The Biology of Allogeneic Hematopoietic Cell Resistance

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At the most basic level, success of an allogeneic hematopoietic cell transplantation (HCT) procedure relies upon the engraftment of recipients with donor hematopoietic stem cells (HSCs) that will generate blood formation for the life of that individual. The formula to achieve durable HSC engraftment involves multiple factors including the recipient conditioning regimen, the nature of the genetic disparity between donor and recipient, and the content of the hematopoietic graft. Animal and clinical studies have shown that the biology of host resistance is complex, involving both immune and nonimmune elements. In this article, we review the factors that contribute to host resistance, describe emerging concepts on the basic biology of resistance, and discuss hematopoietic resistance as it relates specifically to patients with severe combined immunodeficiencies (SCID)—disorders that bring unique insights into the dynamics of cell replacement by allogeneic HSCs and progenitor cells.

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HEMATOPOIETIC STEM CELLS CONSTITUTE THE ENGRAFTING POPULATION

Hematopoietic grafts are composed of mixtures of cells at different stages of development. Contained within these grafts are rare populations of hematopoietic stem cells (HSCs) that constitute <0.1% of the total cells [1]. Because HSCs are the only cells capable of self-renewal and of giving rise to all blood cell lineages, they are the only cells that must engraft in transplant recipients to achieve true durable hematopoiesis. Under conditions of normal hematopoiesis adult HSC reside in a specialized microenvironment or niche within the bone marrow (BM), which provides the necessary factors and molecular cues to maintain the cycle of self-renewal, proliferation, and differentiation [2]. Thus, engraftment is only achieved when the donated HSCs traverse the peripheral circulation and tissues and find their way to marrow niche where they settle to productively resume the task of blood formation.

IMMUNE-MEDIATED RESISTANCE

Immune-mediated resistance is the first hurdle that an incoming HSC must overcome on its journey to the BM. The surface molecules expressed by an HSC determine which element of host immunity can target and eliminate these cells. Although largely unappreciated as a population that can confer resistance, macrophages and other phagocytic cells of the reticuloendothelial system (RES) are a first line of defense in clearing cells from the blood stream. A recent report [3] suggests that CD47 expression confers protection of HSC from macrophage killing. CD47 is an immunoglobulin-like cell surface protein expressed on a wide range of blood cells, including HSC. CD47 interacts with its receptor on macrophages, SIRPα, inhibiting phagocytosis of healthy cells. It was shown that mobilizing cytokines and inflammatory stimuli induce the transient upregulation of CD47 expression on mouse HSCs and progenitors just prior to and during their migratory phase. Furthermore, the level of CD47 on these cells determined the probability that the HSC would be engulfed in vivo. This mechanism of macrophage evasion by CD47 expression appears to be a general strategy applicable to either autologous or allogeneic HSC. The role of host phagocytic cells as specifically applied to allograft resistance is largely unexplored.

Beyond the RES, T lymphocytes and natural killer (NK) cells are the primary immune mediators of allogeneic HSC resistance [1]. When transplant pairs are fully matched at the major histocompatibility complex (MHC) loci, that is, in humans the HLA-encoded
surface molecules are identical between donor and recipient, T cell immunity predominates. However, if HLA disparities exist, the most extreme example being haplo-identical transplantations, NK cells also play an important role. The prominent role of NK cells in HSC resistance is unique to the hematopoietic system, as NK cells do not appear to participate significantly in the rejection of solid organs. The differential effect of T versus NK cells on resisting hematopoietic grafts in the setting of the distinct genetic combinations is underscored by the observations made in patients with severe combined immunodeficiencies (SCID). The SCID syndrome is variable with regard to the lymphocyte defects. SCID recipients that lack both T and NK cells engraft more readily when HLA-incompatible donors are utilized compared to those that have T cell defects only; and overall, the former have significantly better outcomes than patients with functional NK cells [4].

Although it is acknowledged that T and NK cells are responsible for immune resistance, precise delineation of their antigenic targets and mechanism(s) of how donor HSC are eliminated is incomplete. Via their antigen specific T cell receptor (TCR), T cells recognize and respond to minor antigens presented as peptides bound to MHC molecules, and respond even more vigorously to foreign MHC molecules. Presentation of these alloantigens can be carried out by antigen presenting cells (APCs) derived from either donor or host. Control of NK reactivity is distinctly different from T cells. Unlike T cells, NK cells possess an array of surface molecules, rather than a single dominant one, which are either MHC class I specific or non-MHC class I specific. Engagement of these receptors can result in NK cell activation or inhibition. Because NK cells are known to respond strongly to cells that do not express self-MHC molecules, one hypothesis based on the pattern of NK reactivity observed in experimental models, is that NK cells destroy MHC-disparate HSCs if the HSC targets do not express the MHC-alleles that would inhibit an NK clone’s activity.

Whether or not the initial antigen presentation events trigger downstream aggressive inflammatory and cytotoxic activities, which result in elimination of the donor cells, is determined by the cytokine milieu in which the antigen interaction takes place and, in the case of T cells, the costimulatory signals provided by the APCs. Animal studies suggest that multiple cell and effector pathways are capable of causing the ultimate abolishment of donor HSC [5]. CD4+ and CD8+ T cells, together or separately, as well as NK cells have been demonstrated as the chief cause of engraftment resistance in different animal models. In addition to the type of genetic disparity between donor and recipient, and the type of conditioning regimen employed (myeloablative [MA] or nonmyeloablative [NMA]), the recipient’s state of antigen exposure is another factor in determining which host effector T cell subpopulation will dominate. Not surprisingly, patients unintentionally sensitized (i.e., aplastic anemia [AA] [6]) or experimental animal recipients intentionally sensitized to donor antigens demonstrate stronger barriers to engraftment compared with recipients who are naive with respect to donor antigens [5]. Mouse models that are MHC-matched primed against recipient antigens to induce memory T cells have been shown to resist hematopoietic allogeneic grafts with enhanced kinetics compared with unsensitized recipients [5].

To date, no single cytotoxic pathway has been identified as the primary mechanism of donor cell clearance. Studies by us [7] and others [5] have shown that recipient mice genetically deficient in components of the major cytotoxicity pathways (perforin, FasL, granzyme B) or multiple cytokines show little to no diminution in the ability to eliminate allogeneic hematopoietic cells. These negative studies direct attention to nonspecific pathways of inflammation as key effector components of the engraftment barrier. There is growing appreciation of the cellular dynamics that occurs during the process of engraftment between “conventional” T cells that mediate pro-cytotoxic and inflammatory activity and the effects of regulatory cells cells on modulating this alloreactivity. CD4+CD25+FoxP3+ (Treg) cells derived from either host or donor have been shown to assist in the engraftment of allogeneic hematopoietic cells [8-11]. Because there appears to be little downside to exploiting the activities of Tregs, it is anticipated that the use of this population for engraftment facilitation (and for the amelioration of graft-versus-host disease [GVHD]) will be rapidly translated to clinical trials.

NICHE SPACE

Host immunity is generally thought to constitute the largest barrier to HSC engraftment. However, successful engraftment is critically dependent upon HSCs making their way to the correct microenvironment and establishing a foothold in that milieu. The notion that BM “space” must be created by cytoreductive agents or radiation to accommodate transplanted HSC was first proposed over 30 years ago [12]. This hypothesis is based on the concept that HSC numbers and behavior are regulated by physically discrete locations or niches within the BM. Experimental studies by us [13,14] and others [15] have led to the conclusion that space in this regard is occupation of the niche by host resident HSC, and unless these cells are unseated, donor engraftment will not take place. Although the precise identities of the niche cells are still largely unknown and controversial [16,17], a large amount of data
indicate that HSCs are retained within the niche through the use of specific adhesion molecules and chemokine gradients [2]. Through these interactions, HSCs can be assured of receiving the appropriate supportive signals that allow them to retain their stem cell identity.

Counterbalanced against these studies are data suggesting that recipient BM cells can be readily displaced by syngeneic transplanted BM in an efficient and linear dose-dependent manner, even in the absence of conditioning [18,19]. Although these studies did not directly assess HSC replacement, the data were interpreted as more consistent with a model wherein HSCs do not reside locked into fixed locations in the BM, but instead receive their regulatory signals through limiting quantities of freely diffusible factors. To clarify these divergent views, we and others performed studies to test the ease by which host HSCs can be replaced by purified HSCs, rather than simply total marrow replacement [13-15,20]. Unfractionated BM is known to contain a number of different cell types that have been reported to influence engraftment and replacement (see next section), such as host-reactive T cells and stromal cells [21-23]. We found using purified HSC innocula that a low level of HSC replacement does indeed occur in normal mice, even in the absence of cytoreductive conditioning, but not to the degree previously reported.

A number of studies have shown that HSCs and/or progenitor cells circulate under physiologic conditions [24-26]. Thus, we hypothesized that the low level of HSC engraftment reflects a steady-state egress of HSCs from their niches allowing engraftment of donor HSCs. In this model, transplanted HSCs do not directly displace host HSCs that are stable residing within the niche, but engraft only in niches that had been vacated through the physiologic egress of host HSCs. By cell surface phenotype and transplantation of unfractionated blood, it was determined that a calculated 1% to 5% of the total pool of HSCs enter into the circulation of mice each day. Bromodeoxyuridine (BrdU) feeding experiments showed that HSCs in the peripheral blood (PB) incorporate BrdU at the same rate as do HSCs in the marrow, suggesting that egress from the marrow to the blood can occur without cell division and can leave behind vacant HSC niches. To test the hypothesis that small numbers of niches are continuously vacated, repetitive daily transplantations of small numbers of HSCs were performed over the course of 7 days. This approach led to significantly higher levels of engraftment than did large single bolus transplantation of the same numbers of HSCs [27]. Thus, these data provide insight as to how HSC replacement can occur despite the residence of endogenous HSCs in niches, and further suggest therapeutic interventions that capitalize upon physiologic HSC egress.

To further demonstrate that transplanted HSCs are limited by occupancy of appropriate niches by endogenous HSCs, an antibody-based approach was developed to eliminate host HSCs prior to transplantation [20]. Administration of ACK2, an antibody that blocks c-Kit function, led to the transient removal of >98% of endogenous HSCs in immune-deficient mice. Subsequent transplantation of these mice with donor HSCs led to chimerism levels up to 90%, whereas transplantation of mice without preconditioning led to engraftment levels of at most 3%. Further support of the concept that the space limitations in the marrow result from endogenous HSCs that hold tenure within the niche, come from studies using the pharmacologic agent AMD3100, a CXCR4 inhibitor. AMD3100 rapidly induces the egress of HSCs out of the marrow, and has been shown to improve the levels of donor HSC engraftment relative to untreated recipients [28]. It is anticipated that these types of specific approaches that facilitate the creation of HSC niche space while avoiding DNA damage and other toxicities will be incorporated as part of the conditioning regimens of the future.

**NON-HSC CELLS IN A GRAFT FACILITATE HSC ENGRAFTMENT**

Although host-versus-graft (HVG) responses and endogenous HSC in recipients present the barrier to incoming donor cells, an unmanipulated hematopoietic graft carries its own cellular armamentarium capable of lowering engraftment resistance. The concept that immune cells contained in an allograft aid in HSC engraftment arose from studies in patients. Following the determination that T cells are the primary mediators of GVHD, strategies to purge BM cells of T cells were applied to clinical protocols [29,30]. Unfortunately, purging of graft T cells resulted in increased engraftment failures along with significantly increased deaths from this complication [29,30]. These results are one reason why T cell depletion is not routinely performed for HLA-identical grafts. Animal studies using T cell-depleted grafts confirmed the phenomenon of engraftment failure [31] and mouse models have been utilized to identify and characterize the role of facilitating cells in marrow engraftment. Several groups [32-35] have identified non-HSC populations that express the CD8 molecule as able to facilitate allogeneic HSC engraftment across MHC barriers. Interestingly, some of these studies suggest that both CD8+ conventional T cells (CD8α+ TCRα) and a non-T cell population(s) (CD8α+ TCRα) function to enhance engraftment. In a model wherein animals received purified HSC cotransplanted with facilitating populations it was observed that grafts of HSC plus CD8+ TCR+ cells led to significantly higher
levels of donor CD3+ chimerism compared to mice that received HSC alone or HSC plus the CD8+ TCR− population, suggesting that one mechanism by which the former group facilitates HSC engraftment is by clearing host immune cell populations that confer resistance [33]. That CD8+ TCR+ cells also target host HSC is an important corollary to these findings, but has not yet been definitely proven.

The increasing evidence that nonspecific inflammation is a cause of allograft resistance suggests that more than one mechanism of facilitation exists. In support of this idea are reports that cotransplantation of donor Treg cells facilitate engraftment [8,9,11]. On the flip side, but further reinforcing the effect of donor cells on host responses, are the studies showing that allogeneic marrow engraftment is retarded if recipients are treated with agents (i.e., cytosine–phosphorothioate–guanine oligodeoxynucleotides [CpG ODNs]) that bind to Toll-like receptors (TLRs) and trigger innate immune cell activation [36]. TLR agonists induce the expression of costimulatory molecules and multiple chemokines and cytokines. Interestingly, these studies revealed an unexpected outcome that donor APCs and not host cells activated by TLR9 agonists CpG ODNs promoted BM rejection. Thus, donor and host may be differentially affected by activating signals, underscoring the complex dynamics that occur following an allograft infusion. Future directions for study include the details of the location, timing, and regulation of these cellular events.

**CLINICAL LESSONS ON ENGRAFTMENT—SCID**

SCID comprises a number of rare monogenic disorders with the common characteristic of blocking T cell differentiation and direct impairment of B cell immunity. Some forms of SCID also lack NK cells. The molecular defects that underlie SCID include more than 14 distinct genetic variants. Four main mechanisms have been described for the pathophysiology: (1) premature cell death caused by accumulation of purine metabolites, as seen in adenosine deaminase (ADA) deficiency; (2) defective cytokine-dependent survival signaling in T cell precursors; (3) defective V(D)J rearrangement of the TCR and B cell receptor genes; and (4) defective pre-TCR and TCR signaling. The clinical presentation of the different SCID conditions is fairly uniform and characterized by the early onset of life-threatening infections. The severity of these clinical manifestations makes SCID a medical emergency that, in the absence of treatment, leads to death within the first year of life. Although subsets of SCID patients can benefit from enzyme replacement (ADA type) or gene therapy, at present the only known cure for most children with SCID is allogeneic HCT.

Because SCID patients demonstrate profound defects in T cell and/or NK immunity—the known mediators of allogeneic hematopoietic cell resistance, transplantation of these children provides unique insight into immune resistance and these patients are considered appropriate for protocols using haploidentical grafts. Clinical experience shows that the optimal donor is an HLA-matched sibling or parent. However, haploidentical parental transplantations are often performed, as most children do not have such an HLA-matched relative available and because of the length of time needed to identify an unrelated donor. Studies performed over the years comparing SCID recipients of haploidentical, T cell-depleted grafts with those that undergo transplantation from an HLA-identical donor unfortunately show that overall survival (OS) is uniformly poorer in the former patient group [37,38], with the principle obstacles being graft failure or graft loss, GVHD, and a delayed time course to T cell development. Furthermore, for patients with functional NK cells, HLA differences between donor and recipient increase the risk of graft failure and poor outcome. Clinical studies have consistently reported that long-term survival of NK+ SCID patients is roughly half that of the NK− SCID patients [4].

**THE NEED FOR NICHES**

Children with SCID have been shown to benefit from the infusion of allogeneic cells without conditioning. However, whether or not SCID patients should receive conditioning and what type should be administered is controversial. Certain forms of SCID, that is, those forms arising from mutations in DNA repaired genes, show increased sensitivity to ionizing radiation and alkylating agents—a major concern when considering transplant conditioning. Although children that receive no conditioning experience less upfront toxicity and demonstrate functional T cell immunity provided by donor cells, many demonstrate delayed or absent B cell recovery, and in general, myeloid and erythroid cells remain of recipient origin. Thus, in the absence of conditioning, either no or very low level true HSC engraftment occurs, again pinpointing the importance of emptying niche space that is the presumed effect of conditioning. As a further impetus to achieve HSC engraftment, we found that patients that are unconditioned or that receive reduced-intensity conditioning (RIC) demonstrate very low levels of new T cell production by 10 years after transplantation [39].

Approaches to create HSC niche space with limited morbidity are currently under development. Recently, the validity of targeted therapy using
antibodies was shown in patients with primary immunodeficiency that were conditioned with a novel minimal-intensity conditioning (MIC) regimen consisting of two rat anti-CD45 monoclonal antibodies (mAbs) [40]. Fifteen of 16 patients engrafted, and 11 of these patients achieved full or high levels of mixed chimerism in both lymphoid and myeloid lineages. Another promising strategy in this regard is the anti-c-Kit antibody used in immunodeficient mice [20] (discussed above), which permitted high levels of engraftment of donor HSC without additional conditioning. For NK+ SCID patients, such an approach could be partnered with antibodies that target NK cells specifically.

In both experimental animal models and in clinical transplantation, the SCID defects have been particularly instructive on highlighting the importance of niche space that exists at all levels of hematopoietic development. Several studies show that defects in immune-deficient animals are more easily corrected by donor cells if there is niche availability at specific stages of lymphocyte development. Perhaps the best examples are Rag-1-deficient mice, which exhibit high numbers of double-negative (DN2/DN3) thymic cells. These mice are refractory to thymic reconstitution following HCT in the absence of conditioning because of competition for thymic niche seeding. In contrast, γc-deficient mice in which DN T cells cannot proliferate, are well reconstituted [15]. Our laboratory reported similar results [41] for B lineage cells, indicating that the efficiency of B cell reconstitution in mice after nonconditioned HCT depends on the available of niches/resources in the BM environment. This study compared transplants in unconditioned mice that were severely deficient in B cell precursors in the BM (Rag-1γc or FL.T3 IL-7Rα double knockout mice) with B cell-deficient animals that contain normal numbers of pro-B cells (Rag2−/− or Rag2 IL-2Rβ double knockout mice). B-cell reconstitution was limited in recipient mice containing a normal pro-B cell pool, whereas immature and mature B cell numbers reached wild-type levels in mice with compromised early B cell precursors. This idea of opportunistic expansion of progenitors in a permissive niche environment has been borne out in patients wherein superior T cell reconstitution was observed in SCID patients with the γc defect [39].

PERSISTENT IMMUNE DEFICIENCY AND STRATEGIES FOR THE FUTURE

A key obstacle that persists in haploidentical HCT is the severe, long-lasting immunodeficiency that follows infusion of CD34 selected cells—a condition that is sometimes worsened by the occurrence of acute or chronic GVHD (aGVHD, cGVHD). Thus, although engraftment is achieved, it is as yet unclear why neotymopoiesis is markedly delayed (6-12 months in pediatric patients). The consequences of this long-lasting immunodeficiency are opportunistic viral infections that account for ~30% of mortality observed in patients receiving an allograft from HLA-partially incompatible donors [38,42]. To reduce the frequency of these complications, several adoptive immunotherapy strategies have been or are being tested in the clinic. The basic idea is to provide the recipient with either mature T cells devoid of specific, antihist alloreactivity or pathogen-specific mature donor cells. One limitation of pathogen-specific immunotherapy is that the monospecificity of the sorted T cells will be a limitation in multi-infected patients. Other feasible approaches include the use of Notch ligands to preferentially expand lymphoid progenitors. Additional strategies known to be effective in mouse models and in clinical trials include the use of thymopoietic factors (e.g., keratinocyte growth factor) or thymopoietic factors (e.g., androgen ablation using lenprolide, IL-7 administration and growth hormone administration). These strategies have been, and will continue to be, translated into clinical trials in the near future. In parallel with these transplantation studies are the development of gene therapies, which may become important alternatives when faced with very young, severely affected patients who lack an HLA-compatible donor [43].

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REFERENCES


