replace missing blood components. The identification and characterization of hematopoietic stem cells by Drs. Till and McCulloch revolutionized the field and soon after, non-hematopoietic stem and progenitor cells were characterized from the blood and bone marrow. Some of these cell types and various blood-derived cell lineages are involved in the repair of various types of tissue damage that span the spectrum of medical disorders. The goal of this book is to provide an up-to-date review of the various types of blood-derived cells with regenerative capacity, identify opportunities for intervention by examining specific clinical applications, and recognize the regulatory environment that will encompass future therapies in regenerative medicine.

Through the contributors to this volume, we have succeeded in providing insight on numerous blood-derived cell types, including endothelial progenitors, mesenchymal stromal/stem cells, umbilical cord blood-derived undifferentiated somatic stem cells and others. Further, the concept of using umbilical cord blood is discussed throughout the book and several authors describe the current status of regenerative therapy for cardiac disease and neurological disorders. Technical and conceptual issues such as ex vivo expansion and the generation of induced pluripotent stem cells are covered and regulatory insight from various jurisdictions provides a degree of clinical relevance that may shape the immediate future of regenerative medicine.

We wish to thank the many contributors for their tremendous commitment and their precious time in preparing the insightful chapters that comprise this book. Some are long-time friends and contacts while others are new and welcome collaborators. All the contributors are dedicated to advancing our collective knowledge regarding the field of regenerative therapy. The cooperation and contributions from our colleagues and fellow authors has been inspirational. The guidance and support from the series editor, Dr. Kursad Turksen has been most valuable and the staff at Springer has been especially helpful in making this project a reality. In particular, we are indebted to the administrative assistance and invaluable editing performed by Monica Farrell and Stéphanie Rochette.

We hope this book will stimulate enquiring minds and future investigation in this exciting and evolving field of research. The community of dedicated researchers and health care providers will need to engage at all levels to continue the push towards viable treatments that improve the lives of patients around the globe.

Ottawa, Canada — David S. Allan
Graz, Austria — Dirk Strunk

Contents

Contributors

Francisca R. Agbanyo Biologics and Genetic Therapies Directorate, Health Products and Food Branch, Ottawa, ON, Canada

David S. Allan Regenerative Medicine Program, Ottawa Hospital Research Institute, Blood and Marrow Transplant Program, The Ottawa Hospital, Ottawa, ON, Canada

Departments of Medicine and Biochemistry, Microbiology & Immunology, University of Ottawa, Ottawa, ON, Canada

Helen Baldomero Department of Hematology, University of Basel, Basel, Switzerland

Gillian I. Bell Department of Physiology & Pharmacology, The University of Western Ontario, Vascular Biology Group, Krembil Centre for Stem Cell Biology, Robarts Research Institute, London, ON, Canada

Yuan Chung Regenerative Medicine Program, Ottawa Hospital Research Institute, Ottawa, ON, Canada

Jan Cornelissen Department of Hematology, Erasmus University Medical Center, Rotterdam, The Netherlands

Daniel L. Coutu Division of Experimental Medicine, McGill University Lady Davis Institute for Medical Research, Montreal, QC, Canada

Nathalie Etchart Stem Cell Research Unit, Department of Hematology and Stem Cell Transplantation, University Clinic of Blood Group Serology and Transfusion Medicine, and Medical University of Graz, Graz, Austria

Moïra François Division of Experimental Medicine, McGill University Lady Davis Institute for Medical Research, Montreal, QC, Canada

Peter R. Ganz Biologics and Genetic Therapies Directorate, Health Products and Food Branch, Health Canada, Ottawa, ON, Canada

Jacques Galipeau Department of Hematology & Medical Oncology and Department of Pediatrics, Emory University, Atlanta, GA, USA

Liz Anne Gillham-Eisen Biologics and Genetic Therapies Directorate, Health Products and Food Branch, Health Canada, Ottawa, ON, Canada

Reinhard Henschler Institute of Transfusion Medicine and Immune Hematology, German Red Cross Blood Center, Frankfurt, Germany

David A. Hess Department of Physiology & Pharmacology, The University of Western Ontario, Vascular Biology Group, Krembil Centre for Stem Cell Biology, Robarts Research Institute, London, ON, Canada

Benjamin Hibbert Vascular Biology Laboratory, Division of Cardiology, Department of Biochemistry, University of Ottawa Heart Institute, Ottawa, ON, Canada

David A. Ingram Department of Pediatrics, Herman B Wells Center for Pediatric Research, Indiana University School of Medicine, Indianapolis, IN, USA

Armand Keating Cell Therapy Program, Princess Margaret Hospital, University of Toronto, Toronto, Canada

Division of Hematology, Department of Medicine, University of Toronto, Toronto, Canada

Jennifer Klowak Regenerative Medicine Program, Ottawa Hospital Research Institute, Ottawa, ON, Canada

Gesine Kögler Institute for Transplantation Diagnostics and Cell Therapeutics, University of Duesseldorf Medical School, Duesseldorf, Germany

Charles Krieger Division of Neurology, Department of Medicine, Neuromuscular Disease Unit, VHHSC, Vancouver, BC, Canada

Laura Labonté Regenerative Medicine Program, Ottawa Hospital Research Institute, Blood and Marrow Transplant Program, The Ottawa Hospital, Ottawa, ON, Canada

Departments of Medicine and Biochemistry, Microbiology & Immunology, University of Ottawa, Ottawa, ON, Canada

Katarina Le Blanc Karolinska Institute, Division of Clinical Immunology, Karolinska University, Stockholm, Sweden

Coral-Ann B. Lewis Department of Biomedical Physiology and Kinesiology, Simon Fraser University, Burnaby, BC, Canada

Ivan Martin Biomedicine and Surgery, University of Basel, Basel, Switzerland

Ulrich Martin Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), Department of Cardiac, Thoracic-, Transplantation and Vascular Surgery, REBIRTH-Cluster of Excellence, Hannover Medical School, Hannover, Germany

Julie Mund Department of Pediatrics, Herman B Wells Center for Pediatric Research, Indiana University School of Medicine, Indianapolis, IN, USA

Dietger Niederwieser Division of Hematology and Medical Oncology, University of Leipzig, Leipzig, Germany

Giani Oancea Institute of Transfusion Medicine and Immune Hematology, German Red Cross Blood Center, Frankfurt, Germany

Edward R. O'Brien Vascular Biology Laboratory, Division of Cardiology, Department of Biochemistry, University of Ottawa Heart Institute, Ottawa, ON, Canada

Jakob Passweg Department of Hematology, University of Basel, Basel, Switzerland

David M. Putman Department of Physiology & Pharmacology, The University of Western Ontario, Vascular Biology Group, Krembil Centre for Stem Cell Biology, Robarts Research Institute, London, ON, Canada

Andreas Reinisch Stem Cell Research Unit and Department of Hematology and Stem Cell Transplantation, Medical University of Graz, Graz, Austria

Eva Rohde Stem Cell Research Unit, Medical University of Graz, Graz, Austria

University Clinic of Blood Group Serology and Transfusion Medicine, Paracelsus Medical University of Salzburg, Salzburg, Austria

Fabio M. Rossi Department of Medical Genetics, University of British Columbia, Vancouver, BC, Canada

Michael Rosu-Myles Biologics and Genetic Therapies Directorate, Health Products and Food Branch, Health Canada, Ottawa, ON, Canada

Katharina Schallmoser Stem Cell Research Unit and University Clinic of Blood Group Serology and Transfusion Medicine, Medical University of Graz, Graz, Austria

Trevor Simard Vascular Biology Laboratory, Division of Cardiology, Department of Biochemistry, University of Ottawa Heart Institute, Ottawa, ON, Canada

Dirk Strunk Stem Cell Research Unit and Department of Hematology and Stem Cell Transplantation, Medical University of Graz, Graz, Austria

Sowmya Viswanathan Cell Therapy Program, Princess Margaret Hospital, University Health Network, Toronto, ON, Canada

Beate Wagner Department of Transfusion Medicine and Hemostaseology, Clinics of the Ludwig Maximilians University Munich, Munich, Germany

Mervin C. Yoder Department of Pediatrics, Herman B Wells Center for Pediatric Research, Indiana University School of Medicine, Indianapolis, IN, USA

Chapter 1
Undertaking Regenerative Medicine Studies with Blood Stem Cells

Sowmya Viswanathan and Armand Keating

Abstract In this chapter, we provide a perspective on the advances achieved to date in regenerative medicine, identify some of the challenges confronting the field, and make specific recommendations aimed at hastening the translation of research to effective clinical practice. Regenerative medicine is well positioned to address many of the urgent unmet medical needs of the global community. The stakes are high, but success will come only from the collaboration and mindfulness of specialists from diverse fields and from the focused attention of funding agencies.

1.1 Cells, Secreted Factors, and Mechanisms of Repair

Stem cells have been used to treat a variety of malignant and nonmalignant hematological disorders since the first bone marrow transplantation in 1959 (Thomas et al. 1959). Interest in regenerative medicine, however, increased considerably after the identification of diverse populations of stem/progenitor cells from different tissues and was propelled further by promising results in animal models of injury and disease. Although numerous preclinical studies and early phase clinical trials have shown encouraging results, underlying mechanisms remain poorly understood. The discrepancy in efficacy among various cell sources, clinical trials, indications, and preclinical studies remains challenging and requires further investigation.

S. Viswanathan (✉)
Cell Therapy Program, Princess Margaret Hospital,
University Health Network, Toronto, ON, Canada
e-mail: sowmya.viswanathan@uhnresearch.ca

A. Keating
Division of Hematology, Department of Medicine,
University of Toronto, Toronto, ON, Canada

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The popular notion that cell therapy is synonymous with cell *replacement* therapy persists despite the lack of convincing evidence for the transdifferentiation of adult cells and limited evidence for prolonged donor cell engraftment or cell retention at sites of injury. In most cases, there is no correlation between improvements in functionality and cell dose, suggesting that beneficial effects may not arise solely from the local involvement of donor cells but may be due to other factors such as paracrine effects (Gnecchi et al. 2008). For example, conditioned medium from endothelial progenitor cells (EPCs) can ameliorate hind limb ischemia in rat models (Yang et al. 2010). Also, soluble factors from CD133+ bone marrow cells are neuroprotective in a murine model of brain ischemia (Bakondi et al. 2009). Thus, there is growing interest in the field to move from cell therapy to cell-free therapy using secretomes from stem/progenitor cells.

In some instances, cell replacement therapy is still needed. Embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs) can generate tissue-specific, differentiated cells to replace absent or injured cells. For example, human iPSCs can generate fully functional human platelets (Takayama et al. 2010) that in the future could provide a much needed alternative to costly volunteer donor platelets that can lead to complications such as sepsis (Kruskall 1997). iPSCs may also be useful in treating monogenic diseases by replacing cells harboring disease-causing mutations by gene targeting and correction technology as demonstrated in a mouse model of sickle cell anemia (Hanna et al. 2007).

In many cases, the therapeutic cell of interest may not be a stem cell at all. This is particularly evident in our evolving understanding of the therapeutic role of mesenchymal stromal cells (MSCs) from cells that engraft and give rise to differentiated cells (i.e., stem cells) to cells that secrete anti-inflammatory, antiapoptotic, angiogenic, antifibrotic, and immunomodulatory factors (Singer and Caplan 2011). The therapeutic effects of MSCs can often be reproduced by MSC-conditioned medium (Oh et al. 2008; Ma et al. 2006; Ye et al. 2006), which contains proteins secreted in response to injury signals such as TNF-α–stimulated gene/protein 6 (TSG-6) (Milner et al. 2006; Milner and Day 2003) able to directly promote corneal (Oh et al. 2010) and myocardial (Milner et al. 2006; Milner and Day 2003) tissue repair.

Another issue to consider is the heterogeneity of cell populations used for preclinical and clinical investigations. Bone marrow cells which contain a mixture of hematopoietic stem/progenitor cells, EPCs, and MSCs are most often used in clinical applications as they are easy to obtain and isolate. However, the variable clinical outcomes obtained with these heterogeneous cells are difficult to interpret. This is underscored by the results of several clinical trials with bone marrow–derived cells for treating cardiac diseases including acute myocardial infarction (AMI) and chronic ischemic heart disease (Martin-Rendon et al. 2008; Kang et al. 2008; Donndorf et al. 2011). Systematic and meta-analyses of several clinical trials show slight to modest improvements in hemodynamic parameters including left ventricular ejection fraction (LVEF) (2.99% in Martin-Rendon et al. 2008; 2.88% in Kang et al. 2008, 5.90% in Donndorf et al. 2011) without concomitant changes in short-term clinical events such as arrhythmias, rehospitalization for heart failure, or performance status.

It is unclear whether or not these modest improvements are the result of a functional heterogeneity in the treatment cell population. Might some cell subpopulations give better outcomes? There are some hints that this might indeed be the case. When a more homogeneous population of culture-expanded MSCs were used to treat 34 AMI patients, LVEF improved significantly more (from 49% ± 9% to 67 11%) (Chen et al. 2004) compared with the use of heterogeneous population of mononuclear bone marrow cells to treat AMI patients (LVEF increased from 48.3% ± 9.2% to 53.8% ± 10.29% over the same period) (Schachinger et al. 2006).

If a certain purified cell population is indeed better than a heterogeneous mixture of cells, there is yet no indication which cell type might be best suited for a given indication. A systematic and comparative approach with different cell populations is needed to address this issue. There are two approaches that can be taken clinically: prospective randomized clinical trials to compare different cell populations likely to be effective for a given indication and/or the development of suitable database registries to identify variables and prognostic factors that affect outcome for a given indication. In the cardiac field, both meta-analyses and clinical investigations have focused on specific subpopulations (umbilical cord blood cells, EPCs [CD133 and CD34 positive cells]) although most trials are performed with heterogeneous mononuclear cells from the bone marrow (Scacciatella et al. 2010).

1.2 Tracking the Cells That Facilitate Repair

A key question yet to be addressed is whether certain cell types can be mobilized endogenously to act on target tissues and organs or whether isolation, ex vivo purification, manipulation, and reinfusion (in the case of autologous therapies) are required. We also need to know the fate of the cells at the injury site and whether they are donor-derived or endogenously mobilized. Cells can be tracked with radioisotope imaging techniques including positron emission tomography (PET) and single photon emission computed tomography (SPECT), magnetic resonance (MR) imaging techniques, optical imaging (OI) and fluorescence, and bioluminescent imaging (BLI). PET and SPECT imaging provide immediate clinical applicability as they can capitalize on the use of FDA-approved labels including the radioisotope ^{111}In (Meller et al. 2004; Brand et al. 2004) and ^{18}FDG (Meier et al. 2008). However, there are limitations: images tend to be of low resolution and high cost, expose patients to radiation, and the tracers decay quickly, within hours for ^{18}GDF and days for ^{111}In. Other tracers with longer half-lives are available but have not been exploited for donor cell tracking (Oude Munnink et al. 2009). Alternatively, cells labeled with iron oxide nanoparticles including FDA-approved ferumoxides (for liver imaging) and ferumoxytol (for iron deficiency) may be tracked by MR (Frank et al. 2003; Wu et al. 2007) to yield relatively high-resolution images with longer persistence of the signal (2–4 weeks). Cells can also be tracked with optical imaging if they are labeled with fluorescent dyes such as the FDA-approved indocyanine green (ICG) (Sutton et al. 2008).

While human cell tracking has been studied predominantly in immunodeficient animal models, biodistribution, homing, and tissue retention in clinical scenarios are largely unknown. For biologics and drugs, pharmacokinetics (PK) studies provide information such as the area-under-the-curve (AUC), biologic or drug bioavailability, and clearance from the bloodstream after a single dose. In the case of cellular "pharmacokinetics," very few such studies have been undertaken. For example, while MSCs have been used clinically to support hematopoietic engraftment in multiple studies (Koc et al. 2000; Le Blanc et al. 2007; Ball et al. 2007), only two studies looked at cell retention and distribution in patients (Le Blanc et al. 2007; Ball et al. 2007). Lazarus at et al. showed that only 2 of 19 adult patients had detectable MSCs in their bone marrow, 6 to 18 months postinfusion (Lazarus et al. 2005). This would suggest that MSC localization may not be critical, at least in this particular application. MSCs have similarly been shown to have a multi-organ clinical response in reducing graft-versus-host disease (Le Blanc et al. 2008; Kebriaei et al. 2009), but again, due to the lack of cellular tracking studies, it remains unclear whether or not localization to specific tissues or lymph nodes is needed for a clinical response.

In other cases, such as treating central nervous system (CNS) diseases, localization may be more relevant; we need to know if cells or soluble factors secreted by them can reach affected areas. A clinical trial in Israel is using iron oxide–labeled MSCs that are intrathecally or intravenously injected into multiple sclerosis patients to obtain just this kind of biodistribution information (NCT00781872 at clinicaltrials.gov). Dendritic cells were similarly labeled with iron oxide particles and ^{111}In and tracked after intranodal administration in eight stage III melanoma patients scheduled for lymph node dissection, providing information on biodistribution to the lymph nodes and interaction with T cells (de Vries et al. 2005). Overall, however, there are very few clinical studies with labeled cells; the limited published studies reflect a gap in our understanding of clinical trafficking, and in the absence of this knowledge, clinical protocols cannot be designed to optimize cell dosing and schedule.

1.3 Building on the Experience in Hematopoietic Stem Cell Therapy

An iterative approach is important to optimize and refine clinical protocols and requires concomitant preclinical studies with appropriate animal models. Selecting the best animal models for safety and efficacy testing of therapeutic cells is critical to the development of new therapies. It is difficult to strike a balance among small, immune compromised rodent models that are convenient but less physiologically relevant, large animal models which take significantly longer to develop and validate and may need immunosuppressive agents, and bedside to bench clinical studies. An excellent illustrative example of this iterative approach is the concomitant clinical investigations and preclinical studies performed in appropriate lethally irradiated mice and canine models to understand bone marrow transplantation. Early

studies with murine models showed that murine leukemia could be treated by sublethal irradiation and marrow grafting (Barnes et al. 1956). This concept was successfully translated only to human leukemic patients who received grafts from an identical twin donor (Thomas et al. 1959); 200 patients who received allogeneic grafts in the 1950s and 1960s did not survive (Bortin 1970), leading to pessimism about this approach. The important discovery of human leukocyte antigens (HLA) occurred around the same time (Dausset 1958; van Rood et al. 1958), but it was not until the field returned to preclinical studies using canine models and discovered that dogs that received marrow grafts from dog leukocyte antigen (DLA)-matched, but not mismatched littermates survived, did this translate into the now-accepted clinical practice of using marrow from HLA matched human siblings (Storb et al. 1968, 1970, 1971). In 1968, the first successful allogeneic transplantations were performed in children with immune deficiency diseases (Gatti et al. 1968; Bach et al. 1968). The field has grown exponentially since those early clinical and preclinical studies with over 400,000 annual bone marrow transplantations now routinely performed worldwide.

1.4 Promise for the Future

The future of regenerative medicine remains bright despite its many challenges, and is beyond what can be accomplished by any single discipline alone (Haseltine 2001). It requires a combination of technological approaches from many experts including stem cell biologists, molecular biologists, developmental biologists, material scientists, engineers, physicists, imaging experts, clinicians, veterinarians, pathologists, and others. Interactions among these specialists are needed as more complex solutions are required to meet the unmet medical needs of patients. For example, the development of 3-D tissue constructs may necessitate the use of cell bioreactors, scaffolds, cells, and microfluidic and electronic controls systems (Domansky et al. 2010). Increasingly, the development of these complex projects also requires multiple types of institutions including academic institutes, industrial partners, hospitals, and nongovernmental funding organizations (Daar and Greenwood 2007).

The future of regenerative medicine will also depend on how the funding model changes with time to meet the evolving need of the community. Funding for cell therapy research, especially translational research that focuses on bridging basic discoveries and novel clinically relevant therapeutics, remains a major bottleneck. This is true despite increased NIH funding in basic research from US$170.9 million in 2002 to US$340.8 million in 2010 for human nonembryonic stem cell research projects (NIH Stem Cell Research Funding 2011), which tend to focus on hypothesis-driven research projects rather than the more routine but critical, safety and validation studies. New initiatives, such as California Institute of Regenerative Medicine with US$3 billion funding over a 10-year period, are helping to overcome the bottleneck. The creation of new grants panel/study sections specifically focused on translational and clinical studies will further help to develop this exciting field.

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Chapter 2
Defining Endothelial Progenitor Cells

Julie Mund, David A. Ingram, and Mervin C. Yoder

Abstract Human cord blood-derived endothelial progenitor cells (EPC) have been defined as circulating cells that express a panel of cell surface markers similar to those known to be expressed by vascular endothelial cells, that home to sites of hypoxia/ischemia upon infusion into experimental animal models, and participate in blood vessel formation (as analyzed by in vitro and in vivo methods). Although no specific marker for an EPC has been identified, a group of markers has been consistently utilized as a surrogate marker for cells purported to display vascular regenerative capacity. Since both hematopoietic and vascular endothelial subsets display many of the same cell surface antigens and both participate in new blood vessel formation, recent analyses have stressed the need to reconsider the use of the term EPC. This chapter reviews our current approaches to identifying human EPC and provides a brief summary statement for a new definition of an EPC.

2.1 Introduction

The identification of circulating cells that displayed the potential to attach to matrix-coated tissue culture plates, lose expression of hematopoietic antigens and upregulate expression of antigens typically thought to be endothelial specific during in vitro differentiation, demonstrate in vitro colony-forming ability, and home to sites of ischemia/hypoxia upon injection into experimentally injured immunodeficient mice, served to herald the existence of human endothelial progenitor cells (EPC) (Asahara et al. 1997). These observations were paradigm shifting for the

J. Mund • D.A. Ingram • M.C. Yoder (✉)
Department of Pediatrics, Herman B Wells Center for Pediatric Research,
Indiana University School of Medicine, Indianapolis, IN, USA
e-mail: myoder@iupui.edu

D.S. Allan and D. Strunk (eds.), *Regenerative Therapy Using Blood-Derived Stem Cells*,
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cardiovascular field which had come to understand that new blood vessel formation in the adult organism is restricted to angiogenesis: the formation of new blood vessels from existing vasculature (Risau and Flamme 1995). The existence of an EPC in circulating blood suggested that some aspects of vessel regeneration may rely upon vasculogenesis: the formation of blood vessels from angioblastic precursor cells that is most typical for the developing embryo (Sabin 1917). Since the original description of an EPC (Asahara et al. 1997), more than 9,500 original papers and reviews (PubMed as of March 1, 2011 using search term "endothelial progenitor cell") have been written about EPC identification, enumeration in healthy and ill subjects, roles in various developmental and disease-related processes, and potential use as a cell therapy for treatment of cardiovascular disorders. Many excellent reviews have been recently published on the topic of EPC (Diller et al. 2010; Dudek 2010; Fadini and Avogaro 2010; Kusuma and Gerecht 2010; Lenk et al. 2011; Luo et al. 2011; Melero-Martin and Dudley 2010; Psaltis et al. 2011; Sen et al. 2011; Yoder 2010). This chapter will focus on a brief overview of the methods to identify an EPC and summarize a call for a change in EPC definition.

2.2 Methods to Identify Human EPC

Despite the vast number of papers written on EPC biology, methods for identifying these cells have largely been restricted to 3 general paradigms. One method for EPC enumeration relies upon the ability of umbilical cord blood or adult peripheral blood mononuclear cell subsets to adhere to extracellular matrix-coated culture dishes (Hill et al. 2003; Ito et al. 1999). The assay requires that mononuclear cells are plated in specific culture medium containing certain endothelial growth factors. Following 5–7 days in culture, the adherent cells are termed EPC.

Two common molecules tested as EPC markers include oxidized acetylated low-density lipoprotein (Ac-LDL) and the plant lectin *Ulex europaeus* (Ulex). The Ac-LDL is recognized and bound by the cell surface scavenger receptor expressed by the putative EPC. While endothelial cells throughout the body are known to express this receptor and to bind Ac-LDL (Voyta et al. 1984), it is now understood that this receptor is also quite widely distributed among hematopoietic cell subsets, particularly of the myeloid lineage (Rohde et al. 2006). Thus, the use of this particular molecule does not discriminate an EPC from many other cells of the cord blood or peripheral blood that may have adhered to the culture dishes.

One cannot rely upon Ac-LDL ingestion to define an EPC since adhesion of circulating hematopoietic cells to the extracellular matrix is a well-recognized method for isolating certain cell subsets, particularly monocytes (Hassan et al. 1986). Is Ulex binding to the putative EPC a more specific endothelial marker than Ac-LDL uptake? Ulex is bound by many circulating blood cells and a variety of epithelial cells in addition to vascular endothelial cells (Graziano et al. 2001; Holthofer et al. 1982; Liu and Li 1996; Schwechheimer et al. 1984). Thus, neither Ac-LDL uptake nor Ulex binding is specific markers for an EPC.