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Immune reconstitution and implications for immunotherapy following haematopoietic stem cell transplantation

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Abstract

Recovery of a fully functional immune system is a slow and often incomplete process following allogeneic stem cell transplantation. While innate immunity reconstitutes quickly, adaptive B- and especially T-cell lymphopoiesis may be compromised for years following transplantation. In large part, these immune system deficits are due to the decrease, or even absence, of thymopoiesis following transplantation. Thereby, T-cell reconstitution initially relies upon expansion of mature donor T cells; a proliferation driven by high cytokine levels and the presence of allo-reactive antigens. This peripheral mechanism of T-cell generation may have important clinical consequences. By expanding tumouricidal T cells, it may provide a venue to enhance T-cellular immunotherapy following transplantation. Alternatively, decreased thymic function may impair long-term anti-tumour immunity and increase the likelihood of graft-versus-host disease.

Keywords

immune reconstitution; cellular immunotherapy; allogeneic stem cell transplant; thymus

INNATE IMMUNE RECONSTITUTION: NATURAL KILLER, NEUTROPHIL AND DENDRITIC CELLS

The innate immune system is comprised of a collection of cells that recognize and eradicate pathogens or aberrant cells without priming or antigen presentation. Natural killer (NK) cells, neutrophils, monocytes, dendritic cells (DCs) and macrophages contribute to innate immunity. NK cells purge tumour or virus-infected cells. By 1 month post transplant, NK cells circulate at normal levels and confer some degree of immune protection (Figure 1).¹⁻⁶ These donor-derived NK cell clones effectively lyse recipient leukaemia in vitro.⁷ Studies have also shown that the number of NK cells early after transplant correlate with remission rates, implicating the function of these early cells in the clearance of residual tumour.⁸⁻¹⁰ Data also suggest that killer immunoglobulin-like receptor (KIR) mismatch may play a critical role in NK-cell-mediated tumour eradication.^{7,9} Many, but not all, studies have associated NK cell KIR mismatch with protection from relapse and graft-versus-host disease (GvHD) in human leukocyte antigen haplotype mismatched and matched unrelated transplants for myeloid and acute lymphoid malignancies.^{9,11,12}

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CONFLICT OF INTEREST STATEMENT

None declared.

Like NK cells, recovery of neutrophils and monocytes is rapid following transplantation; however, the time line for DC reconstitution is delayed and falls between that of innate and adaptive immune recovery.^{3,4,13-20} DCs process and present antigens to the adaptive immune system, while producing inflammatory cytokines important for stimulation of the innate immune system. After stem cell transplantation, although donor DCs can be detected in the peripheral blood within the first few weeks, the total number may not approach normal for more than 1 year in adults (Figure 1).^{21,22} Furthermore, studies suggest that while the peripheral blood DCs are largely of donor origin (> 80% by Day 14), up to 70% of tissue DCs may remain of host origin after SCT.²³⁻²⁵ These host origin tissue DCs may persist for up to a year following SCT.²⁴ Furthermore, the functional competency of these newly derived DCs is not yet well understood in the context of stem cell transplantation. As DC-loaded peptides engender better infectious responses than vaccine alone following transplantation, there is evidence that DC function may be impaired post transplant, although these studies are limited by differing diseases and transplant conditions.²⁶⁻²⁸

Collectively, these data suggest that many of the components of the innate immune system reconstitute relatively quickly and competently in the early post-transplant period. While this quick, functional recovery of innate immunity may be exploited for tumor immunotherapy, to date, few studies have demonstrated that these innate immune cells can be manipulated to maximize graft-versus-leukaemia (GvL) effects, with the one notable exception of acute myeloid leukaemia (AML). Some studies have suggested that a graft with KIR mismatch may predict a high probability of NK alloreactivity, augment the graft-versus-AML effect, and diminish the risk of relapse following transplantation.^{7,9,29} Unfortunately, for most patients and diseases, the data for this NK-alloreactive effect are limited. Additionally, because little is known about the kinetics and function of tissue DCs following transplantation, mechanisms to exploit this biology in vivo have yet to be elucidated. Indeed, the few studies that have investigated anti-tumour vaccination strategies after transplantation have included the administration of DCs loaded with specific peptides to obviate the need for functional donor DCs in vivo (see below).

ADAPTIVE IMMUNE RECONSTITUTION: B AND T CELLS

In contrast to innate immune cells, the lymphoid reconstitution of the adaptive compartment is delayed, resulting in persistent recognized deficits in terms of global immunity. In large part, deficiencies in T- and B-cell function after transplant are due to impaired thymopoiesis. As a result, T-cell reconstitution relies upon an alternate pathway of development, termed 'homeostatic peripheral expansion' (HPE). In HPE, mature donor T cells expand, rendering a limited repertoire of T cells available to recognize and signal B-cell counterparts.³⁰ B and T cells require priming by antigen and engender long-term specific responses using unique receptor sequences. In non-transplant subjects, as B and T cells have the potential for long-term and specific immunity, they are attractive targets for cellular immunotherapy. However, the protracted time line for lymphoid recovery after transplant remains a challenge to this approach following transplantation. Circulating B cells may not reach normal levels for at least 12 months post transplant and T-cell reconstitution may be delayed for more than 2 years.^{4,5,14,19,31-34} Despite this fact, there are aspects of the immediate post-transplant milieu that may be especially conducive to anti-tumour T-cell responses, including high levels of cytokine that can fuel cytotoxic T-cell proliferation and activation. Thus, an understanding of the cytokine- and antigen-driven responses that are possible following transplantation may permit the genesis of a platform for anti-tumour treatments, with cautious consideration to the potential for exacerbation of GvHD.

B-CELL IMMUNE RECONSTITUTION

B-cell development

B cells are lymphocytes generated in the bone marrow from common lymphoid progenitors. B cells possess immunoglobulin receptors that are generated by somatic recombination, whereby genes are re-arranged to create diverse receptor sequences. B cells undergo selection in the bone marrow prior to release into the peripheral blood. Naïve mature B cells emerge from the marrow with surface immunoglobulin (Ig) M and IgD receptors, then migrate to secondary lymphoid structures. Once a B cell encounters an antigen, often through interactions with CD4⁺ T cells or DCs, it becomes activated and releases IgM. The activated B cell then processes the antigen for presentation to T cells. After T-cell stimulation, the B cell may undergo isotype switching such that it expresses IgG, IgA or IgE on the cell surface. The B-cell receptor/immunoglobulin molecule may then undergo further receptor modification in the variable regions (V_H), through somatic hypermutation, increasing the avidity of the antibody/epitope interaction.

B-cell development

Following allogeneic stem cell transplantation, B-cell development recapitulates that of normal ontogeny in terms of circulating cell types and numbers. In normal ontogeny, B-cell numbers rise until the toddler years, after which there is a gradual descent to normal adult levels in the peripheral blood. Following stem cell transplantation, levels of circulating B cells are very low for the first few months, but then reach levels exceeding normal adults by 1-2 years, approaching the range of normal neonates (Figure 1).³² The levels then gradually fall. Post transplant, donor B cells emerge with a naïve phenotype (IgM⁺ IgD⁺) initially, with memory B-cell development up to 5 years later.^{35,36} Consistent with this, IgM levels recover first, by 2-6 months^{6,37}, followed by IgG levels which approach normal between 3 and 18 months following transplantation. Finally, IgA reconstitution may be delayed for up to 3 years.^{6,37} It is important to note that the IgG levels may, in part, reflect residual host plasma cell production, and thus may not be entirely suggestive of donor B-cell competence because host plasma cells are relatively resistant to current preparative regimens and may persist for up to 2 years.^{38,39}

B-cell function following transplantation

The function of B cells remains compromised for 1-2 years following stem cell transplantation. In part, this has been attributed to the T-cell defects during this time frame, with maturation arrest at the naïve stage due to insufficient CD4⁺ T-cell signalling. After transplant, memory B cells that require CD4⁺ help for isotype switching + show a skewed pattern of complementary determining regions 3 (CD3) of the immunoglobulin heavy chain.^{40,41} In addition to the lack of CD4⁺ help, there appears to be an environmental defect following transplantation, because the rate of somatic hypermutation is decreased in mature B cells even in the presence of normal donor CD4⁺ T cells.⁴² Thus, following transplantation, B-cell immunity is impaired due to: (1) prolonged low levels of circulating B-cell numbers; (2) a relative deficit of mature B cells secondary to decreased isotype switching; and (3) a diminished ability to undergo somatic hypermutation. Importantly, this decreased B-cell function following stem cell transplantation has resulted in diminished vaccine responses to infectious antigens, even after normal B-cell numbers have been achieved.^{26,43,44}

T-CELL RECONSTITUTION

Thymic-dependent T-cell reconstitution

The thymus is the primary site for T-cell development. When peripheral T-cell populations are severely depleted, renewed thymic activity can contribute to T-cell reconstitution, producing naïve CD4 helper, CD8 cytotoxic effector and CD4⁺ CD25⁺ regulatory T cells. During

thymopoiesis, bone-marrow-derived T progenitors migrate to the thymus, expand and mature. Developing thymocytes acquire a T-cell receptor (TCR), generated through recombinant re-arrangement of variable (V), diversity (D) and joiner (J) genes. The re-arrangement of these genes within the thymus ensures TCR diversity. Prior to emigration as naïve T cells, these TCRs undergo positive and negative selection, deleting autoreactive clones termed 'central tolerance'.

Renewed thymopoiesis may be assessed by several techniques including thymic imaging, naïve T-cell frequency, TCR excision circle quantity, and spectrotyping. With renewal of thymopoiesis, the proportion of cells with a 'naïve' phenotype increases in peripheral T-cell populations.⁴⁵⁻⁴⁷ Measurement of TCR re-arrangement excision circles (TRECs) provides a means of quantifying thymic productivity.⁴⁸ TRECs are episomal DNA circles that are generated as a byproduct from the spliced DNA elements remaining after re-arrangement of the VDJ genes encoding the TCR α and β chains. TRECs are not replicated during cell division but are retained in the thymic emigrant cell, and thus are diluted with cell division. Robust thymopoiesis will be reflected in high frequencies of TREC-bearing cells, termed 'recent thymic emigrants'. In contrast, with intense peripheral expansion, peripheral TREC frequencies remain low. Finally, thymic function may also be evaluated by spectratyping, a polymerase chain reaction-based analysis of length variation in the CDR3 of the TCR β chain. A productive thymus is reflected in a Gaussian distribution of CDR3 lengths because of the enormous diversity generated by VDJ re-arrangement occurring in maturing thymocytes. In contrast, clonal expansion results in an oligoclonal pattern of limited CDR3 lengths. Renewal of thymopoiesis thus re-establishes polyclonal Gaussian patterns in the CDR3 spectratypes, first in naïve cells and subsequently in memory T cells.^{47,49}

T-cell reconstitution by homeostatic peripheral expansion

An alternate pathway for T-cell development is through the rapid cell division of mature T-cell clones, termed 'HPE'.^{46,50} This was first demonstrated in a murine model in which two very distinct patterns of T-cell reconstitution ensued following the adoptive transfer of syngeneic bone marrow cells and congenic lymph node cells into C57BL/6-irradiated, thymus-intact or thymectomized recipients. Thymus-bearing hosts largely reconstituted with syngeneic marrow-derived T cells through a thymus-dependent mechanism. Thymectomized mice derived the majority of peripheral T cells from the congenic lymph node innocula, via HPE.⁴⁶ HPE drives T-cell reconstitution in lymphopenic hosts and is associated with a shift from naïve to memory/activated phenotype in the proliferating cells.⁵⁰

HPE is dependent upon the support of homeostatic cytokines and cognate antigen-driven and regulatory cellular interactions. Interleukin (IL)-7 and IL-15 are the cytokines that drive HPE of naïve and CD8+ memory T cells, respectively. Regulatory cells and tumour growth factor beta (TGF β) provide cellular constraints on HPE. Finally, antigen presentation can drive HPE in lymphopenic and transplant recipients.

IL-7 is a critical, non-redundant cytokine required for stimulating naïve T-cell expansion and sustaining naïve T-cell survival. Human and animal studies have shown that IL-7 markedly increases naïve CD4+ and CD8+ T cells, diminishing TREC frequency.^{51,52} Conversely, IL-7 depletion restricts T-cell expansion through cytokine depletion by a consumption-based mechanism. Current data suggest that lymphocyte depletion following preparative regimens leads to an increase in cytokine availability (presumably due to lack of cells consuming the cytokine). Upon infusion of donor T cells, the surplus IL-7 drives naïve cells into proliferation until T-cell numbers return to a level at which IL-7 is once more a limiting factor. Human studies initially revealed this inverse correlation between serum levels of IL-7 and T-cell reconstitution after lymphodepletion; high serum levels of IL-7 coincided with severe

lymphopenia, which decreased rapidly with lymphocyte recovery following transplantation.^{53,54}

Recent evidence suggests that IL-15 as well as IL-7 may act as a homeostatic cytokine, supporting HPE in lymphopenic hosts. IL-15 enhances proliferation of human CD8⁺ memory populations in vitro.^{55,56} It is produced constitutively by tissues and antigen-presenting cells, and is upregulated in the setting of inflammation. When lymphocytes have been severely depleted as in the setting of bone marrow transplantation, plasma IL-15 levels increase dramatically and exceed normal levels concomitant with a disproportionate expansion of CD8⁺ + memory cells.⁵⁷⁻⁶⁰ Consistent with this finding, IL-15R expression and responsiveness are highest on activated memory CD8⁺ T cells.⁶¹⁻⁶³

Since IL-15 and IL-7 are produced constitutively and could lead to unchecked T-cell expansion, these are balanced by factors that negatively regulate T-cell subsets. TGF β antagonizes the effect of IL-15, curtailing the proliferation and persistence of CD8 central memory populations.^{64,65} Administration of TGF β to human and murine CD8⁺ memory cells in vitro decreases CD8 proliferation and attenuates effector function.^{66,67} Similarly, regulatory T cells (Tregs) constrain the HPE of naïve CD4⁺ and CD8⁺ T cells, influencing the host reactivity of T cells during immune reconstitution.⁶⁸ Tregs are CD127⁺CD25⁺ CD4⁺ T cells that function to control auto-immune responses.^{69,70} Characterized by the expression of the transcription factor FoxP3 and a high level of glucocorticoid-induced tumour necrosis factor receptor, Tregs have been shown to modulate the peripheral expansion of T cells to low- and high-affinity antigen in lymphopenic hosts.^{68,70} Treg levels appear to be low following transplantation or chemotherapy, but expand rapidly in the first month.^{71,72}

In addition to cytokine and cellular regulation of HPE, antigen presentation can also direct the recovering T-cell compartment. First demonstrated in murine models, peripheral expansion of TCR transgenic cells was increased substantially in the presence of cognate antigen.⁷³ This mechanism was further characterized as a rapid, IL-7-independent expansion of CD4⁺ and CD8⁺ T cells that is dependent on major histocompatibility complex II interactions and CD28 ligation⁷⁴, and termed 'endogenous proliferation' to differentiate it from homeostatic proliferation which is driven by cytokine signals.⁷⁵ In irradiated lymphopenic hosts, both processes could be demonstrated for subsets of CD4⁺ and CD8⁺ cells during immune reconstitution.⁷⁵ In allogeneic transplantation, the influence of endogenous proliferation is best demonstrated by the disproportionate expansion of cytomegalovirus (CMV)-reactive CD8⁺ + T cells observed in CMV-positive individuals.^{76,77} Thus, although homeostatic proliferation permits the expansion of the T-cell compartment as a whole following stem cell transplantation, endogenous proliferation directs T cells to proliferate in response to specific antigen stimuli early in T-cell recovery.

T-cell reconstitution by peripheral expansion in the initial post-transplant period and implications for immunotherapy

In the early post-transplant period, elevated levels of IL-7 and IL-15 drive homeostatic expansion of residual (host) or infused (donor) T-cell subsets. Thymopoiesis is significantly impaired due to thymic injury from the preparative regimen, diminished thymic function in adults, the lack of circulating T-cell precursors, and the absence of critical cytokines or growth factors. This early reliance of the T-cell compartment on HPE has profound consequences. The relative frequencies of the T subsets shift towards a preponderance of activated and cycling memory T cells.⁷⁸ As T cells expand through HPE, the frequency of TRECs declines.⁷⁸ T cells, especially CD8⁺ subsets, expand rapidly in the first weeks, but this expansion is often unstable with longitudinal studies describing a sharp decline in overall T-cell numbers by 3-6 months.⁵⁸ Robust CD8⁺ proliferation and impaired CD4⁺ reconstitution lead to an inverted

CD4:CD8 ratio in the months to years following stem cell transplantation.^{6,33} The TCR repertoire resulting from these early expansions is typically skewed and oligoclonal.

This oligoclonal peripheral expansion of relatively few donor T cells potentially presents an opportunity for cellular immunotherapy. Murine models have shown that severe lymphodepletion establishes optimal conditions to promote graft-versus-tumour due to the high levels of homeostatic cytokines (IL-7 and IL-15) and the depletion of regulatory T cells that impede immune responses. Higher doses of irradiation yielded superior anti-tumour responses when mice were given transgenic melanoma-antigen-specific T cells.⁷⁹⁻⁸¹ Furthermore, this effect was abrogated in the absence of IL-7 and IL-15 when these knock-out mice were used as recipients.⁷⁹ Depletion of regulatory T cells also enhanced the tumouricidal effect.⁸² In the setting of vaccination, while Tregs can also proliferate rapidly and hamper effective anti-tumour responses in lymphoreplete hosts, following stem cell transplantation, the Treg compartment is constrained (by IL-2) while anti-tumour effectors expand and out-compete Tregs.^{83,84} Murine stem cell transplantation models showed that the post-transplant period provides an optimal milieu for cellular immunotherapy, demonstrating specific anti-melanoma immunity and tumouricidal effect when either a vaccine and cytokine adjuvant, a tumour lysate DC pulsed vaccine, or GM-CSF-producing tumour vaccine were administered in the post-transplant lymphopenic setting.^{80,85,86}

Human studies have corroborated murine data, showing maximal anti-tumour T-cell expansion in the setting of severe lymphopenia. Studies in the highly immunogenic tumour melanoma have demonstrated the potential for this platform. Ex-vivo-expanded melanoma-infiltrating lymphocytes administered with IL-2 after cytoreductive therapy led to clinical responses in up to 50% of patients evaluated.^{87,88} Furthermore, a single study has even demonstrated that T cells can be genetically engineered to target melanoma and affect clinical responses when given during intense lymphopenia.⁸⁹

Vaccination strategies using peptide-pulsed DC vaccines for melanoma have also demonstrated T-cell-specific responses with some clinical responses.⁹⁰⁻⁹² It should be noted that these DC-pulsed peptide vaccines did not incorporate lymphodepletion in the regimens. In haematological malignancies, this framework for vaccines has only recently been incorporated into clinical trials, with few studies reported to date. In contrast to melanoma, when peptide-pulsed vaccines were administered in the post-transplant setting (autologous or allogeneic) for multiple myeloma or AML, some assays could detect an immunological response but clinical responses were absent.⁹³⁻⁹⁵ Notably, these studies administered the vaccines months following transplantation, after exponential T-cell proliferation has likely occurred and IL-7 and IL-15 cytokine levels would be expected to be low. These studies suggest that peptide-pulsed vaccines may be used safely after stem cell transplantation, and that alternative timing strategies may actually lead to a clinical response. Given the successful strategies in melanoma, future trials may seek to incorporate infusion of tumour-targeted T cells and/or the administration of DC vaccines in the early post-transplant period to capitalize on the cytokine storm and absent Treg populations to potentiate tumouricidal effects.

T-cell reconstitution by thymopoiesis following transplantation and implications for cellular immunotherapy

While early post-transplant T-cell reconstitution relies upon HPE, an optimal T-cell armamentarium with a diverse TCR repertoire is only achieved through the (delayed) recovery of thymopoiesis (Figure 2).^{47,48,96-100} This may have important implications for cellular immunotherapy, particularly in adults who demonstrate limited residual thymic activity. Without renewal of thymopoiesis, naïve T cells and total CD4 + T-cell reconstitution is impaired indefinitely.^{19,45,47,48} In paediatric patients, CD4+ naïve T cells are delayed for 1 year or more until thymic activity has resumed.³¹ Even 20 years following transplantation,

deficits in the naïve CD4 population persist in patients with inadequate renewal of thymopoiesis.³⁴

While the CD8+ rich, memory, oligoclonal population of T cells due to HPE may be beneficial as a platform for vaccination therapy, the delay in thymopoiesis following transplantation may ultimately restrict vaccine efficacy. The lack of diversity of TCRs could diminish the likelihood of an appropriate TCR for tumour antigen recognition. Murine models have demonstrated that decreased TCR diversity due to age or thymectomy correlated with diminished response to an infectious antigen.¹⁰¹ Furthermore, since CD4+ T cells are necessary for longlasting B- and CD8+ T-cell immunity, the persistent defects in CD4+ T cells likely contribute to impaired responses to infectious and tumour vaccines following transplantation. Murine data suggest that optimal vaccination responses occur in the setting of active thymopoiesis.^{19,102} In human trials, impaired thymic function has been correlated with increased incidence of relapse, suggesting that renewed thymopoiesis may enhance anti-tumour immunity.^{103,104}

In addition to mitigating the effects of cellular immunotherapy, thymic defects are associated with increased risk of acute and chronic GvHD which further impedes de-novo immune responses.¹⁰⁵⁻¹⁰⁹ In a paediatric population, GvHD and low TREC levels indicative of poor thymic function even conferred an increased risk of mortality.¹⁰⁹ Post-transplant thymic dysfunction contributes to the onset and persistence of GvHD. While thymic damage permits the escape of alloreactive T cells that may engender GvHD^{46,110}, the thymus is also a target of these allo-reactive T cells, limiting thymic renewal.^{111,112} Thus, the regeneration of thymic structures is stunted and denovo diverse T production is impaired indefinitely.^{111,112}

Of critical importance when considering T-cell immunotherapy in the context of stem cell transplantation is the barrier that factors that support GvL may worsen GvHD. In murine studies, high doses of IL-7 and IL-15, through exogenous administration or transgenic models, increased the severity and frequency of GvHD.¹¹³⁻¹¹⁵ In human studies, persistence of high IL-15 levels following allogeneic stem cell transplantation was correlated with severe acute GvHD.^{116,117} In contrast, factors that reduce peripheral expansion can reduce GvHD. Regulatory T cells could control homeostatic expansion in lymphopenic mice and prevent acute GvHD in murine models.^{68,118} Similarly, in clinical trials, the level of FoxP3+ T regulatory cells in the donor inoculum was inversely correlated with development of acute GvHD.⁷¹

STRATEGIES TO OPTIMIZE CELLULAR IMMUNOTHERAPY FOLLOWING TRANSPLANTATION

As T- and B-cell reconstitution is critical to successful immunotherapy approaches following transplantation, maximal anti-tumour responses are likely to be elicited when lymphoid reconstitution is both rapid and functional. In part, efforts should be directed towards enhancing thymic recovery while potentially directing the immediate post-transplant HPE towards tumour-specific antigens. Thymic function is influenced by: age; stem cell source; GvHD (see above); the degree of damage due to the preparative regimen; and possible cytokine, hormonal and growth factors. Host age affects thymic recovery significantly, with each successive decade reducing the rate and potential for thymic renewal.^{47,48,100,106,119} In many older adults, recovery of naïve cells through thymopoiesis occurs 3-5 years following transplantation; in some, naïve cells remain below normal levels for decades after stem cell transplantation.^{34,120} While host age is an immutable challenge to thymic recovery, other factors may be influenced by transplant design. To reduce thymic damage, less intensive preparative regimens have enhanced CD4+ T-cell reconstitution and T-cell repertoire diversity, although these may also reduce the levels of HPE cytokines available for early T-cell expansion.^{13,121,122} Increasing the availability of functional T-cell progenitors has enhanced thymopoiesis in murine models.^{123,124} In human studies, umbilical cord stem cells resulted in higher TREC

frequencies than adult bone marrow stem cells¹²⁵, possibly due to a higher frequency of T-cell progenitors in these marrows. Furthermore, manipulation of thymic cytokines, growth factors or hormones has improved thymic function after stem cell transplantation. As IL-7 is an important survival factor for developing thymocytes, administration of this cytokine in murine transplant models has enhanced thymopoiesis following bone marrow transplantation.^{126,127} Similarly, keratinocyte growth factor (KGF) has shown promise in boosting thymic productivity in murine hosts.^{128,129} Human studies utilizing KGF have not confirmed these data; however, they were not designed to evaluate enhanced thymopoiesis.¹³⁰ Finally, androgen withdrawal and growth hormone have been associated with enhanced thymopoiesis in murine and human studies.¹³¹⁻¹³⁶

In addition to the factors that affect thymic recovery, the transplant design may enhance immunotherapy by targeting the initial expansion of allo-reactive T cells in the graft. Stem cell source has been studied separately and shown to influence HPE-derived early T-cell reconstitution. Peripheral blood stem cell grafts initially reconstitute peripheral T-cell numbers more rapidly.^{3,5} T-cell depletion and GvHD have also been shown to delay T-cell reconstitution.¹⁰⁵ Degree of mismatch may also influence early T-cell recovery; partially matched or unrelated donors demonstrate delayed T-cell reconstitution with skewed repertoires and very low TREC frequency.^{1,137}

SUMMARY

Immune reconstitution following stem cell transplantation does not recapitulate that of ontogeny. This has important implications for the development and maintenance of tumour immunotherapy. While most of the innate immune system recovers quickly and competently, lymphoid reconstitution may be quite delayed in terms of cell number and global function. In large part, lymphoid recovery is hindered by the lack of thymopoiesis. As a result, T-cell reconstitution relies upon an alternate pathway of development that expands infused or residual cells in response to cytokines and antigens. Furthermore, donor DC renewal may also occur late after transplant, minimizing the capacity for these cells to present tumour antigens effectively and alert the developing immune system to the presence of minimal residual disease. These delays in lymphoid and DC reconstitution have significant clinical ramifications, permitting the exploitation of the initial T-cell expansion while challenging effective long-term immunity in the absence of thymopoiesis, appropriate B-cell signalling (due to the absence of CD4+ T cells) and functional donor DCs.

However, these components of immune reconstitution may be manipulated to aid GvL effects. Optimal stem cell transplantation involves eradication of residual tumour, long-term anti-tumour immunity, and minimal GvHD. For rare patient and donor pairs, the early functional recovery of NK cells affords considerable anti-tumour benefit. However, for most graft-host pairs, the optimal transplant entails achieving a balance between the peripheral expansion of anti-tumour T cells and thymic reconstitution, rendering a continuous infusion of de-novo T cells for tumour immunosurveillance with a diverse repertoire to minimize the likelihood of severe GvHD. To maximize stem cell transplantation for immunotherapy, future studies may alter the donor graft to direct the initial homeostatic T-cell expansion towards tumour eradication, either by infusing genetically engineered anti-tumour T cells, expanding naturally occurring GvL T cells prior to infusion, and/or administering DC vaccines loaded with peptides to drive a specific anti-tumour T-cell response. For the best effect, current immunological data suggest that these therapies should be given in the immediate post-transplant period to capitalize on the surfeit of cytokine levels. Alternatively, one could consider administering IL-7 or IL-15 after the first month to raise the levels and drive HPE of anti-tumour T cells after the cytokine surplus is depleted, although this should be considered cautiously in allo-transplantation because of the heightened risk of GvHD. While CD8+ T cells are typically

targeted for these therapies, treatments that employ CD4⁺ T cells may lead to longer lasting immunity and permit a more effective B-cell response. In the initial period, administration of CD4⁺ T cells may help to achieve B-cell responses, although the greatest immunotherapy benefit is likely to be obtained when thymopoiesis is augmented as well, replenishing naïve CD4⁺ T cells. Hopefully, future studies will advance thymic renewal while directing the initial homeostatic T-cell expansion towards tumour eradication. In this way, both pathways of T-cell development can be maximally exploited towards improved GvL immunotherapy without stimulating GvHD, leading to long-term fully functional lymphoid immunity and successful tumour and infection clearance.

Practice points

- innate immunity, including neutrophils and NK cells, recovers quickly and competently
- donor DC competence is delayed
- T-cell and, to some extent, B-cell immune reconstitution is delayed following stem cell transplantation
- the initial post-transplant period is dominated by cytokine-driven peripheral expansion of mature donor T cells
- recovery of thymopoiesis is impaired after stem cell transplantation and contributes to T- and B-cell incompetence

Research agenda

- the initial post-transplant period may provide a platform for successful T-cell immunotherapy strategies due to the robust peripheral expansion during this time frame
- strategies to aid in thymic renewal may improve long-term tumour immunosurveillance

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Adult Immune Reconstitution Following Stem Cell Transplant

Figure 1.

Representative time lines of immune reconstitution in adult patients without extensive graft-versus-host disease, infections or relapse. NK, natural killer; DC, dendritic cells.^{1,2,4,18,20-22,31,103,105}

Figure 2.

Diagrammatic representation of the clinical consequences of the two types of T-cell reconstitution evident in the post-transplant period. Early after stem cell infusion, homeostatic proliferation drives the T-cell repertoire due to the high levels of cytokines and delayed thymic recovery. As a result, T cells in the donor product expand and mature into the memory pool, leading to an expanded memory pool with decreased diversity in both naïve and memory lymphocytes. Given the rapid proliferation of few T cells, this immediate post-transplant period presents an opportunity for immunotherapy, through the administration of tumour-targeted T cells or through vaccine administration. Later, in patients with the potential for thymic recovery [including younger patients without graft-versus-host disease (GvHD)], renewed thymopoiesis enriches the naïve T-cell pool. Due to the T-cell receptor re-arrangement in the thymus, these recent thymic emigrants enhance the diversity of the naïve, and subsequently the memory, T-cell repertoire (diagrammatically shown as multi-patterned cells). This has significant implications for long-term immunity. Not only are these patients less prone to life-threatening infections and GvHD, the enhanced T-cell diversity and renewed CD4⁺ cells likely confer improved tumour immunosurveillance, as shown by decreased rates of tumour relapse. HPE, homeostatic peripheral expansion; TREC, T-cell receptor re-arrangement excision circle; IL, interleukin; Treg, regulatory T cells.