Hematopoietic Stem Cell Transplantation for the Pediatric Hematologist/Oncologist

Valerie I. Brown
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Part I

Introduction, History and the Basic Principles of Pediatric Hematopoietic Stem Cell Transplantation (HSCT)

Introduction

1

Valerie I. Brown

Abstract

This book stemmed from the concept of a symposium I organized and moderated a few years ago for the annual meeting of the American Society of Pediatric Hematology/Oncology. As the only member of the program committee who had expertise in pediatric hematopoietic stem cell transplantation (HSCT) at the time, I was charged to organize the joint Pediatric Blood and Marrow Transplantation Consortium (PBMTC)/ASPHO symposium for the following year's annual meeting. Generally, we try to make this session less esoteric in order to address the educational needs of a broader audience beyond pediatric HSCT specialists. At that time, the other committee members and I really appreciated that there can be a significant disconnect between the pediatric HSCT subspecialist and the rest of the pediatric hematology/oncology community with the biggest issues being which patients should be referred to the pediatric HSCT subspecialist for consideration of HSCT and when this referral should be made. Bridging this gap has become more and more important as the indications and accessibility to HSCT continue to expand. The symposium in its final format had one pediatric HSCT subspecialist present the data regarding the indications and the timing of evaluation for HSCT of pediatric patients with malignant conditions. The other symposium speaker addressed the same topics but for patients with nonmalignant disorders for which HSCT may be a treatment option. Overall, this symposium was well attended and well received. Based upon the positive responses received from attendees, this book was conceived and subsequently written with similar objectives

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and target audience in mind: This book is to provide an in-depth reference guide for not only pediatric HSCT subspecialists but also pediatric hematology/oncology specialists, fellows, residents, nurses, and advanced practitioners.

In general, this book is organized chronologically in terms of the "HSCT course." However, it is important to understand the past in order to understand the present practices and the challenges to moving the field of HSCT forward, and so Chap. 2 summarizes the "history" of HSCT from both the nonclinical and clinical aspects, starting with the first fundamental discoveries in immunology that led to the scientific understanding of immunology and transplantation biology and the development of HSCT in humans. While research in the areas of transplantation biology and immunology were being conducted in the first half of the twentieth century, it was not until the 1940s with the detonation of the two atomic bombs and the dawn of the Atomic Age that concerted efforts to apply transplantation biology to feasible patient care accelerated. The focus of research and funding transitioned to the investigation of the effects of exposure to radiation at varying levels on humans and how to treat these exposures. With the harnessing of radiation, physicians and scientists were exploring strategies to utilize radiation in a controlled fashion to treat a variety of diseases. This pioneering work led the way to the landmark clinical trials of the late 1960s and 1970s that are summarized in Chap. 2. It was also around this time that scientists and physicians who were pioneers of this burgeoning field began to form national and international organizations. In 1972, the International Bone Marrow Transplant Registry (IBMTR) was established and evolved into the Center for International Blood and Marrow Transplant Research (CIBMTR) in 2004. In 1974, the European Society for Blood and Marrow Transplantation was started. Organizations such as these were established in order to provide a formal mechanism by which these investigators could exchange their findings and pool their HSCT-related data in order to accelerate advances in HSCT. The 1980s and early 1990s were the times when alternative donors, such as umbilical cord blood and mobilized peripheral blood stem cells as well as graft manipulation and alternatives to myeloablative conditioning regimens, were being explored in order to expand the donor pool while reducing life-threatening side effects that can accompany HSCT. As a result of all of these efforts, more than 50,000 HSCTs are performed annually worldwide with much success currently.

Because HSCT is an immunotherapy, a basic understanding of the fundamental principles of hematopoiesis and transplantation biology is presented in Chap. 3. This chapter includes a discussion of hematopoiesis, the hematopoietic stem cell niches contained within the bone marrow, and how this bone marrow microenvironment that is hospitable to HSCs is created and maintained. This chapter also covers the fundamentals of transplantation biology with a focus on the immune response to allografts and the mechanisms of allograft rejection and tolerance. The pathophysiology of graft-versus-host disease can be found in Chaps. 18 and 19.

Part II of this book focuses on topics related to the pre-HSCT period and includes a discussion of the indications and timing of HSCT (Chap. 4). Chapter 5 is dedicated to the impact that minimal residual disease (MRD) status has on HSCT. In addition, this section of the book addresses other important pre-HSCT topics including how a potential patient is determined to be a suitable HSCT recipient (Chap. 6) and how the most suitable donor and donor HSC source are selected (Chaps. 7 and 8). Finally, this section concludes with an in-depth discussion of the need for conditioning prior to HSCT, the different types and intensities of conditioning regimens, and how the appropriate conditioning regimen is selected for an individual patient (see Chap. 9).

Part III centers on the key events that occur during the peri-HSCT period which spans the pre-engraftment period (days 0–30 post-HSCT)

through the period of early post-engraftment (days 31–100). The principles of engraftment and donor chimerism are discussed in Chap. 10, whereas potential complications encountered during this peri-HSCT period are covered in Chaps. 11, 12, 15, and 16 (including complications associated with engraftment as well as hepatotoxicity and renal toxicity), whereas Chaps. 13 and 14 focus more on the supportive care that is needed during these periods of the HSCT process (i.e., nutrition and the management of pain and mucositis). The prevention and treatment of infections are extremely important in HSCT, particularly during the peri-HSCT period. Chapter 17 is solely dedicated to the prevention and treatment of the most common and/or life-threatening infections encountered by pediatric HSCT patients. Finally, the last chapter of this section (Chap. 18) covers acute graft-versus-host disease (GvHD) which is a very common anticipated consequence of HSCT that can range from being self-limited to life-threatening. In addition to discussing the clinical features, diagnostic studies, and management of acute GvHD, this chapter addresses the approaches used for the prevention of acute GvHD.

Complications that occur during the late post-engraftment period (>100 days post-HSCT) are covered in Part IV of this book. The first chapter of this section (Chap. 19) focuses on chronic GvHD. Chronic GvHD can affect every organ system in the body but most commonly involves the skin, the eyes, the upper and lower GI tract, and the liver. The incidence, risk factors, clinical features, diagnostic studies, grading, treatment, and outcomes of chronic GvHD are detailed in this chapter. The remainder of the chapters (Chaps. 20–25) cover other common late post-engraftment complications and are organized by organ system. These include hematologic, pulmonary, renal, cardiac, and neurologic complications. In addition, non-GvHD-related issues of the skin, hair, and nails are addressed in Chap. 25.

Many medical issues may persist long after the actual infusion of the hematopoietic stem cells (i.e., the transplant). Part V of this book focuses on these topics. Chapter 26 details the immune reconstitution in terms of the different components of the immune system as well as the factors that impact this reconstitution. In addition, this chapter offers recommendations regarding revaccination post-HSCT. Chapter 27 provides a comprehensive review of long-term compilations of HSCT and how to approach the care of long-term survivors of HSCT.

Finally, Part VI contains a very comprehensive table of medications and agents that are commonly used in pediatric HSCT patients. It presents the medications by indications and provides pediatric dosing and schedule (where available) as well as common indications, side effects, and other relevant information for each agent. The information contained within this chapter is evidence-based. However, it provides general guidelines, and so the authors of this chapter and I strongly recommend that the reader follow their own institutional guidelines.

While other HSCT books are very comprehensive and "content" dense, this book was specifically designed to be a detailed guide to be used by all medical providers whose practice will intersect with a pediatric HSCT candidate, recipient, or donor. The authors and I hope that this book begins to bridge the disconnect that can exist between the pediatric HSCT specialists and other medical providers and trainees and promote a dialogue between these two groups.

Historical Overview of Pediatric HSCT

2

Valerie I. Brown

Abstract

With a better understanding of transplantation biology and immunology derived from animal models, hematopoietic stem cell transplantation (HSCT) in humans has become possible. Attempts at HSCT in humans were first reported as early as the 1930s. However, with the detonation of two atomic bombs at the end of World War II and advent of the "Atomic Age," interest in HSCT as a treatment modality for the effects of exposure to sublethal and lethal doses of irradiation on bone marrow function was reignited. Before the mid-1970s, the majority of HSCTs in humans were performed for nonmalignant conditions with 40% for severe aplastic anemia and 15% for primary immunodeficiencies. While attempts were made to treat advanced, refractory acute leukemia patients with HSCT, they were generally unsuccessful and used identical twin sibling as the donor initially. The first reports of successful sustained engraftment occurred in the early 1960s, but these patients died from complications associated with what is now known as graft versus host disease. It was not until 1968 that there were reports of three infants with primary immunodeficiency conditions that were long-term survivors after matched sibling donor bone marrow transplantation. Of note, all three patients are still alive today. In the late 1970s, Thomas and his colleagues reported their findings that of 100 patients with refractory acute leukemia, 13 were alive and leukemia-free 1–4.5 years after undergoing HLA-identical sibling donor bone marrow transplantation. These results showed that some patients with advanced acute leukemia could be cured of their disease with HSCT and that HSCT should be undertaken in the first or

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second remission (i.e., not with active disease) if the patient has an HLA-matched sibling donor because outcomes would be predictably better. Thus, by the mid-1980s, approximately 75% of all allogeneic HSCTs were performed to treat leukemia, and the vast majority were with HLA-identical sibling donors. As supportive care improved, drugs (such as calcineurin inhibitors) became available for graft versus host disease (GvHD) prophylaxis, and the use of alternative donor HSCTs (including matched unrelated donors, mismatched related and unrelated donors, familial haploidentical donors, and umbilical cord blood) was investigated, HSCT became a viable option for many more patients. Furthermore, the development of less intensive conditioning regimens and use of alternative hematopoietic stem cell sources made HSCT a feasible treatment modality for those who would otherwise be ineligible for HSCT. Nowadays, HSCT is a very important treatment modality for both pediatric and adult patients for a wide range of malignant and nonmalignant disorders. This chapter is divided into two major sections with the first part focusing on the seminal discoveries in transplantation biology and immunology using animal models (scientific and preclinical perspective) and with the second part highlighting key human clinical reports related to HSCT (clinical perspective).

Introduction

In the late 1860s, the pathologists, Neumann in Prussia (now Russia) and Bizzozero in Italy, independently reported the observation that mammalian blood cells are derived in the red, spongy areas of the bone, i.e., the bone marrow, and the blood cells exit the bone marrow via small blood vessels that traverse the bone cortex to the peripheral blood. Various attempts at replacing the bone marrow in patients who were perceived as having a deficiency in their bone marrow occurred early on; for example, in 1939, an attempt at treating a patient with aplastic anemia by injection of a few milliliters of the ABO-compatible bone marrow into the patient's sternum was reported. Of note, the patient had no response. It was not until a better understanding of transplantation biology and immunology was achieved first by using animal models and then in human clinical trials did HSCT become successful and eventually become a feasible treatment modality as we know it today.

The first half of this chapter focuses on the landmark observations and discoveries using animal models that led to the successful development of HSCT as we know it today, while the second half of this chapter highlights the studies in humans from the initial attempts at HSCT through the development of alternative donor and hematopoietic stem cell (HSC) transplants and the recognition and improvements in supportive care as well as other barriers to expanding HSCT to the majority of potential patients and how they were overcome.

Another aspect that has significantly contributed to the advancement of HSCT in humans has been the formation of national and international organizations to track and monitor HSCT. These include the establishment of the Center for International Blood and Marrow Transplant Research (CIBMTR) in 1972. It was formed with the goal of setting up a systematic method of collecting HSCT outcome data through collaboration. At the time, there were less than 50 patients who had been transplanted at 12 centers worldwide. Shortly thereafter, the European Society for Blood and Marrow Transplantation (EBMT) was established in 1974 to provide an organization in which scientists and physicians could cooperate to develop HSCT-related clinical studies. In 1993, the American Society for Blood and Marrow Transplantation (ASBMT) was formed. This association was established as a scientific and professional society for those dedicated to the advancement of HSCT. In conjunction with the International Society for Cellular Therapy (ISCT), the ASBMT cofounded the Foundation for the Accreditation of Cellular Therapy (FACT) in 1996.

FACT is a worldwide recognized accreditation program for HSCT centers.

In 1986, the U.S. Navy established the National Bone Marrow Donor Registry (now called the National Marrow Donor Program, NMDP) to establish an organization to facilitate the identification of unrelated HSC donors. Initially, 10,000 potential donors were registered, and the first donor search was performed in 1987. In 2004, CIBMTR and NMDP joined together. To date, the NMDP has facilitated over 74,000 marrow and umbilical cord blood transplants, with almost 6400 transplants a year. NMDP consists of over 150 HSCT centers and over 90 donor centers. Today, CIBMTR represents a large network of centers in over 50 countries and has collected data on more than 425,000 patients.

Scientific and Preclinical Perspective

Pre-World War II to the Mid-1940s

In the early twentieth century, Alexis Camel and colleagues noted that skin and organ transplants function for a time but were eventually rejected after 1–2 weeks. In the 1930s, Gorer, Snell, and colleagues were beginning to investigate the immunologic basis of tumor transplantation in mice; their work led to the discovery of the H2 antigen transplantation system [1, 2]. In the 1940s, Medawar and colleagues established the immunological basis of allograft rejection [3]. Owen et al. developed the concept of "immune tolerance," noting that freemartin bovine dizygotic twins had a mixture of red blood cells from each partner [4]. In the same decade, Billingham, Brent, and Medawar showed that donor-specific tolerance could be induced by injection of donor cells into newborn mice [5]. These seminal discoveries set the stage for further preclinical work performed after World War II that led to the feasibility of HSCT in humans.

Post-World War II to the Mid-1950s

Hematopoietic stem cell transplantation really started to take form post-World War II (late 1940s to early 1950s) when Jacobson et al. found that mice could survive lethal irradiation if the spleen were shielded (i.e., protected) with lead [6, 7]. Based upon this work, Jacobson and his colleagues proposed that humoral factors accounted for these observations that they termed the "humoral hypothesis." In contrast, Lorenz et al. [8] showed that lethally irradiated mice and guinea pigs could survive if they received a retroperitoneal injection of spleen or bone marrow cells that were harvested prior to the irradiation, thus supporting the "cellular hypothesis." These two reports spurred a great debate regarding the drivers of bone marrow recovery, i.e., humoral versus cellular mechanisms.

Further support for the cellular hypothesis was provided by the work of Barnes and Loutit in the mid-1950s. Their experiments showed that bone marrow recovery after spleen or bone marrow infusion was due to living cells and not "humors" [9]. In 1955, Main and Prehn [10] showed that cellular reconstitution (versus humoral factors) was protective against irradiation. They showed that mice that were lethally irradiated and rescued by an autologous bone marrow infusion did not reject subsequent skin grafts indefinitely even across major histocompatibility complex barriers. These experiments provided the proof of acquired tolerance and that this acquired tolerance was conferred by the transfer of living cells. In the following year, Trentin et al. [11] showed that tolerance of the skin graft was specific for the donor strain. Ford et al. [12] went on to show that the bone marrow of lethally irradiated mice rescued by the donor bone marrow or spleen cells displays the cytogenetic characteristics of the bone marrow donor; this was also the first report that used the term "radiation chimera" while referring to the resultant transplanted mouse. Also, in support of the cellular hypothesis, Nowell et al. [13] found that rat bone marrow protects mice against lethal irradiation. They found donor rat bone marrow cells in the bone marrow of the transplanted mice, indicating that the infused donor bone marrow cells can home to and take up residence in the host's bone marrow. In 1956, van Bekkum determined that intravenous administration of hematopoietic stem cells is the optimal route of administration to repopulate the bone marrow and

answered the key question of how to get bone marrow cells to grow in the recipient bone marrow.

Around the same time, Barnes et al. [9] successfully treated murine leukemia with supralethal doses of irradiation and normal bone marrow grafting, suggesting that bone marrow grafting could be used to treat human patients with leukemia. Barnes and colleagues went on to speculate that donor immune cells may have the capacity to destroy residual leukemia cells. This is the first written speculation of the concept of the graft versus leukemia effect.

The Late 1950s to the Late 1960s

The late 1950s ushered in a decade-long period of productive research in transplantation biology and immunology utilizing animal models, ongoing in mice and then in canine models. In 1957, Uphoff et al. [14] discovered that genetic factors control the severity of the immune reaction of donor cells against the host. They also showed that methotrexate can ameliorate the graft versus host reaction that had been noted in mice after they had received lethal irradiation followed by bone marrow or spleen infusion [15]. Billingham and Brent [16] also noted this phenomenon of engrafted donor cells mounting an immune reaction against the host, and they termed it "secondary disease." It was also referred to as "wasting syndrome" because of its associated symptoms of significant weight loss; the presence of poor, unhealthy fur; and generalized scruffiness [17]. This syndrome was subsequently recognized as graft versus host disease (GvHD). Shortly thereafter, Lochte et al. [18] demonstrated that methotrexate could be used not only to treat GvHD but also to prevent it. However, it was not until the development of better immunosuppressants, such as calcineurin inhibitors, that the control of GvHD became feasible.

In the early 1960s, Till and McCulloch [19] showed that the bone marrow contains clonogenic precursors capable of self-renewal and multi-lineage differentiation, i.e., hematopoietic stem cells (HSCs). Also, in the early 1960s, the important roles that the thymus, T-cells, B-cells, and other lymphoid subsets play in transplantation biology were beginning to be recognized by multiple investigators [20–22]. Eventually, Berenson et al. [23] found that the $CD34^+$ cell surface protein was a marker of a subpopulation of bone marrow cells enriched for the ability to engraft and give rise to all cell types of hematopoietic origin, i.e., $CD34^+$ is a marker for HSCs. In 1994, Korbling et al. reported that HSCs can be separated and purified based upon their $CD34^+$ expression of the cell surface [24].

While work continued in transplantation biology with inbred murine models, the use of outbred canine models expanded greatly during the 1960s, and this research yielded critical observations of not only basic transplantation biology but also of improvements in supportive care and better recognition and understanding of complications associated with HSCT. In the early 1960s, investigators showed that dogs could survive two to four times above lethal doses of total body irradiation (TBI) if they were given back previously harvested (fresh or frozen) autologous bone marrow cells after the exposure to TBI. As part of the work done with dogs, Cavins et al. [25] found that HSCs could be obtained from not only the bone marrow but also the peripheral blood.

By the late 1960s, research using dog models that determined the dog leukocyte antigen (DLA) system (which is equivalent to MHC in mice and HLA in humans) was critical in determining the outcomes of allogeneic bone marrow engraftment [26]. Irradiated dogs receiving DLA-mismatched bone marrow from littermates after lethal irradiation died from graft rejection or GvHD, whereas those who received DLA-matched bone marrow from littermates followed by post-HSCT methotrexate were long-term survivors [27–29]. Storb et al. [30] also found that dogs could successfully engraft after receiving chemotherapy alone (i.e., no TBI), with either cyclophosphamide or busulfan. Most of the dogs showed only donor cell engraftment, but some were healthy mixed chimeras. This work suggested that HLA-matched "sibling donor HSCTs" could lead to long-term, healthy chimeras.

The 1970s

As the 1970s began, studies continued to focus on understanding the causes of graft failure. For example, Storb et al. observed in dog models

that blood transfusions from the donor (related or unrelated) prior to transplant can result in sensitization of the recipient to donor transplantation antigens, resulting in graft failure [31].

Clinical Perspective

Post-World War II to the Mid-1960s

Early attempts in human HSCT were only successful in syngeneic HSCTs. In 1949, a report from Poland described the use of bone marrow infusion as a treatment in children with leukemia and other blood disorders [32]. The discovery of human leukocyte antigen (HLA) groups and the development of techniques to perform tissue typing were critical advances to the development of HSCT. In 1954, Miescher and Fauconnet [33] first described antibodies that were induced by transfusion or pregnancy that react with antigens on human white blood cells. In 1958, two other groups (Dausset et al. and van Rood et al.) observed that HLAs were inherited in codominant fashion [34, 35]. Serotyping was initially developed in dogs by Epstein et al. and was eventually developed for HLA [26, 36]. Shortly after these reports describing serotypes were published, international HLA workshops were held during which investigators exchanged reagents, standardized antigen definitions, established a common nomenclature, and developed standardized testing techniques. The advancements in typing techniques are discussed further in Chap. 7. These international workshops have continued on, and today, over 12,000 Class I and over 4500 Class II alleles have been identified [37].

The first attempts of treating humans with supralethal TBI followed by bone marrow grafting were reported in 1957 by Thomas et al., using an identical twin sibling as the donor [38]. In 1959, the same transplant group in Seattle reported the treatment of two patients with advanced leukemia with high-dose irradiation followed by a bone marrow infusion from their respective identical twin sibling [39]. The two patients engrafted and were "leukemia-free" for 4 months. However, they relapsed and succumbed to their disease. These landmark studies showed that TBI followed by an infusion of compatible bone marrow could reconstitute hematopoietic function as well as produce a snit-leukemic effect, albeit not durable, in these cases. Between the mid-1950s and the mid-1960s, approximately 200 HSCTs had taken place worldwide with generally dismal long-term outcomes, as reviewed in the landmark paper by Bortin which was published in 1970 [40].

These early failures were due to a lack of knowledge regarding human histocompatibility and typing, the use of inadequate radiation dosing to provide adequate immunosuppression, the lack of drugs to effectively prevent and treat GvHD, and the selection of patients with such advanced disease to undergo HSCT [41]. In addition, the lack of adequate supportive care such as effective antibiotics and antiviral agents as well as inadequate transfusional support with platelets contributed to these poor outcomes early on in HSCT. In a report from Leiden, the Netherlands, a child with severe combined immunodeficiency disease (SCID) was transplanted with a "matched" unrelated donor bone marrow infusion and four fetal thymuses [42]. While he appeared to show hematopoietic recovery, the child died 2 weeks later of fulminant bacterial pneumonia and possibly GvHD or a generalized autoimmune reaction. In another report from this same era, the Seattle HSCT group transplanted a patient with chronic myelogenous leukemia (CML) in blast crisis [43]. He was conditioned with TBI and received a bone marrow infusion from his "matched" sibling (who was later noted to be a one-antigen mismatch donor). The patient engrafted, but he subsequently died of cytomegalovirus (CMV) pneumonia. As a result, opportunistic infections in the post-HSCT patient population were recognized as a serious, life-threatening barrier for HSCT to succeed. The development of antiviral agents such as ganciclovir and sensitive CMV detection methods positively impacted HSCT outcomes significantly [44–46].

The Late 1960s to the Late 1970s

While the reports of the use of HSCT for the treatment of advanced leukemia in humans were very discouraging early on, the use of HSCT for non-malignant disorders, particularly primary immu-

nodeficiencies, showed promise by the late 1960s. In 1968, Gatti et al. [47] reported the first successful allogeneic bone marrow transplant (BMT) in an infant with severe combined immunodeficiency disease (SCID) using an HLA-identical sibling as the donor. Two other reports of successful HLA-identical sibling BMTs for the treatment of a primary immunodeficiency were published shortly thereafter [48, 49]. All three of these patients remain long-term survivors today. Thomas et al. reported the first successful matched sibling donor allogeneic HSCT for severe aplastic anemia in 1972 [50]. HSCT was being attempted in very few pediatric patients with leukemia prior to 1975 because it was felt that there would be very little chance of a cure in this patient population that had been so heavily pretreated resulting in leukemia that would be very resistant to further treatment.

As the 1970s progressed, consistent donor bone marrow engraftment was achieved in patients with a variety of indications for HSCT. In a two-part series published in the *New England Journal of Medicine* in 1975, Thomas et al. reported the outcomes of the first 100 patients transplanted in Seattle [51, 52]. Of the 100 patients, 73 had advanced leukemia, whereas 37 had severe aplastic anemia. Overall, 50% of the patients with severe aplastic anemia had successful outcomes, and the number of patients with advanced leukemia who achieved remission post-HSCT was increasing. However, the consistent, successful allogeneic donor engraftment resulted in an increased incidence of GvHD, with acute GvHD occurring in approximately 50% of patients despite the long-term use of methotrexate. While the entity of GvHD had been recognized as a serious, potentially life-threatening consequence of allogeneic transplantation as a "wasting syndrome" in mice some 20 years prior, GvHD was not recognized as a serious barrier to moving HSCT forward in humans until the early 1970s with the advent of consistent successful engraftment of allogeneic matched sibling donor bone marrow in humans. Then, patients were dying from GvHD despite the use of methotrexate which was found to prevent GvHD in only about 50% of HSCT patients. It was not until 1978 when Powle et al. [53] described the first use of the calcineurin inhibitor cyclosporine A to treat GvHD in humans that the option of allogeneic HSCT in humans became more accessible to a larger group of patients.

In 1982, Deeg et al. [54] reported the successful use of a short course of methotrexate with cyclosporine A as GvHD prophylaxis in dogs. In 1986, Storb et al. [55] reported that a short course of methotrexate (days 1, 3, 6, and 11) in combination with daily cyclosporine A decreased the incidence of acute GvHD in matched sibling donor transplants to 20–30%. This combination is still considered the "gold standard" for GvHD prophylaxis today. Other approaches to reduce the incidence of GvHD were investigated at this time. In 1981, Reisner et al. [56] reported that T-cell depletion of the donor graft could decrease the risk of GvHD.

The Mid-1970s to the Late 1970s: Allogeneic HSCT Is Curative for Leukemia

In the first half of the 1970s, the advancement of HSCT as a viable treatment modality was somewhat stalled because the patients undergoing HSCT were typically patients with otherwise incurable, end-stage leukemia, and they either died from their disease or succumbed to GvHD or opportunistic infections, as described above. However, the HSCT group in Seattle published the results of 100 patients with advanced leukemia who had undergone matched sibling donor BMT [57]. Of these 100 patients, 13 were long-term survivors, demonstrating that some patients with end-stage, advanced leukemia could be cured with allogeneic HSCT. Thus, it was hypothesized that patients who undergo HSCT in the first remission (and not waiting until they relapse) may have a better chance for a cure. In Germany, in the late 1970s, the Berlin-Frankfurt-Munster (BFM) and the CoALL groups took this approach of transplanting patients with relapsed leukemia shortly after achieving a remission immediately after completion of induction chemotherapy [58]. Thomas et al. reported the successful treatment of patients with acute myeloid leukemia (AML) or with acute lymphoblastic leukemia (ALL) in the first remission with matched sibling donor HSCT [59, 60]. Dopfer et al. [61] reported that this treatment strategy was more beneficial for pediatric

patients with relapsed leukemia. Subsequent trials supported this observation. Now, it is well established that the lower tumor burden (i.e., low or no minimal residual disease (MRD) detected) is associated with superior outcomes [62].

Acute leukemias treated with HSCT were not the only type of leukemia being investigated. In 1979, Fefer et al. reported the disappearance of Ph+ chromosome in four patients with chronic myelogenous leukemia who were treated with chemotherapy and irradiation followed by an identical twin sibling donor BMT [63]. Two subsequent studies demonstrated that the treatment of CML in the chronic phase with chemotherapy and TBI followed by allogeneic BMT from a matched sibling donor was successful [64, 65]. Two large studies supported the successful outcomes of patients with CML treated with allogeneic HSCT [66, 67]. Treatment with HSCT of CML in the chronic phase was the standard of care until the development of BCR-ABL-targeted therapies such as imatinib and dasatinib. Nowadays, HSCT for CML patients in blast crisis is still considered the standard of care.

By the late 1970s, multiple reports noted that there was a decreased incidence in relapse of leukemia in patients with GvHD, and in a few patients, decreasing immunosuppression could lead to a remission of leukemia post-HSCT. These were the first inklings in humans of the concept of the "graft versus leukemia effect" in which the immune cells from the donor are capable of recognizing the leukemia cells as "bad" and eliminate (or at least disarm) them. This is the same concept proposed by Barnes after analyzing his leukemic mice studies over 40 years earlier [9]. The concept that HSCT serves as an immunotherapy (and not just a method to eliminate tumors cells) was supported by the observation that the infusion of donor lymphocytes along with the discontinuation of all immunosuppression can induce a remission. This approach of donor lymphocyte infusion (DLI) was first utilized successfully in CML and then in Epstein-Barr virus post-transplant lymphoproliferative disease (EBV-PTLD) [68, 69]. Kolb et al. reported that relapse of CML post-HSCT could be successfully placed back into remission with donor lymphocyte infusions (DLI) [70]. In 1994, Papadopoulos et al. reported the successful use of DLI for the treatment of EBV-PTLD [69]. These reports were among the first to suggest that the donor HSC graft not only "replaces" the recipient's immune system that is eliminated by myeloablative conditioning but also acts as immunotherapy for the treatment of the underlying malignancy, i.e., creating a graft versus malignancy effect. It is now a well-established practice to use DLI if a post-HSCT patient shows signs of impending relapse (such as decreasing donor chimerism) or has a frank relapse of his/her leukemia (see Chap. 11).

The 1980s to the Present: Expansion of the Application of HSCT, Refinement of Conditioning Regimens, and Development of Alternative Donor HSCT

Expansion of the Application of HSCT: While HSCT was being actively investigated for the treatment of leukemia, HSCT was also being explored for the treatment of nonmalignant conditions beyond primary immunodeficiencies. It was not until the 1980s that HSCT was tested in humans as a curative treatment for hemoglobinopathies. In the early 1980s, the first successful matched sibling donor BMTs for thalassemia were performed. In 1982, Thomas et al. [71] reported the successful transplantation of a patient with thalassemia major using an allogeneic matched sibling donor. In 1984, Lucarelli et al. [72] reported the first successful outcomes of treating children with thalassemia with BMT. In that same year, Johnson et al. reported the case of an 8-year-old girl who underwent allogeneic matched sibling donor BMT for AML [73]. She also had sickle cell disease. The HSCT not only cured her of AML but also cured her of sickle cell disease. Currently, matched sibling donor HSCT is performed routinely for patients with thalassemia major and for patients with sickle cell disease with certain high-risk factors. Alternative HSCTs, such as familial haploidentical HSCTs, are currently being investigated (see Chap. 4).

Refinement of Conditioning Regimens: Initially, total body irradiation (TBI) was delivered as a single fraction alone as the conditioning

regimen. However, it was shown that TBI delivered in multiple fractions at a lower dose was superior to delivering it as a high-dose, single fraction [54, 74]. Because irradiation can cause such devastating long-term sequelae, particularly in young patients, conditioning regimens that avoid TBI such as busulfan/cyclophosphamide (Bu/Cy) were being explored in the early 1980s [75, 76]. In the following decade, multiple reports of the use of non-TBI conditioning regimens were reported but with mixed results. Clift et al. in 1994 reported no difference in a Bu/Cy versus a cyclophosphamide/TBI (Cy/TBI) regimen in event-free survival (EFS) [77]. In contrast, the first HSCT studies for patients with AML showed that outcomes were better with Cy/TBI versus cyclophosphamide/busulfan conditioning regimens; it is notable that this study was performed before the availability of intravenous busulfan [78]. In a subsequent study from 1997, Long et al. showed that cyclophosphamide/etoposide/TBI as a conditioning regimen demonstrated efficacy in patients with high-risk leukemia [79].

In the late 1990s, the concept of non-myeloablative (NMA) conditioning was being actively explored. Giralt et al. [80] demonstrated that patients conditioned with a purine analogue-based (i.e., fludarabine), NMA conditioning regimen resulted in successful engraftment. Shortly thereafter, multiple groups reported the use of NMA conditioning followed by HSCT in elderly patients with hematologic malignancies who would otherwise not tolerate a myeloablative conditioning regimen [81]. The use of NMA conditioning was also being actively investigated for patients who would have no benefit from the graft versus malignancy effect (and thus no need for GvHD), such as patients with primary immunodeficiencies or hemoglobinopathies [82]. However, many of the initial studies in patients with hemoglobinopathies were not very successful because a significant proportion of patients lost their grafts and reverted back to their chronic disease state despite initially engrafting.

In 2001, Giralt et al. introduced a conditioning regimen of fludarabine/melphalan as a reduced-intensity conditioning (RIC) regimen [83]. In 2005, Rao et al. [84] showed a significant survival advantage after RIC (versus myeloablative conditioning) followed by matched unrelated donor HSCT in children with primary immunodeficiency. Time to engraftment, chimerism, immune reconstitution, and incidence of GvHD were comparable. Of note, RIC was associated with increased viral reactivation.

Development of Alternative Donor HSCT: Because not every patient who may benefit from a HSCT has a suitable donor, alternative sources of donor HSCs have been actively pursued. These alternative donor sources needed to be safe and not result in increased morbidity and mortality. These types of HSCTs are referred to as alternative donor HSCTs (i.e., an alternative to matched sibling donors). In order for alternative donor HSCTs to be effective in humans, the mechanisms by which HSCs are regulated and donor grafts are rejected needed to be elucidated, and the pathophysiology of GvHD needed to be better understood. Work in these areas became actively investigated when Knudtzon et al. [85] first reported the in vitro growth of HSCs isolated from human umbilical cord blood in 1974. The first use of umbilical cord blood (UCB) as the HSC source for allogeneic matched sibling donor HSCT occurred in 1988 [86]. The patient had Fanconi anemia. In 1995, Broxmeyer proposed the use of unrelated UCB as an alternative HSC donor source. Shortly thereafter, UCB unit banks were established across the world. The first study of the use of unrelated UCB as the HSC source in 25 children with a variety of indications was reported in 1996 [87]. This study demonstrated that unrelated mismatched UCB HSCT was a feasible alternative donor HSCT. Because a UCB unit has a fixed HSC dose, its use was initially limited to children and small adults to minimize the risk of graft failure. However, Barker et al. reported the use of two unrelated UCB units in the same patient with no untoward effects [88]. These findings resulted in the expansion of UCB HSCT to adults as well as pediatric patients.

While it was known that HSCs can be found in the peripheral circulation, the absolute number of HSCs is low. However, it was observed that this number would be higher in a cancer patient's peripheral blood when recovering from chemotherapy. Investigators took advantage of this rebound effect and used low-dose cyclophospha-

mide to promote the release of HSCs into the peripheral blood, while others used endotoxin to evoke a similar response [89, 90]. With the availability of the cloned hematopoietic growth factors, granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF), mobilization of HSCs, and collection by apheresis became feasible [91–93]. Juttner et al. [94] reported the use of peripheral blood HSCs for autologous HSCT for AML. Shortly thereafter, it was shown that GM-CSF [92] and G-CSF [95] could be used in humans to stimulate and mobilize $CD34^+$ HSCs into the periphery for pheresis and then used for autologous HSCT. Nowadays, growth factor-mobilized peripheral blood HSCs are used as the stem cell source for both autologous and allogeneic HSCTs from both related and unrelated donors.

In addition, the late 1990s marked the advent of the use of partially mismatched donors [96, 97]. Furthermore, Reisner et al. [56] reported the use of a familial haploidentical donor as the HSC source for a patient with SCID that is now being actively investigated for multiple indications, including sickle cell disease, severe aplastic anemia, and leukemia, for patients who do not otherwise have a suitable donor.

Key Points

- With a better understanding of transplantation biology and immunology using animal models, HSCT in humans became feasible.
- With the detonation of the two atomic bombs at the end of World War II in the 1940s, interest in HSCT as a treatment for exposure to lethal doses of irradiation reached prominence.
- While HSCT was attempted as early as the 1940s, it was not until the late 1960s that HSCT resulted in long-term, disease-free survivors which consisted of three infants with primary immunodeficiency.
- In the late 1970s, allogeneic matched sibling donor HSCT was demonstrated to induce long-term remissions in a fraction of patients with advanced, end-stage leukemia, suggesting that HSCT may be more effective if performed in patients in the first or second remission. This proved to be true.
- In the 1980s and 1990s, advances were made that resulted in HSCT becoming widely available for patients who would otherwise be ineligible for HSCT. These advances include improvements in supportive care, the development of less intensive conditioning regimens, and the availability of alternative donors and HSC sources.

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3 Brief Introduction to the Basic Scientific Principles of Hematopoietic Stem Cell Transplantation (HSCT)

Valerie I. Brown

Abstract

The primary function of the immune system is to provide essential defense mechanisms against all foreign pathogens. The immune system has evolved in such a way that different immune responses are optimized to recognize and then eliminate or contain different types of foreign antigens which are expressed or secreted by foreign pathogens. It provides not only efficient and effective killing of microbes/pathogens via innate immunity but also specific long-lasting immunity against a particular microbe/pathogen to be triggered if the foreign microbe's antigen is encountered in the future via adaptive immune responses. Immunologic mechanisms are intimately involved in engraftment, engraftment rejection, graft versus host disease, and graft versus malignancy effect. In addition, immunologic tolerance is key for allogeneic immune reconstitution post-hematopoietic stem cell transplantation (HSCT). Because of a better understanding of the immune system and its different immune properties and responses, physicians and researchers have been able to perform successfully and safely HSCT in humans. While many of the concepts of basic immunology and transplant biology are intertwined into other chapters of this book, this chapter focuses on providing the fundamental principles of basic immunology and transplant biology, including the development of the components of the immune system (i.e., hematopoiesis), the molecules, cells, tissues, and organs that make up the immune system as well as their structural and functional organization and the types of immune responses along with their cardinal features. Key concepts related to HSCT including antigen presentation, alloreactivity, and tolerance and how these processes relate to HSCT

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will be described in brief. Firstly, though, this chapter begins with the definitions of some key terms and concepts related to basic immunology and transplant biology in order to establish the "vocabulary" of the immune system.

Introduction

The overarching function of the immune system is to serve as vital defense against foreign substances. Different mechanisms of defense have evolved against different pathogens. Knowledge of the immune system and its different responses has permitted physicians and researchers to successfully perform hematopoietic stem cell transplantation (HSCT) with long-term engraftment and success in humans. This chapter presents the major tenets of hematopoiesis, the organization of the immune system, different immune responses, and how this information relates to HSCT, starting with definitions of some of the key molecules, cells, functions, and concepts of the immune system.

Definitions

A vocabulary has been developed to describe the immune system and all of its components and processes. This section provides the definition of some of these keywords and concepts.

Immunity: Immunity refers to the protection against disease, particularly infections, that is mediated by a collection of cells, tissues, and molecules called the immune system. Immunity also refers to the ability to respond to any foreign substance, infectious and noninfectious.

Immune system: The immune system consists of the highly integrated collection of all the cells, tissues, organs, and molecules that provide protection against foreign organisms and substances. The immune system is responsible for immunity.

Immune response: An immune response refers to the collective and highly orchestrated response by immune molecules and cells to a foreign substance (e.g., microbes and their components), although noninfectious agents, such as proteins, polysaccharides, and chemicals, can elicit an immune response. An autoimmune response is the pathologic immune response to self-molecules that very often has detrimental effects.

Innate immunity: Innate immunity provides protection against infection via rapid, pre-existing responses to microbes with the same reaction (with the same intensity, time to initiation, and duration) to repeated infections. Components of innate immunity include cells (phagocytes, e.g., neutrophils, macrophages, and NK cells) and cytokines (predominantly produced by dendritic cells and mononuclear phagocytes), the complement system, and epithelial barriers.

Adaptive immunity: Adaptive (or acquired) immunity is stimulated by exposure to foreign substances and is characterized by exquisite sensitivity, specificity, and memory. Its specificity for distinct macromolecules and its memory allows for a more rapid and vigorous response with repeated encounters to the same foreign pathogen; it is mediated by lymphocytes.

Humoral immunity: Humoral immunity is a type of an adaptive immune response that is the principal defense against extracellular microbes and their toxins. Humoral immune responses are mediated by antibodies that are produced by activated B-cells.

Cell-mediated immunity: Cell-mediated (or cellular) immunity provides defense against intracellular microbes that either have infected a host cell or have been ingested by a phagocyte. Cell-mediated immune responses are mediated by T-cells predominantly of two different phenotypes: $CD4^+$ helper T-cells that mediate phagocyte activation and $CD8^+$ cytotoxic T-cells that are responsible for directly killing infected cells.

Homeostasis: Homeostasis is the state of the adaptive immune system that maintains a constant number and diverse repertoire of lymphocytes. It is a balance that is achieved by the regulation of death, inactivation, and expansion/proliferation of lymphocytes.

Tolerance: Tolerance is characterized by the unresponsiveness to antigens by the adaptive immune system that leads to inactivation or death of antigen-specific lymphocytes. Tolerance is the mechanism by which the immune system tolerates (or ignores) self-antigens and does not attack

self-tissues whereas tolerance of foreign antigens may be induced under certain circumstances and may be detrimental in the long term.

Antigen: A molecule that induces a specific immune response or is recognized by T-cells or B-cells as well as antibodies is an antigen. An antigen binds to an antibody or the T-cell receptor (TCR). An antibody can bind to an antigen alone whereas most TCRs bind to an antigen peptide fragment only when it is complexed with "self" MHC molecules.

Cytokine: Any secreted protein that regulates, stimulates, suppresses, and/or coordinates the activities of cells of the immune system is all classified as cytokines. Cytokines also mediate inflammatory reactions. Cells of the immune system secrete at least one cytokine and express specific signaling receptors for several cytokines. This expression is dynamic and often stochastic. Interleukins, chemokines, tumor necrosis factor (TNF), and interferons are all considered cytokines.

Chemokine: Chemokines are subsets of cytokines that regulate cell movement, migration, and chemotaxis. Chemokines maintain the localization of T-cell subsets and APCs within lymphoid organs.

Major histocompatibility complex (*MHC*)/*human leukocyte antigen* (*HLA*): Major histocompatibility complex (MHC) is the large genetic locus that contains the highly polymorphic genes which encode the peptide-binding molecules most commonly recognized by the T-cell receptor on the cell surface of T-cells. The human leukocyte antigen (HLA) locus is the equivalent to MHC in humans, and this locus is located on the short arm of chromosome 6 in humans. MHC molecules are expressed on the cell surface. The two major classes of MHC are Class I and Class II. MHC Class I molecules are polymorphic proteins that help to display peptide fragments of protein antigen derived from the cytosol on the cell surface of APCs for recognition by T-cells. This antigen peptide-MHC Class I complex is typically recognized by $CD8^+$ T-cells. MHC Class I molecules are expressed mostly on all nucleated cells. In contrast, the antigen peptide-MHC Class II complex, which is made up of polymorphic heterodimeric proteins, is also located on the cell surface but restricted to dendritic cells, macrophages, and B-cells, i.e., antigen-presenting cells. It displays antigen peptides derived from extracellular proteins that have been digested, processed into small peptide fragments, and then displayed on the cell surface of APCs for recognition by $CD4^+$ helper T-cells.

Alloantigen: An alloantigen is an antigen that is expressed on cells or tissues from one individual that is recognized as foreign by another individual.

Alloreactive: T-cells or antibodies that recognize and react to antigens (alloantigens) on cells or tissues from another individual are said to be alloreactive.

Effector cell: An effector cell is an immune cell with effector functions during an immune response, killing microbe-infected cells ($CD8^+$ cytotoxic T-cells), killing microbes (macrophages), secreting cytokines to enhance an immune response ($CD4^+$ helper T-cells), and secreting antibodies (differentiated B-cells).

Cluster of differentiation (*CD*) *nomenclature*: The cluster of differentiation (CD) nomenclature was established initially to name uniformly cell surface molecules in order to characterize cells of a particular lineage or stage of differentiation. They leave a defined structure and are recognized by a cluster of monoclonal antibodies. Each cell surface molecule is designated by CD. A specific constellation of CD molecules can identify a specific immune cell subtype, termed immunophenotype. For example, CD3 represents the T-cell receptor and is considered a marker for T-cells. While both helper and cytotoxic T-cells express CD3 on the cell surface, the expression of CD8 distinguishes cytotoxic T-cells from other T-cell subsets, whereas helper T-cells are $CD3^+$, $CD4^+$, and $CD8^-$. This CD marker system is used beyond cells of the immune system and is used to uniformly name molecules found on all cell types in the body.

Hematopoiesis, Its Regulation, and Cells of the Immune System

Hematopoiesis

Hematopoiesis refers to the highly regulated process by which all mature blood cells (i.e., leukocytes, erythrocytes, and platelets) are produced from pluripotent stem cells. Figure 3.1 represents a depiction of the lineage differentiation tree [1].

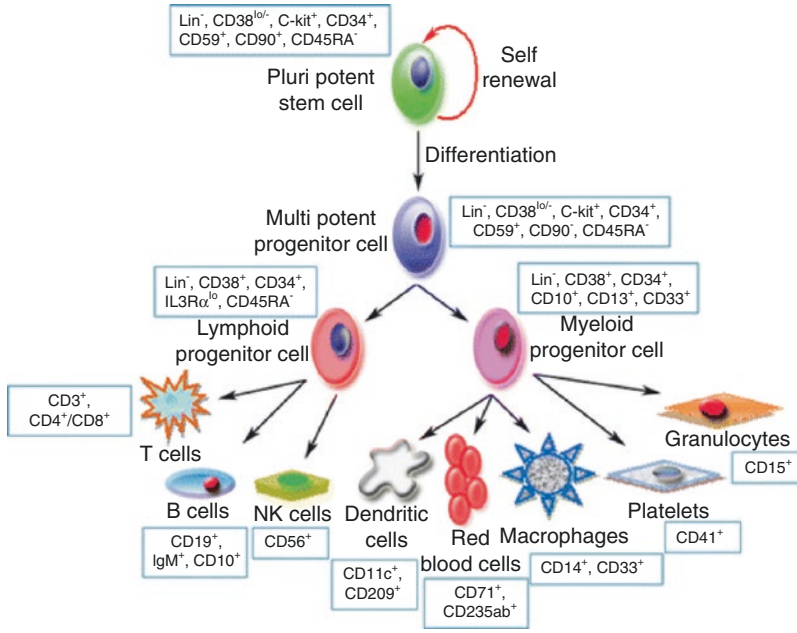

Fig. 3.1 Hematopoietic stem cell differentiation. Differentiation of hematopoietic pluripotent stem cells into multipotent progenitor cells which then differentiate into distinct hematopoietic lineages. This is the best example of stem cell differentiation, and the niche defines specific differentiation events in the bone marrow, spleen, or liver (Reprinted from: Vira, Darchni, Basak, Saroj K., Veena, Mysore S., Wang, Marilene B., Batra, Raj K., Srivatsan, Eri S. Cancer stem cells, microRNAs, and therapeutic strategies including natural products. Cancer and Metastasis Reviews. 31(3): 733–751, 2012, with permission from Springer) [1]

In humans, primitive hematopoiesis starts at d18 of gestation in the blood islands in the yolk sac. Only nucleated erythroblasts and, to a lesser extent, macrophages and megakaryocytes are produced there. Then, hematopoiesis moves to the aorta-gonad-mesonephros (AGM) region in the embryo where primitive hematopoietic stem cells (HSCs) are exposed to a microenvironment that promotes the transition to definitive HSCs. From there, these definitive HSCs migrate to the fetal liver where they undergo extensive expansion. During the second trimester, HSCs migrate to their specific niches within the bone marrow where they reside for the remainder of a person's life. Thus, humans are born with "adult" HSCs. At birth, hematopoiesis takes place in virtually all of the bones, but, as we age, hematopoiesis becomes more restrictive. By puberty, hematopoiesis occurs exclusively in the bone marrow of the flat bones, i.e., the sternum, vertebrae, iliac bones, and ribs. While the majority of hematopoiesis occurs in the bone marrow with the majority of HSCs residing in the bone marrow, HSCs can function and provide hematopoiesis in extramedullary sites, primarily in the liver and spleen (see Fig. 3.2, [61]).

HSCs that have the two properties of reconstituting and self-renewal capacity are referred to long-term hematopoietic stem cells (LT-HSCs) and are identified by the immunophenotype of Lin^-, $CD34^+$, $CD38^-$, $CD90^+$, and $CD45RA^-$. The self-renewal property is defined as follows: when a stem cell divides, one of the daughter cells goes on to differentiate, while the other daughter cell does not go on to differentiate, but instead maintains the properties of a stem cell. In the homeostatic state, the majority of the cells that make up the LT-HSC pool are quiescent with only a small proportion undergoing cell division. Cellular senescence is the state in which cells no longer divide although they remain metabolically active. Senescence is governed by telomere length. Telomerase maintains the ends of chromosomes to protect telomere shortening that would otherwise occur with each cell division. Most mature cells do not express telomerase, and thus telomere shortening is associated with aging

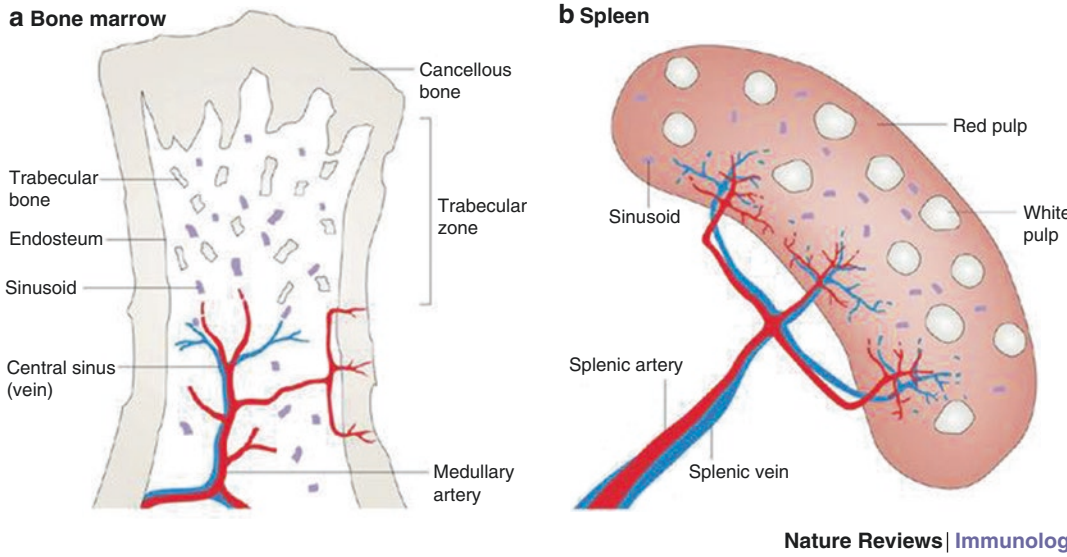

Fig. 3.2 Anatomy of the adult hematopoietic organs, bone marrow, and spleen. (**a**) Hematopoietic stem cells (HSCs) reside primarily within the bone marrow during adulthood. The bone marrow is a complex organ containing many different hematopoietic and non-hematopoietic cell types. Hematopoiesis occurs within the medullary cavity, surrounded by a shell of vascularized and innervated cancellous bone. Minute projections of the bone (trabeculae) are found throughout the trabecular zone of the bone, such that many cells in this region are close to the bone surface. The interface of the bone and bone marrow is known as the endosteum, and this is covered by bone-lining cells that can differentiate into bone-forming osteoblasts. Bone-resorbing osteoclasts are also present at the endosteum. Arteries carry oxygen, nutrients, and growth factors into the bone marrow, before feeding into capillaries and then sinusoids, which coalesce to form the venous circulation. Sinusoids are specialized venules that form a reticular network of fenestrated vessels that allow cells to pass in and out of circulation. (**b**) HSCs can also be found at low levels in extramedullary tissues such as the spleen and liver throughout adult life. When bone-marrow hematopoiesis is impaired by age, cancer, or myeloablation, expanded numbers of HSCs can engage in extramedullary hematopoiesis in the spleen. HSCs reside around sinusoids in the red pulp of the spleen, but not in the white pulp, which contains lymphocytes and antigen-presenting cells (Reprinted by permission from Macmillan Publishers Ltd: Kiel MJ and Morrison SJ. Uncertainty in the niches that maintain hematopoietic stem cells. Nature Reviews Immunology. 8:290–301, 2008 [61])

and cell senescence. Normal HSCs exhibit telomere shortening with serial transplantations [2]. These LT-HSCs give rise to multipotent cells referred to as short-term (ST-) HSCs. ST-HSCs have a limited to no capacity of self-renewal but can provide multilineage reconstitution, albeit transient. A higher percentage of ST-HSCs enter the cell cycle daily as compared to LT-HSCs.

ST-HSCs can go on to become the oligopotent progenitors, common myeloid progenitors (CMP) and common lymphoid progenitors (CLP). After multiple steps of differentiation, CMPs and CLPs will ultimately give rise to all terminally differentiated components of blood. Differentiation of CMPs will eventually lead to the development of platelets, erythrocytes, granulocytes, and macrophages, whereas all mature B-, T-, and NK cells are derived from CLPs. Dendritic cells can be derived from either CMPs or CLPs.

Hematopoiesis is a process that is strictly regulated by highly orchestrated interactions of molecular (noncellular) and cellular constituents. The regulation of proliferation and differentiation of these progenitor and precursor cells (i.e., hematopoiesis) is driven, for the most part, by cytokines and growth factors that are secreted by stromal cells and macrophages contained within the bone marrow. The major cytokines with their source, principal targets, and principal cell type induced are enumerated below and summarized in Table 3.1.

Key Cytokines of Hematopoiesis

Below is a list of cytokines that play important roles in hematopoiesis:

SCF: Stem cell factor (SCF) (otherwise known as c-kit ligand) is secreted by stromal

Table 3.1 Summary of key cytokines and growth factors that regulate hematopoiesis

	SCF	IL-3	IL-7	GM-CSF	G-CSF	M-CSF	Flt-3 ligand
Principal cellular source(s)	• Bone marrow stromal cells	• T-cells	• Fibroblasts • Bone marrow stromal cells	• Activated T-cells • Macrophages • Endothelial cells • Fibroblasts	• Activated T-cells • Macrophages • Fibroblasts • Endothelial cells	• Macrophages • Endothelial cells • Bone marrow cells • Fibroblasts	• Bone marrow stromal cells
Principal progenitor/ precursor target(s)	• HSCs	• Immature progenitors	• Immature lymphoid progenitors	• Immature and CMPs • Mature macrophages	• Committed granulocyte progenitors	• Committed progenitors	• HSCs • Dendritic cell progenitors • B-cell progenitors
Principal cell type(s) induced	• All cell types	• All cell types	• T-cells • B-cell precursors	• Granulocytes • Monocytes • Macrophage activation	• Granulocytes	• Monocytes	• Classical and plasmacytoid dendritic cells • B-cells

SCF stem cell factor, *HSCs* hematopoietic stem cells, *IL-3* interleukin-3, *IL-7* Interleukin-7, *GM-CSF* granulocyte-macrophage colony-stimulating factor, *CMPs* common myeloid progenitors, *G-CSF* granulocyte colony-stimulating factor, *M-CSF* macrophage colony-stimulating factor

cells of the bone marrow. It acts on pluripotent hematopoietic stem cells, inducing maturation of all hematopoietic lineages. Its receptor is KIT.

IL-3: Interleukin-3 (IL-3) is principally secreted by T-cells and targets immature hematopoietic progenitor cells to induce the maturation of all hematopoietic lineages.

IL-7: Interleukin-7 (IL-7) which is preferentially secreted by fibroblasts and bone marrow stromal cells plays an important role in the proliferation of B- and T-cell precursors as well as differentiation of B- and T-cells. It also regulates the survival of naïve and memory T-cells.

GM-CSF: Granulocyte-macrophage colony-stimulating factor (GM-CSF) is produced by activated T-cells, macrophages, endothelial cells, and fibroblasts within the bone marrow stroma. The primary functions of GM-CSF are to stimulate the proliferation of macrophages, monocytes, and neutrophils.

G-CSF: Granulocyte colony-stimulating factor (G-CSF) is produced by activated T-cells, macrophages, and endothelial cells at the site of inflammation and/or tissue damage that acts on the bone marrow to stimulate proliferation and mobilization of neutrophils to replace those that have been consumed in inflammatory reactions.

M-CSF: Monocyte colony-stimulating factor (M-CSF) is secreted by macrophages, endothelial cells, bone marrow cells, and fibroblasts. It acts on committed hematopoietic progenitors to induce the maturation of monocytes. Its receptor is CSF1R.

Flt-3 ligand: Flt-3 ligand is secreted by bone marrow stromal cells. It targets HSCs as well as progenitors of dendritic cells and B-cells to induce the maturation to classical plasmacytoid dendritic cells and B-cells. Flt-3 ligand binds to the Flt-3 tyrosine kinase receptor on precursors of dendritic and B-cells.

Cells of the Immune System

Phagocytes (*neutrophils and macrophages*): The primary role of phagocytes is to ingest and destroy microbes as well as eliminate damaged tissue. Phagocytes are part of innate immunity. They respond in the same way to repeated exposures of the same microbe(s) in a stepwise fashion. After recruitment to the site of infection or tissue damage and the recognition of microbes, phagocytes are activated, resulting in the ingestion of microbes by phagocytosis and then the destruction of the ingested microbes. Phagocytes also play a role in the effector phase of some adaptive immune responses. Phagocytes consist of neutrophils (also called polymorphonuclear leukocytes) and mononuclear phagocytes.

Neutrophils: Neutrophils are the most abundant white blood cell type in the blood circulation. The cytoplasm of neutrophils is loaded with two types of granules filled with molecules that are poised to destroy ingested microbes and damaged cells. The majority of these granules are called specific granules. These granules are filled with lysozymes, collagenase, and elastase. The other predominant type of granule is the azurophilic granule which is a lysosome that contains enzymes along with other molecules (including defensins and cathelicidins which are microbicidal). Neutrophils typically are short-lived, just 1–2 days.

Macrophages: Mononuclear phagocytes include monocytes which are circulating mononuclear phagocytes and differentiate into macrophages when they reside in tissues. The most abundant type of monocyte is the classical monocyte. Classical monocytes are rapidly recruited to sites of infection or tissue damage and secrete abundant amounts of inflammatory mediators. In contrast, nonclassical monocytes promote tissue repair after injury and patrol along endothelial surfaces looking for areas in need of repair. Macrophages are derived from circulating monocytes and mature into specified macrophages once they migrate from the circulation. Once they enter a tissue, they become long-lived and specialized according to their tissue of residence. The most common tissues include those of the liver, brain, spleen, lungs, peritoneal cavity, and skin (see Fig. 3.3) (reviewed in [3]). For example, Kupffer cells are macrophages that live in the sinusoids of the liver, whereas microglial cells are macrophages

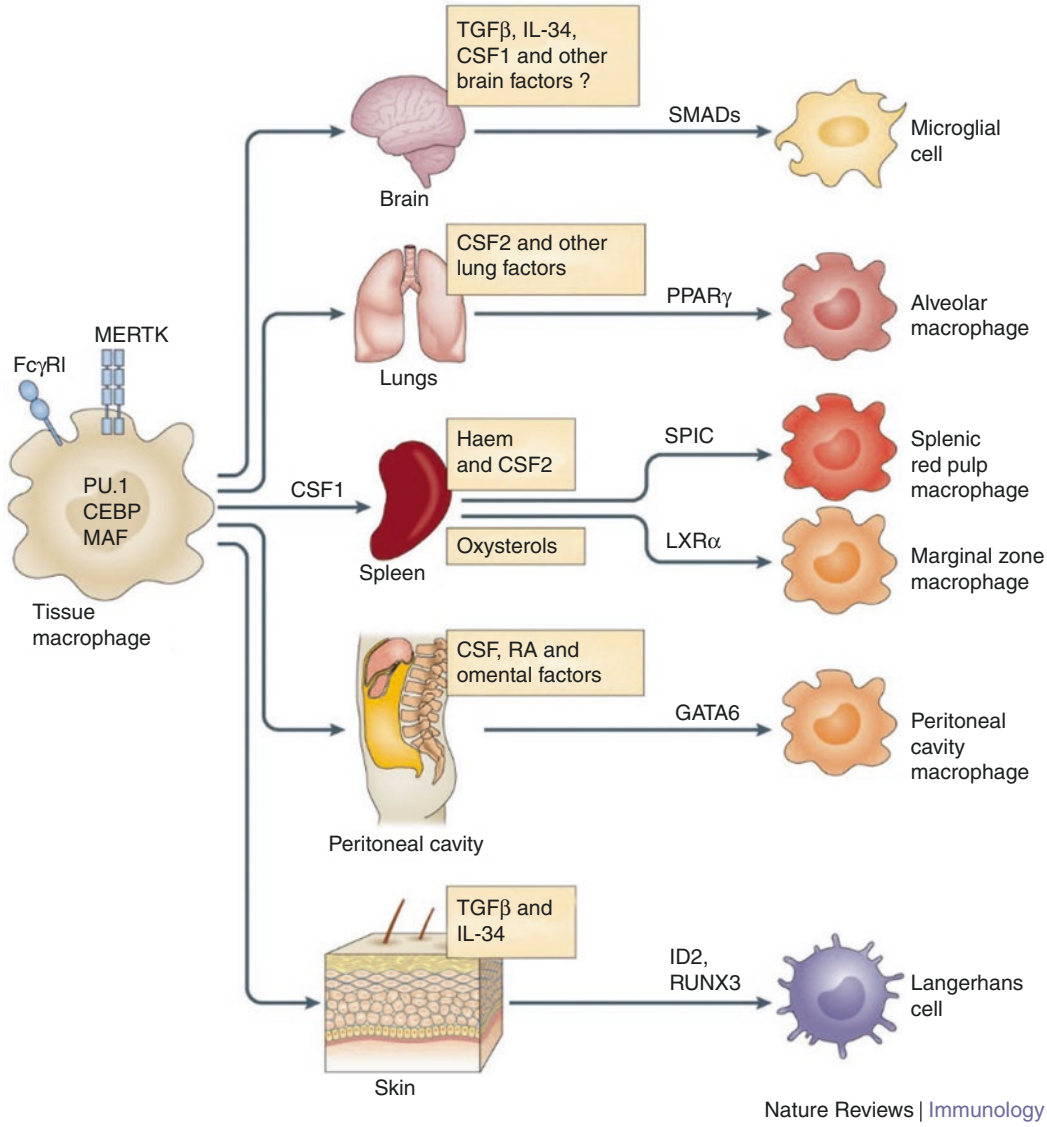

Fig. 3.3 The tissue microenvironment determines macrophage differentiation cues. During embryonic development, macrophages enter the tissues where they self-renew and proliferate. Macrophages in all tissues are characterized by expression of the cell surface marker FcγRI (also known as CD64), tyrosine-protein kinase MER (MERTK), and the transcription factors PU.1, CCAAT/enhancer-binding protein (CEBP) family members, MAF, and MAFB. In the tissues, macrophage identity and functions are shaped by cytokines and metabolites that are produced in the local environment and drive specific transcription factor expression. In the brain, incoming yolk sac-derived cells are exposed to locally express transforming growth factor-β (TGFβ), which drives Smad phosphorylation and the expression of genes that are unique to microglia. In the lungs, fetal monocytes that are exposed to colony-stimulating factor 2 (CSF2) express peroxisome proliferator-activated receptor-γ (PPARγ), which drives their differentiation into alveolar macrophages. In the spleen, haem drives SPIC expression, which controls the differentiation and maintenance of red pulp macrophages and the expression of key splenic red pulp macrophage-specific molecules, including vascular cell adhesion molecule 1 (VCAM1). In the marginal zone of the spleen, macrophage maintenance depends on liver X receptor-α (LXRα)-mediated signals. Retinoic acid (RA) and omental factors induce the expression of GATA-binding protein 6 (GATA6), which promotes the differentiation of peritoneal cavity macrophages. ID2, inhibitor of DNA binding 2; IL-34, interleukin-34; RUNX3, runt-related transcription factor 3 (Reprinted by permission from Macmillan Publishers Ltd: Lavin Y., Mortha A., Rahman A., Merad M. Regulation of macrophage development and function in peripheral tissues. Nature Reviews Immunology. 15:731–744, 2015 [3])

that reside in the brain. Splenic red pulp and marginal zone macrophages reside in the spleen and alveolar macrophages in the lungs. It was thought that Langerhans cells in the skin were macrophages, but data have shown that they are actually derived from dendritic cells (reviewed in [4]). The major function of macrophages is to ingest and destroy molecules by producing reactive oxygen and nitrogen species that are toxic to microbes and proteolytic degradation. Macrophages also ingest dead cells as well as apoptotic cells before they can release their toxic contents and trigger an inflammatory response. Activated macrophages secrete cytokines that promote recruitment of more monocytes and neutrophils into the infected and/or injured areas to amplify the immune response. Macrophages can also act as antigen-presenting cells (APCs) (see "Antigen-Presenting Cells" section below). In addition, they also promote the repair of damaged tissues, stimulating angiogenesis and fibrosis. Macrophages can undergo classical or alternative activation. Classical activation results in macrophages that are efficient in the ingestion and killing of microbes, whereas alternative activation results in macrophages that promote tissue remodeling and repair. Unlike neutrophils, macrophages are not terminally differentiated, and they can divide at the site of inflammation.

Mast cells, *basophils*, *and eosinophils*: Mast cells, basophils, and eosinophils make up a small percentage of white blood cells (or leukocytes) and are called granulocytes because their cytoplasm contains abundant granules filled with various inflammatory and microbicidal substances. Mast cells mediate allergic reactions. Their cytoplasmic granules are filled predominantly with histamine and are fused with the cell membrane. When activated, they release histamine extracellularly, inducing inflammation. They are located in the skin and mucosal epithelia with very few in the circulation. Basophils act similarly to mast cells but are not normally present in tissues. They make up less than 1% of leukocytes in the blood. They play a role in anaphylaxis, asthma, atopic dermatitis, and hay fever. They secrete histamine, proteoglycans, and serotonin to produce inflammation. They can perform phagocytosis. In contrast, eosinophils are known to play a key role in immune responses against parasites. The cytokines, GM-CSF, IL-3, and IL-5, promote myeloid precursors to differentiate into eosinophils. Eosinophils are found normally in the mucosal linings of the lungs, GI tract, and GU tract. Their numbers are increased in the setting of inflammation.

Antigen-presenting cells (*APCs*): Antigen-presenting cells (APC) are a critical component of adaptive immune responses. Professional APCs (e.g., dendritic cells, macrophages, and B-cells) ingest pathogens and foreign substances and then process these antigens into peptide fragments. These peptide fragments are then bound to MHC Class II molecules and displayed on the cell surface of APCs to naïve T-cells. If a T-cell's antigen receptor (the TCR) recognizes an antigen peptide-MHC Class II complex presented on the cell surface of an APC, then the T-cell is activated. An additional co-stimulatory signal is needed before full T-cell activation can occur. APCs also secrete cytokines that stimulate and induce the maturation of naïve lymphocytes (T- and B-cells). Dendritic cells are the predominant cell subtype of APCs that initiate T-cell-mediated immune responses. Macrophages and B-cells are also part of cell-mediated and humoral immune responses, respectively.

Dendritic cells as APCs: Dendritic cells play a key role in the activation of naïve T-cells and in innate immune responses to infections (reviewed in [5]). They also link innate and adaptive immune responses together. They arise from the myeloid lineage, directly from a precursor cell that can also differentiate into monocytes (but not granulocytes). The cytokine Flt-3 ligand induces differentiation into dendritic cells. Dendritic cells have long projections in order to ingest microbes and present antigens complexed to MHC molecules to naïve T-cells efficiently. Dendritic cells tend to reside within the skin, mucosal epithelia, lymphoid tissues, and organ parenchyma. Classical (or conventional) dendritic cells migrate to lymph nodes after ingesting microbes in order to display the processed antigen peptide fragments to naïve T-cells that are residing in the lymph node and stimulate the T-cells that recognize specifically the antigen peptide fragment-MHC complex. In contrast, plasmacytoid dendritic cells, which are another subtype of dendritic cells, are involved in immune responses to