Stem Cell Biology and Regenerative Medicine

Harold S. Bernstein *Editor*

Tissue Engineering in Regenerative Medicine

Humana Press

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This book is dedicated to my father,
Wallace Carl Bernstein (1923–2010),
who taught me to ask questions.

Preface

Over the past decade, significant advances in the fields of stem cell biology, bioengineering, and animal models have converged on the discipline of regenerative medicine. Significant progress has been made leading from preclinical studies through phase 3 clinical trials for some therapies. This volume provides a state-of-the-art report on tissue engineering toward the goals of tissue and organ restoration and regeneration. Examples from different organ systems illustrate progress with growth factors to assist in tissue remodeling; the capacity of stem cells for restoring damaged tissues; novel synthetic biomaterials to facilitate cell therapy; transplantable tissue patches that preserve three-dimensional structure; synthetic organs generated in culture; aspects of the immune response to transplanted cells and materials; and suitable animal models for nonhuman clinical trials.

Tissue regeneration, and even stem cell therapy, is not a new concept. As discussed in the cautionary first chapter, efforts toward bone and marrow transplantation have been underway for almost half a century. Steady progress has been made in understanding the criteria for successful cell transplantation, and developing a robust structure for clinical oversight. More recently, pluripotent stem cells, with their capacity for self-renewal and tissue-specific differentiation, have become a prime candidate for tissue engineering and regenerative therapies. More than 100 clinical trials have examined the use of mesenchymal stem/stromal cells. Biochemical and mechanical interactions between the extracellular matrix and cell surface receptors, as well as physical interactions between cells, are now recognized as essential for stem cell self-renewal and differentiation. New technologies for scaffold engineering and fabrication have taken advantage of these observations, and hold promise for repairing tissues requiring a highly specialized niche, such as skeletal muscle. These discoveries have led to clinical trials with bioengineered vascular conduits in children with congenital heart disease, complete hollow organs, and complex organs such as bioartificial livers. An evolving understanding of innate and adaptive immune responses, including the foreign body response, has led to novel approaches to modulating the immune system that facilitate tissue repair. Finally, the development of small animal models for discovery, and large animal models for studies of safety and efficacy, has propelled the field of tissue engineering toward the clinic.

The chapters of this book are organized into six sections: Stem Cells, Biomaterials and the Extracellular Environment, Engineered Tissue, Synthetic Organs, Immune Response, and Animal Models. Each section is intended to build upon information presented in the previous chapters, and set the stage for subsequent sections. Throughout the chapters, the reader will observe a common theme of basic discovery informing clinical translation, and clinical studies in animals and humans guiding subsequent experiments at the bench.

I thank the members of my laboratory for their helpful discussion, and my colleagues in Pediatric Cardiology for their support – we all strive to improve the lives of our patients. I appreciate always the encouragement I receive from Tricia Foster, Nathaniel Bernstein, and Katharine Bernstein. I am grateful to the 54 colleagues who have contributed their expertise to this project. We hope that this first edition of *Tissue Engineering and Regenerative Medicine* will serve as an introduction and guide for students of the field at all levels.

San Francisco, CA Harold S. Bernstein

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Part I
Stem Cells

Chapter 1
Hematopoietic Stem Cell Transplantation: Reflections on Yesterday and Thoughts for Tomorrow

Andrew D. Leavitt

Abstract Biomedical science is entering a new era with exciting prospects for using cellular therapy to treat a wide spectrum of human diseases from nerve injury to diabetes, myocardial infarction, and more. Hematopoietic stem cell (HSC) transplantation has been used to treat patients for nearly half a century. The experiences and lessons learned over those 50 years are both informative and encouraging. This chapter distills the history of HSC transplantation to provide an orientation to the past that can be used to more wisely navigate the future of cell therapy. The details presented help the reader appreciate that developing novel cell therapy can be a struggle and that chance will likely continue to play a role in future success. However, it also becomes apparent that attention to fundamental details, such as choice of cell type or types, where to obtain the cells, how to handle and process the cells, how to prepare and select patients, how to evaluate success and failure, and how to organize the biomedical community to serve the good of patients, are all critical for new cell therapy to become a reality.

Abbreviations

BMT	Bone marrow transplantation
GVHD	Graft-versus-host disease
GVL	Graft-versus-leukemia
HLA	Human lymphocyte antigen
HSCs	Hematopoietic stem cells

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PBSCs Peripheral blood stem cells
UCB Umbilical cord blood

1.1 Introduction

HSCs are the most studied and well-understood of all adult stem cells, and they provide a model system and paradigm for the more global understanding of stem cell biology [1]. HSCs have also been used clinically for nearly 50 years, with over 55,000 HSC transplants performed around the world in 2009 alone [2]. HSCs and their clinical application, therefore, provide an excellent reference point for discussing the future of stem cell therapy, be it the use of embryonic stem cells and their derivatives or the direct use of tissue-specific adult stem cells. This chapter presents a brief history of HSC transplantation to give perspective and to help inform and orient the reader to issues that will likely be faced as biomedical scientists begin developing tomorrow's stem cell therapies. Accounts of the history of HSC transplantation have been summarized by others, including a personal account by E. Donnell Thomas who shared the 1990 Nobel Prize in Medicine for his pioneering role in the development of BMT [3, 4].

1.2 Radiation: A Double-Edged Sword

Marie Curie (born Maria Sklodowska) shared the 1903 Nobel Prize in physics with Pierre Curie and Henri Becquerel "in recognition of the extraordinary services they have rendered by their joint researches on the radiation phenomena discovered by Professor Henri Becquerel." She also won the 1911 Nobel Prize in Chemistry "in recognition of her services to the advancement of chemistry by the discovery of the elements radium and polonium, by the isolation of radium and the study of the nature and compounds of this remarkable element." Tragically, she died on July 4, 1934, from marrow toxicity, reported in various sources as aplastic anemia and/or leukemia, but almost certainly secondary to the chronic radiation exposure she received during her early pioneering studies related to naturally radioactive substances. The bone marrow toxicity of ionizing radiation was appreciated only after much of her initial exposure, and interestingly the field of clinical marrow transplantation relied for decades on the use of ionizing radiation as a preparative regimen to both eradicate underlying malignant disease and to immunosuppress the recipient to facilitate marrow engraftment and HSC repopulation.

The highly deleterious effects of radiation on bone marrow were appreciated well before World War II [5], but development and use of the atomic bomb in the 1940s highlighted the marrow toxicity of radium, uranium, and other sources of ionizing radiation. Classified government research to develop treatments for bone marrow toxicity due to atomic bomb radiation exposure was performed in the 1940s under the auspices of the Atomic Energy Commission, but it was not published until

1950 [6]. Those studies sought "to determine what benefits, if any, may be derived from the transplantation of normal bone marrow in animals that have suffered damage to their bone marrow as a result of single dose roentgen irradiation." The studies failed to achieve their goal of allogeneic engraftment or to demonstrate any clinically useful effect of marrow transplantation. However, failure was most likely secondary to inadequate radioablation of the recipient animal's immune system required to achieve engraftment. So, even though the studies failed to achieve their goal [6], they highlighted one of the critical aspects of HSC transplantation – the host is not naturally receptive to foreign cells and the host's immune system needs to be suppressed to overcome this barrier to cellular therapy. This critical fact is important to consider when developing any future form of allogeneic stem cell therapy.

1.3 Bone Marrow Transplantation: It Is the Cells

In 1949, independent investigators reported that lead shielding of the spleen protected mice from the mortality of total body irradiation [7]. Interestingly, it was thought that the beneficial effect was humorally mediated. Even after a 1951 report demonstrated that intravenous or intraperitoneal injections of bone marrow cells protect mice and guinea pigs from the mortality of total body radiation [8], the humoral theory remained the prevailing theory to explain radioprotection. It required an innovative experiment reported in 1955 to begin to convince the research community that radioprotection stemmed from the bone marrow cells themselves engrafting into the recipient [9]. In brief, the investigators knew that skin grafts would not survive if performed between H2-incompatible mice, but the authors showed that skin grafts could survive across H2-incompatible strains if the recipient was first transplanted with marrow from the skin donor [9]. Moreover, skin graft survival required that the irradiated recipient mouse receive marrow from the same mouse strain that provided the skin graft. These findings, as the authors concluded, "are consistent with the cellular repopulation theory of radiation protection." In 1956, using the then novel technique of genetically traceable donor marrow cells, it was convincingly shown that the radioprotective effect of BMT correlated with engraftment of donor marrow cells in the recipient [10]. The essential role of the marrow cells in radioprotection had finally been established, as had the fact that allogeneic marrow transplantation could work.

1.4 A Rough Clinical Start: Patients Are Always a Bigger Challenge than Mice

With the animal transplant data in hand and knowing that radiation could kill leukemic cells, it was only natural for investigators to try to connect these two observations for therapeutic benefit. A 1956 report demonstrated that radiation could be used to

eradicate leukemia in mice and that bone marrow transplant could rescue the host from the marrow-damaging effects of the radiation treatment [11]. One year later, in 1957, Thomas et al. published the first report of infusing allogeneic marrow into humans when he described his experience with six patients – three with hematologic malignancies (chronic myelogenous leukemia, multiple myeloma, and chronic lymphocytic leukemia), one with ovarian carcinoma, one with metastatic cancer of uncertain origin, and one who had suffered a massive central nervous system bleed [12]. The five patients with malignancies had each received chemotherapy and/or radiation therapy shortly before the marrow infusions.

This initial report clearly focused on evaluating the safety and toxicity of the marrow cell infusions and not their therapeutic benefit [12]. There were no deaths attributed to the infused cells, and great effort was taken to assess for pulmonary emboli, which were not found to be a problem clinically or when evaluated at postmortem exam. One case suggested transient engraftment based on circulating blood cell analysis, but no long-term engraftment was demonstrated. The major conclusion was that anticoagulated suspensions of allogeneic marrow cells, strained through fine mesh to remove particulate matter, can be safely given to human recipients, as had been previously demonstrated in animals [13]. In addition to demonstrating relative safety (i.e., no major obvious untoward effects) in a very small number of patients, the authors raised important fundamental issues that are important to consider when developing any type of cell therapy in the future. They discussed the need to establish a clinically relevant cell dose, to develop a preparative regimen to treat the recipient so that their immune system does not reject the allograft, and to define a detailed monitoring system that allows for accurate assessment of toxicity and benefit.

The same group reported in 1959 the successful, albeit temporary, eradication of acute lymphocytic leukemia in a patient treated with total body irradiation (Co^{60}) followed by allogeneic BMT from an identical twin [14]. While the patient relapsed 12 weeks later, the case demonstrated that lethal radiation followed by BMT could achieve a remission, even in advanced disease, and it highlighted the importance of immunologically matched donors for efficient engraftment [14]. The authors concluded that transplants of syngeneic marrow are readily achieved in humans, that 1,000 rad of whole body radiation administered properly does not produce troublesome acute radiation sickness in humans, and that whole body irradiation at the 1,000 rad level produces a remission but not a cure of leukemia when followed by infusion of syngeneic marrow. Chemotherapy (cyclophosphamide) was soon added to total body irradiation to help eradicate the underlying disease when employing allogeneic BMT to treat patients with acute leukemia, a preparative regimen that remained in use for several decades.

Reports of allogeneic BMT rose steadily over the next few years, with over 60 such transplants reported in 1962. However, enthusiasm rapidly declined as toxicity was clear and success was hard to find; only a few transplants were reported annually through the late 1960s [15]. A 1970 review of all 203 reported allogeneic transplants through 1968 highlighted the dismal state of the field, with few if any true successes. In fact, 125 of the 203 recipients did not even demonstrate evidence of

engraftment, including 66 of 73 patients with aplastic anemia [15]. Interestingly, the other seven aplastic anemia patients received allogeneic marrow from a syngeneic twin, five of whom had clinical recovery from their disease. This subset of patients provided hope for BMT as a clinical intervention, and the outcome with the identical twins reemphasized the critical importance of immunologic match for a successful engraftment of donor bone marrow. It also highlighted the difference between treating a disease that has a dominant phenotype that is likely to recur, such as leukemia, versus one with a recessive phenotype, such as aplastic anemia.

While the late 1950s through the early 1970s was not a good time for clinical success within the BMT field, significant headway was made in critical areas of transplant immunology through the use of animal studies. The advances grew out of studies in the early 1950s that actively developed immune tolerance in young mice [16]. By the mid-1960s, runt disease in mice [17, 18], which is essentially what we call GVHD in the human transplant setting, was becoming well-understood, at least from the perspective of factors related to its development [19, 20]. For example, it was not associated with the injection of syngeneic cells but required antigenic differences between donor and host, and the more pronounced the differences, the more severe the disease. Moreover, persistence of the allogeneic cells was required for persistent disease, and injection of presensitized cells could worsen the problem. Also, one could tolerize the animal prior to transplant and avoid runt disease. These findings continue to influence the field of HSC transplantation today as investigators seek to control GVHD while maintaining therapeutic success, in particular when treating malignant disease. However, as discussed below, the relationship between GVHD and therapeutic success differs with the disease being treated.

In parallel with the work in mice, others were using dogs to better understand issues of engraftment, rejection, and GVHD [21, 22]. Dogs, while having a clear disadvantage due to their size and cost of housing, had a distinct advantage in being outbred and large enough for the types of surgical procedures needed to be performed at the time. Dog models demonstrated graft rejection and GVHD, but some became long-term engrafters, true HSC transplant successes, and the search was on to understand why. Ultimately, dog models were used to develop immune serum to allow for the identification of matched allogeneic donors, and it was in this setting that the use of methotrexate to reduce GVHD was developed. By the end of the 1960s, the dog model system had been used to develop a nearly 90% success rate from immunomatched allogeneic outbred donors identified using the serum reagents developed by the investigators [23–25]. They had shown quite clearly in a large animal model that lymphocyte immunophenotyping was critical for the success of allogeneic transplants, something that was proven to be true in human transplants and that continues to be of central clinical importance to this day.

GVHD remains a great cause of morbidity and mortality following allogeneic HSC transplantation. Improved antileukemic preparative regimens have made disease recurrence less problematic. However, it is now appreciated that GVHD is a double-edged sword when treating leukemia with allogeneic HSC transplantation. GVHD is itself deleterious, but allogeneic HSC transplant also provides a GVL

effect that is beneficial and contributes to overall survival. Attempts to separate these two immunologic phenomena are under intense study.

1.5 Finally Some Encouraging Results

The disappointing results summarized in 1970 [15] saw many investigators leave the field, but some persevered. They believed that success was possible if they could answer a few key questions – cell dose, patient preparation that can both treat disease and prevent graft rejection, and how to reduce the problem with GVHD. A 1972 publication described four patients with aplastic anemia treated with HLA-A matched sibling donors, giving BMT a much-needed boost. All were opposite sex transplants, so standard karyotyping could determine if blood count return post-treatment was due to endogenous marrow recovery or allogeneic marrow engraftment. One patient died from GVHD with a cellular marrow at 45 days posttransplant, another rejected the transplant and died 67 days after transplant, but two were alive with a robust functioning allogeneic marrow at the time of the report, 138 and 215 days out from transplant.

In 1975, the BMT team in Seattle published a two-part review [26, 27] that extensively outlined the scientific rationale for performing BMT and the requirements for successful BMT, including details on the care of the patient, the importance of immunosuppression to allow for engraftment and prevent rejection, the need to eradicate underlying malignancy, and the need for HLA matching. It also established a marrow-nucleated cell count dose that should be met for successful transplant and defined many clinical aspects of GVHD. The review also presented the authors' results treating 37 patients with aplastic anemia and 73 with end-stage leukemia. While the survivorship was low for the patients with end-stage leukemia, the fact that any were alive 2 years posttreatment was a remarkable success that energized the BMT field. Patients were alive that would otherwise have died if it were not for their BMT. However, the field really took off following a 1977 report describing the outcomes of 100 consecutive patients treated with chemotherapy, total body radiation, and sibling-matched allogeneic transplants for end-stage recurrent leukemia. Thirteen of the patients were apparent "cures" as defined by no recurrence of disease at 2 or more years (some over 4 years) posttransplant [28].

The authors and others realized that success might be much higher if leukemia patients were treated before they relapsed and reached end-stage status of their disease. In 1979, two groups reported on matched, related, allogeneic transplantation for leukemia, demonstrating a nearly 50% survival at 2 years [29, 30]. Bone marrow transplant had worked. Patients were benefiting, and over the next 15 years such transplants became part of mainstream medical care. It is estimated that roughly 60,000 transplants were performed around the world in 2010. The Center for International Blood and Marrow Transplant Research maintains a worldwide database of HSC transplants, including source of cells, underlying disease, and outcome (http://www.cibmtr.org).

1.6 Not All GVHD Is Bad

GVHD was rapidly appreciated to be a major complication of allogeneic transplants, and detailed clinical information on how to define this disorder was included in the 1975 two-part report [26, 27]. However, even as far back as the 1950s, it was speculated that the allogeneic donor cells might also provide a beneficial effect when treating malignant diseases such as leukemia [11]. That is, maybe the same immunologic attack of the normal host tissue could also play a role in destroying the diseased cells. This has turned out to be true, with higher cure rates associated with moderate GVHD. This idea was further supported by findings from identical twin (syngeneic) transplants [31]. It was originally thought that an identical twin would be the ideal donor because of the lack of or minimal GVHD. However, patients with acute myelogenous leukemia who received an allogeneic donation from an identical twin had a significantly higher relapse rate than those who received marrow from an HLA-matched sibling [31].

The twin data highlighted that HLA (-A, -B, -DR, and DQ) matching does not match all immunologic differences, and the ones that remain are sufficient to allow for clinically important GVL effect. This immunological therapeutic value of the allogeneic HSC transplant, GVL, remains a critically important contributor to the cure rate for allogeneic transplants for malignant hematologic diseases. However, it is important to remember that there is no beneficial role for graft-versus-disease when using allogeneic transplantation to treat nonmalignant diseases, such as sickle cell anemia [32] and thalassemia [33]. Innovative approaches to reduce GVHD will be essential if we are to bring this valuable treatment to more patients with nonmalignant hematologic disorders [34].

Congenital immunodeficiencies represent yet another group of disease that can be treated with allogeneic transplantation. As with other nonmalignant diseases, GVHD needs to be minimized at all costs. However, these patients allow for greater HLA mismatch in "the other" direction because the recipient immune system is often unable to mount a host-versus-graft response to reject the marrow. Consequently, more gentle conditioning regimens can often be employed, which translates to less therapy-related toxicity. The immunocompetence of the recipient could have a large impact on trial design and clinical outcomes when identifying initial candidates for novel cell therapies developed in the future.

1.7 Source of Hematopoietic Stem Cells

While increasing numbers of people now use the name "hematopoietic stem cell transplantation," from the start and for many years it was called BMT for obvious reasons. In the original 1957 report entitled "Intravenous infusion of bone marrow in patients receiving radiation and chemotherapy," the cells infused into the six patients were obtained from fetal ($n=1$) or adult ($n=1$) cadavers, ribs removed at surgery ($n=1$), or the anterior or posterior iliac crest aspiration of a living donor ($n=3$).

By the 1970s, iliac crest marrow aspiration was the standard method for obtaining bone marrow cells for transplantation, a procedure that requires general anesthesia.

While the HSCs are required for long-term, sustained engraftment, it is well-appreciated that the transplanted marrow includes many more hematopoietic cell types than just HSCs. The importance of the non-HSC cells for assisting with engraftment remains uncertain, but it is quite clear that the non-HSC progenitor cells play a critical role in providing a more rapid production of circulating allogeneic blood cells following infusion. This aspect of progenitor cells helps protect the patient from infection and bleeding, complications of neutropenia and thrombocytopenia, respectively [35]. Given that transplant morbidity and mortality are directly related to the duration of posttransplant cytopenia, the non-HSC cells in the transplanted material clearly play an important and favorable clinical role. Consequently, as cellular therapy moves to other tissues, it is important to consider the value of cells beyond the stem cells proper. It could be that an overly reductionist or "pure" cell population has less benefit than one that contains critical accessory cells.

It became clear in the late 1980s that adequate numbers of HSCs could be obtained from the peripheral blood of patients following administration of newly available human cytokines, such as G-CSF or GM-CSF [36]. Interest grew rapidly in the clinical use of such PBSCs as source material for HSC transplantation, and reports of their use became common in the mid-1990s [35, 37–42]. Clinical trials confirmed their safety and efficacy, and PBSCs rapidly expanded as an HSC source for allogeneic and autologous transplants. G-CSF rapidly became the mobilizing agent of choice [43, 44]. More recently, a CXCR4 inhibitor has been approved as an alternate method for mobilizing PBSCs in a subset of patients. Curiously, the use of PBSCs posed a nomenclature problem for the field. How could PBSC transplants be called bone marrow transplants when the cells were not collected from the bone marrow? Fortunately, BMT is also the acronym for "blood and marrow transplantation," which is how it is commonly used today.

While there is not a simple clinical method to quantify the true HSC content of a PBSC product, standard of care is to use CD34 surface expression as a surrogate marker for HSCs and to dose PBSC transplants based on a desired number of $CD34^+$ cells/kg that ensures engraftment. This contrasts with marrow samples, where the clinical adequacy of the collection is based simply on a nucleated cell count/kg. In either case, it is important to realize that no clear enumeration of HSCs is applied to determine the adequacy of an HSC collection, yet the use of surrogate markers has proven productive and safe for many decades.

The limited availability of related, matched allogeneic donors became a problem as the sophistication of HLA matching and the use of transplants grew. While in principle one has a one-in-four chance of finding a sibling match, success is even less in real life. Therefore, the majority of patients who can benefit from a BMT do not have an acceptable sibling donor. The first report of a successful, unrelated HLA-matched (HLA-A, -B, -C, and -DR; four loci, which means eight total alleles) allogeneic transplant for leukemia was reported in 1980 [45]. Finding a match was made possible through the advent of more sophisticated HLA phenotyping, but the

success in finding this particular donor was the result of pure luck and circumstance. The matched donor was a technician at the Seattle transplant center, where everyone had been HLA typed as part of the center's studies in HLA typing.

The first matched, unrelated allogeneic transplant highlighted the potential value of developing a robust mechanism for identifying unrelated HLA-matched donors. As a direct outgrowth of this particular experience and productive lobbying of the US government by concerned and involved individuals, federal funding was eventually allocated for the development of the National Marrow Donor Program (http://www.marrow.org) in the USA. The program has grown dramatically over the ensuing 25 years, is now linked to other similar programs in Europe and elsewhere, and unrelated donors are identified for thousands of patients each year through the sophisticated international systems. It is a great example of how national boundaries and differences can become invisible when health care and humanity are placed above politics. As a testament to the importance and the success of these programs, more unrelated than related allogeneic transplants were performed in the USA in 2009.

UCB HSCs [46] were first demonstrated as a clinically useful option for HSC transplants in 1989 [47] when they were used to treat a patient with Fanconi's anemia, a nonmalignant, congenital blood disorder. UCB has a number of advantages over other HSC sources, including the lack of risk or discomfort to the donor and the ability to store the product in large banks. The latter point means that one can avoid the need to isolate the HSC product from a donor in a timed fashion relative to the patient's treatments. It also means that intercurrent health issues do not delay or prevent a donation as they can with a living donor. There is also an apparent advantage related to greater tolerance of HLA mismatching [48]. On the other hand, the limited number of cells in most UCB units precludes their use in older adolescents and adults, a fact that has led to the use of multiple UCB units to treat an adult [49]. Regardless, UCB now occupies a legitimate seat at the table of HSC sources for patients of all ages in need of allogeneic HSC transplantation, and the future establishment of organized public UCB banks will be a big step forward in making UCB cells available to more patients in need [50]. While many efforts have been undertaken, human HSCs have not yet been convincingly generated from human embryonic stem cells, so the clinical application of hESC-derived HSCs remains theoretical.

1.8 Autologous HSC Transplantation

Allogeneic HSC transplantation was for many years the primary focus for HSC transplantation, and the most common application was to treat hematologic malignancies. However, it was clear from the beginning that autologous transplants may prove useful if antileukemia regimens could eradicate the disease, thereby making unnecessary the GVL effect achieved with allogeneic transplants. The use of combined chemotherapy and total body irradiation preparative regimens provided such an opportunity, as did subsequent use of all chemotherapy preparative regimens,

and autologous HSC transplant was found to be curative in a number of patients with acute leukemia [51–53].

While autologous transplants are performed in the setting of clinical remission, there was great concern that relapse could be due to reinfusion of leukemia clones with the transplanted cells. This question was addressed with some of the very first gene therapy trials in which viral vectors were used to mark harvested cells prior to their reinfusion. If the viral vector marked relapsed disease, investigators would know that it came from the harvested and reinfused cell product. Such studies showed that a fraction of relapsed disease does in fact come from reinfusion of malignant cells [54–56]. The risks of autologous and allogeneic transplants differ, with the former having a much higher risk of relapse and no risk of GVHD-related morbidity and mortality. In contrast, allogeneic transplants have a much lower risk of relapse but a significant risk of GVHD-related morbidity and mortality. As risk stratification has evolved, different subsets of patients are preferentially treated with one or the other approach.

1.9 Regulatory Agencies

BMT grew up in an era quite different from today when it comes to regulation and oversight. In fact, one might wonder if HSC transplantation could have ever gotten off the ground in today's regulatory environment. For many decades, procedural decisions and standards were established by individual transplant programs without outside scrutiny. However, as programs grew and more centers opened, it became important for professional organizations to establish rules to guide the field. From this appreciation was born the Federation for Accreditation for Cellular Therapy, the major professional organization that now accredits BMT programs, and accreditation has become an important goal for all centers in the USA.

The Federation for Accreditation for Cellular Therapy, originally called the Foundation for the Accreditation of Hematopoietic Cell Therapy, was established in 1996 to develop and implement the inspection and accreditation program of the parent organizations, the International Society for Hematotherapy and Graft Engineering and the American Society of Blood and Marrow Transplant. Training of inspectors began in September 1996 and the first on-site inspections began in September 1997. The Foundation for the Accreditation of Hematopoietic Cell Therapy changed its name to the Federation for Accreditation for Cellular Therapy in December 2001 when it became clear that cellular therapy was growing beyond traditional hematopoietic stem and progenitor cells.

The Federation for Accreditation for Cellular Therapy inspects an entire program, including collection, laboratory, and clinical care. The Joint Accreditation Committee of the International Society for Cellular Therapy and the European Group for Blood and Marrow Transplantation launched their first official inspection programs in January 2004, providing Europe a similar accreditation program. The American Association of Blood Banks also inspects and accredits BMT laboratories.

The Federation for Accreditation for Cellular Therapy and like organizations have done much to make BMT programs safer and more responsive to patient needs.

In addition to professional accreditation agencies, such as the Federation for Accreditation for Cellular Therapy and the American Association of Blood Banks, BMT programs in the USA must have all or part of the program licensed with state health care agencies and be registered with the Food and Drug Administration. The governmental organizations work to ensure good practices and to provide an avenue to disseminate information relevant to maintaining a safe operation. They make on-site inspections on a regular basis to ensure that procedures are in place and followed and that clinical outcomes and support are consistent with high-quality care. It behooves the cellular therapy community to put energy into professional organizations that provide oversight of any new cellular therapies that develop. Self-policing by informed and interested professionals is the best way to ensure safety and reproducibility and to avoid unwanted and unproductive regulations from outside agencies. For BMT programs in the USA, the Federation for Accreditation for Cellular Therapy and the American Association of Blood Banks provide excellent avenues for working with the states and with the Food and Drug Administration to ensure rational and productive systems.

1.10 Conclusions

The history of HSC transplantation offers an informative glimpse into the past, providing a number of experiences that can help guide the future of stem cell therapy. First and foremost is the appreciation that HSC transplantation did not "work" right away. In fact, it took decades before people could speak of meaningful clinical success. However, unlike today's stem cell activities, the field of HSC transplantation grew up in relative anonymity, a truth that made its initial struggles less likely to derail its efforts. Therefore, the first issue for the stem cell field is to not oversell its product or its timeline for success and to articulate clear and simple goals.

While the field of HSC transplantation took a while to gather momentum, there were observations even in the early years that proved informative. For example, the relatively early successful transplant of patients with immunodeficiency syndromes highlighted the fact that some patients provide a more receptive environment for transplant engraftment than do others. Such experiences demonstrate the significant impact of highly selected patient populations on successful outcomes. People developing new cellular therapies need to keep this in mind because nothing breeds success and maintains public support like success.

Unlike the development and application of HSC transplantation, most novel cellular therapies being considered today are for nonmalignant diseases. This is an advantage because it typically means not having to eradicate a phenotypically dominant disease and replace it with a normal (phenotypically recessive) new stem cell population. For example, replacing injured nerves or destroyed pancreatic islet cells does not require therapy to remove the diseased cells. However, it could be that the

environment, i.e., cellular niche where the new cells need to engraft, is damaged or altered in the diseased state leaving it less receptive to new cells, such as in myocardial infarction or diabetes. Consequently, understanding the health and makeup of the engraftment location might be critical for success.

The field of cellular therapy, both stem cell and other, also needs to keep in mind that the fundamentals are the key. Just like for HSC transplant, one needs to determine the (minimum) number of cells needed to achieve one's goal and how to best prepare the patient to receive and accept the transplanted cells. It is envisioned that some cellular therapies will ultimately be developed through modification of autologous cells, but that will not happen tomorrow, so selective immunomodulation will be just as important as it is for current day tissue and organ transplantation. Moreover, consideration should be given to the possible use and benefit of accessory cells, much as the non-HSC progenitor cells help with the clinical success of HSC transplants. Of course, well-designed systems to monitor for toxicity and efficacy are essential to keep the field developing productively.

Modern stem cell therapy is growing up under an intense public spotlight. The better the cell therapy community polices itself, the more care it takes to learn from the accreditation and inspection organizations that have developed within the HSC transplant community, the more trust it will be given by the public. Involved members of the scientific community must actively engage regulatory agencies and develop professional oversight groups, much like the HSC transplant community has done. This has resulted in better and safer HSC transplant programs, better data monitoring, and it affords the involved community an efficient mechanism for communication and engagement with government organizations. The future for cellular therapy is promising and exciting, and lessons learned along the way must be carefully and actively used to everyone's advantage.

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