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National Institutes of Health Hematopoietic Cell Transplantation Late Effects Initiative: The Immune Dysregulation and Pathobiology Working Group Report

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Abstract

Immune reconstitution following hematopoietic stem cell transplantation (HCT) beyond one year is not completely understood. Many transplant recipients who are free of graft versus host disease (GVHD) and not receiving any immunosuppression more than a year after transplant seem to be able to mount appropriate immune responses to common pathogens and respond adequately to immunizations. However, two large registry studies over the last two decades seem to indicate that infection is a significant cause of late mortality in some patients, even in the absence of concomitant GVHD. Research on this topic is particularly challenging for several reasons. First, there are not enough long term follow-up clinics able to measure even basic immune parameters late after HCT. Second, the correlation between laboratory measurements of immune function and infections is not well known. Third, accurate documentation of infectious episodes is notoriously difficult. Finally, it is unclear what measures can be implemented to improve the immune response in a clinically relevant way. A combination of long-term multicenter prospective studies that collect detailed infectious data and store samples as well as a national or multi-national registry of clinically significant infections (e.g., vaccine-preventable severe infections, opportunistic infections) could begin to address our knowledge gaps. Obtaining samples for laboratory evaluation of the immune system should be both calendar driven and eventdriven. Attention to detail and standardization of practices regarding prophylaxis, diagnosis and definitions of infections would be of paramount importance to obtain clean, reliable data. Laboratory studies should specifically address the neogenesis, maturation and exhaustion of the adaptive immune system and in particular how these are influenced by persistent alloreactivity, inflammation and viral infection. Ideally, some of these long-term prospective studies would collect information on long-term changes in the gut microbiome and their influence on immunity. Regarding enhancement of immune function, prospective measurement of the response to vaccines late after HCT in a variety of clinical settings should be undertaken to better understand the benefit as well as the limitations of immunizations. The role of intravenous immunoglobulin is still not well defined, and studies to address it should be encouraged.

Keywords

immune reconstitution; late infections; immunization; intravenous immunoglobulin

Introduction

The National Institutes of Health Blood and Marrow Transplantation Late Effects Initiative, comprised of pediatric and adult HCT health care providers, administrators, researchers, advocates and survivors across federal and non-federal groups and sponsored by the National Cancer Institute and National Heart, Lung and Blood Institute, aims to identify knowledge gaps, develop practice recommendations and formulate important research questions to improve transplant survivor monitoring and management (cite commentary).

The Immune Dysfunction and Pathobiology Working Group, established as one of 6 working groups within this initiative, convened in September 2015 with the goal of providing recommendations for immune function and infection control in the field of HCT survivorship. The working group focused on identifying trends in late infections, describing immune reconstitution in the lab and reviewing interventions to improve immune function in HCT survivorship studies. These findings and recommendations for research were presented at a public meeting in June 2016, including over 150 participants with expertise across HCT survivorship. The findings were revised based on audience comments and are presented here.

A major goal after allogeneic hematopoietic stem cell transplantation (HCT) is to achieve optimal immune reconstitution, which we define operationally (in the case of allogeneic HCT) as: the restoration of functional pathogen-specific immunity and establishment of anticancer immunity in the absence of immune dysregulation (e.g., GVHD and/or HCT-associated autoimmunity). Late after transplant (i.e., > 1 year) variable degrees of immune recovery are observed in different patients, and the data are limited.

This paper will review what is currently known about immune function late after HCT, identify knowledge gaps and propose research priorities to fill those gaps, with an emphasis on what is arguably the most important function of the immune system: protection against infection.

Section 1. Late infections after Hematopoietic Stem Cell Transplantation (HCT)

Historically, infection is one of the 3 leading causes of death after HCT (along with relapse and graft versus host disease (GVHD))¹. Most infections occur during the first year and different types of infectious syndromes predominate at various times^{2,3}. Multiple factors influence the pace of immune recovery and the risk for and type of infectious complications. These factors include patient age, underlying disease, antecedent immunosuppressive state, prior infections, conditioning regimen, type of donor, degree of match, stem cell source, immunosuppressive regimen used to prevent GVHD, anti-infective practice, the occurrence of post-transplant GVHD and viral infections, and use of certain post-transplant therapies to prevent disease relapse that alter immune recovery⁴⁻⁸ (Table 1).

By one year, immune reconstitution is well underway for many HCT recipients¹⁵. However, some immunologic deficits are detectable in many patients using sensitive immunologic assays at 1–2 years, and even beyond 10 years^{16,17}. Patients with GVHD or CMV infection or recipients of HLA mismatched donors frequently have delayed, incomplete, or dysregulated immune reconstitution. Chronic GVHD is associated with multiple deficits in different arms of immunity and many types of protective responses are dysregulated¹⁸⁻²¹. Late infections are common complications and causes of death in patients with persistently active GVHD²². Functional asplenia has been reported to predispose to rapidly developing sepsis from *S. pneumoniae*, that can lead to mortality among GVHD patients²⁰. Older studies suggested the use of unrelated donors (with or without GVHD) was also associated with an increase in late infections^{22,23}, although many of those patients were likely

mismatched since low resolution typing methods were in use then. In the absence of active GVHD, persistently low CD4 counts and persistently low immunoglobulin levels have been associated with the risk for late infectious morbidity ^{2, 24}.

Thus, the risk of late infection for patients with ongoing GVHD and prolonged immunosuppressive therapy remains substantial. In contrast, in most patients without GVHD the incidence of life-threatening infection is much lower and continues to decline with passing time after transplant.

Two large retrospective CIBMTR studies have investigated late deaths (defined as beyond 2 years) of allogeneic HCT survivors. The first one, with more than 6,000 two-year survivors and a median follow-up of 6.6 years, estimated a risk of death from infection in the absence of GVHD of approximately 6% ²⁵. Half of the infections were bacterial. A similar study ten years later of more than 10,000 two-year survivors with a median follow-up of 9 years estimated that 10–20% of all deaths were caused by infection in the absence of active GVHD ²⁶. Proportions of deaths due to infection were similar in all major categories of diseases for which the transplant was performed. Generally, the risk of infectious death decreased over time after transplant, less after ten years compared to 2–4 years. Unfortunately, this kind of large retrospective registry study lacks the capability to capture and analyze fine details regarding specific infections and risk factors.

In some small retrospective studies, pneumonia appeared to be the predominant type of late serious infection ^{21, 22, 27}. For example, in a small single-center study, two-thirds of infectious deaths were due to pneumonia, and a pathogen was detectable in 57% of pneumonias with *Aspergillus* and CMV predominating ²¹. Concomitant GVHD was present in many of the patients with infection in such reports, limiting our ability to determine rates of infections in the absence of GVHD. Other risk factors were CMV infection, mismatched or unrelated donor grafts, and use of TBI ²¹. Older reports noted the importance of late varicella-zoster virus infection ²², but today with routine prolonged acyclovir prophylaxis this is much less common except in patients with persistent GVHD ²⁸. Limitations of these older studies include small numbers, unique center-specific transplant practices, varying follow-up practices, and different case mix that might affect both types and frequency of infection as well as risk factors. In a preliminary (unpublished) analysis of CIBMTR survivors who died from late infection, antecedent GVHD had occurred in most, suggesting persistent immune deficits after recovery from GVHD. This study is ongoing.

Persistent viral infections often lead to additional clinically important complications late after HCT. Persistent immune deficits after CMV infection can confer susceptibility to other infectious pathogens. Of note, non-relapse mortality in patients with early CMV infection continued to increase beyond one year in a large CIBMTR analysis, with infection being a major cause of death, suggesting that there may be long-lasting immune deficits after CMV infection that predispose patients to later infection ^{12, 13}. Other viral infections also may lead to complications late after transplant. Viral hepatitis either before or early after transplant may be associated with late complications. Flares of hepatocellular injury can occur at the time of tapering of immunosuppressive therapy due to deleterious immune responses to viral replication. Chronic infection can also result in complications such as chronic active

hepatitis, cirrhosis, or hepatocellular carcinoma more than 10 years after transplant ²⁹. Improved screening of blood and transplant donors, use of the hepatitis B vaccine, and the use of hepatitis B antivirals and the recent introduction of potent hepatitis C antivirals have resulted in a lessening of the risk for late hepatitis complications. Recent recognition of HPV-associated gynecologic and head and neck carcinomas has led to calls for consideration of the HPV vaccine for prevention ^{30–32}. It is possible that other late viral associated complications will be identified with the increasing number of recipients surviving beyond 10 years and the identification of new viral pathogens and their associations with transplant complications.

The variability in the reporting of infectious disease data in the HCT population has several causes. First, the definition of infectious syndromes is complex, and changes as new diagnostic assays are developed. Important distinctions may be missed by transplant clinicians and data managers who are not familiar with the most current definitions. Second, there is no standardization between centers in the application of infectious disease diagnostic algorithms and variability of anti-infective practices, with some centers relying heavily on prophylaxis or empiric anti-infective therapies, while other centers pursue infectious disease diagnoses aggressively. Such variability may lead to confounding due to ascertainment bias. Third, the variability late after HCT is even greater. Clinical care of the HCT survivor after one year is not typically performed by many transplant centers, particularly in the U.S. Community practices, to which these patients have returned, may not find it important to capture detailed infection data in clinic encounters. In many cases aggressive diagnostic testing is not used or even possible and empirical therapy for suspected or presumptive therapies predominates. This is confounded moreover by inconsistent availability of knowledgeable personnel to collect follow-up data. The variability in the quality of such data is substantial and leads to even greater ascertainment bias. The net effect of these limitations is that audits of infection data reports frequently find errors in under-reporting of infectious events and in some cases over-reporting due to lack of use of standardized definitions. ^{8, 33}

Key research priorities and recommendations

1. A long-term, multicenter prospective study of late infectious events after HCT is highly desirable. One-year HCT survivors should be enrolled and followed up to 5 years to determine the incidence of serious infection, types of pathogens, the risk factors, and the immunologic correlates. The sample size should be large enough to capture important differences between key variables that influence infection risk. To provide valid, actionable information we recommend the following:
 - a. only centers with enough commitment and resourced to provide high quality infection data should participate
 - b. standardized definitions of infection events should be applied
 - c. standardization of the anti-infective practices and diagnostic approaches should be implemented
 - d. standardized follow-up protocols should be used

- e. audits of data should be performed.
 - f. Samples should be collected for immunologic correlate testing to allow analysis of both clinical and immunological risk factors.
2. There is a growing recognition of the important role of the gut microbiome on the host immunity. Studies have identified associations between changes in the microbiota early after HCT on early infectious complications, GVHD, transplant-related mortality, and relapse^{34–36}. However, there are no studies on how long-lasting such early patterns of microbiota are on both late microbiota and late infection risk. Thus, a second research priority would be a prospective study to examine the association of early and late microbiota changes after HCT and the association of such changes with late infections and immunity. Such a study could be incorporated into the prospective study of late infections described above.
 3. Knowledge about the occurrence of certain specific late infections after HCT is lacking. For example, the effectiveness of consensus infection control guidelines on infection is of great interest. Are centers adhering to the vaccine guidelines; are they effective in reducing infection; what are the barriers that impede effectiveness? A third research priority is to create a registry of vaccine-preventable and other rare infectious diseases (e.g., late aspergillosis or *Pneumocystis pneumonia*). Case identification should be annotated with key information about risk factors, immunologic parameters and information about vaccination.

Section 2. Immune Reconstitution in the Laboratory

Functional Immune recovery after HCT depends on persistence of adoptively transferred mature donor immune cells present in the graft, and neogenesis of cells derived from donor hematopoietic progenitor cells (HPC).^{37, 38} Early immune recovery following HCT has been studied by quantifying white cell subsets. Early immune recovery proceeds in the following order: NK cells, B cells, CD8 T cells first, followed later by CD4 T cells, plasma cells and dendritic cells. Detailed analyses of lymphocyte subset recovery and thymic function early after transplant have been published but beyond the first post-transplant year the data are limited. Despite normal white blood cell numbers, some HCT patients do not possess normal functional immunity. Methods to determine presence of absence of functional immunity have not been validated, even if CD4 lymphocyte numbers or CD4/CD8 ratios are sometimes considered appropriate surrogate markers³⁹. Validated measures of immune function after HCT are urgently needed. Such methods could eventually guide infection prevention strategies after HCT.

Multiple factors have an impact on the immune parameters that can be measured in the laboratory. Table 2 highlights some of the relevant findings and others will be discussed in the subsections dedicated to T and B cell function. The key point is the dearth of data about immune function late after HSCT.

T cell immune reconstitution

Pathobiology of late immune dysregulation—Impaired thymopoiesis, lymphopenia and antigen exposure all contribute to TCR repertoire dysregulation⁴⁷. Memory skewing of the T cell response and associated impairment of T cell repertoire diversity has been associated with poor control of chronic viral infection⁴⁸ and impaired anticancer immunity⁴⁹. Late memory CD8+ T cells are less able to produce IL-2 in association with other cytokines.⁵⁰

Measures of T cell immune reconstitution—The types of assays currently available to assess T cell immune reconstitution include enumeration of CD4⁺ and CD8⁺ T cells. T cell subset analyses by flow cytometry (naïve, memory, and effector) are performed primarily in the research setting. Furthermore, assessment of thymopoiesis and recent thymic emigrants (by TRECs and phenotyping), TCR repertoire analysis or sequencing, and antigen-specific functional assays including response to vaccines are also not routinely used in clinical settings.

Naïve, central and effector memory and stem cell memory T cells may be enumerated by their expression of CD45 isoforms (e.g., CD45RA) in combination with maturation and homing markers (CCR7, CD31, CD103, CD27, CD62L, CD28, CD95 and CD57). Other critical T cell subsets include CD8+ memory stem cells (CD8+CD161^{hi})⁵¹, regulatory T cells (CD4⁺CD25^{hi}CD127^{low}FOXP3⁺)^{52,53}, and T helper 17 cells⁵⁴.

Estimation of TCR repertoire diversity historically used indirect methods like TCR V β repertoire analysis by microfluorimetry⁵⁵ or assessment of skewing within individual V β regions by TCR β “Spectratyping.”^{56,57} However, rapid evolution of the efficiency and cost of next generation sequencing technologies now allows direct assessment of repertoire diversity within surface marker-sorted T cell subsets or HLA-peptide multimer-sorted subsets of antigen-specific CD8+ T cells.^{58–60}

Several antigen-specific T cell functional assays are available for evaluating virus-specific responses, for example, to CMV^{61–63}, EBV⁶⁴, aspergillus⁶⁵, as well as to tumor antigens like Wilms’ tumor 1 (WT1) and proteinase-3⁶⁶. The relevant assays include cytokine flow cytometry⁶¹, ELISPOT⁶⁷, and HLA-peptide tetramer staining^{63,68}. Cytokine secretion, measured by flow or ELISPOT, elicited CD4+ and CD8+ T cell responses to peptide antigens⁶⁹, proteins⁶¹, or cells⁷⁰ can be detected⁷¹.

Clinical correlates of measured T cell reconstitution—Some studies have found an association between early immune reconstitution and clinically relevant endpoints. Survival was better in children whose CD3+CD8+ counts rose to >5th percentile of age-matched normal levels during the first year compared to children who never attained these levels⁴⁴. Similarly in adults, early reconstitution of CD3+ and CD8+ T cells correlated with improved progression-free survival (PFS)⁴⁵. Not surprisingly, impaired vaccine responses were associated with delayed T immune recovery⁷². In this study, of mostly young adults, vaccine responses to PnCRM7 and HIB tested at a median of 13 months post-HCT were better among those who had achieved CD4+ T cells >200/ μ L and IgG levels >500 mg/dL⁷².

Overall, higher levels of circulating CD4⁺CD45RA cells correlated with improved PNCRM7 response ⁷².

Studies of functional assessments of CMV-specific T cells have primarily been performed in the early post-HCT interval, leading to incomplete understanding of why persistent deficits in CMV-specific immunity lead to viral reactivation in nearly a third of HCT recipients. In a large (n=269) single institution study examining the incidence of late CMV reactivation following allogeneic HCT, the incidence of late reactivation was 31% and was more likely to occur in patients with prior or ongoing GVHD, in recipients of mismatched or unrelated donors, and in individuals transplanted for a lymphoid diagnosis ⁷³. In contrast to studies of CMV- and EBV-specific T cell immunity, little is known about functional immunity to other herpesviruses important in HCT recipients, including HSV, VZV and HHV-6, some of which contribute to late morbidity and mortality in a subset of HCT recipients.

At 2 years post-HCT a CD4⁺ and CD8⁺ T cell defect was observed involving naive, terminally differentiated, memory and competent cells ⁷⁴. At 5 years post-HCT, another study showed that low numbers of CD4⁺ and CD4⁺CD45RA⁺ T cells and reversed CD4/CD8 ratios persisted; CD4⁺CD45RA⁺ T cell numbers were low despite the absence of cGVHD at 2 years ⁴¹.

In a study of patients who were beyond 10 years post-HCT and no longer taking immunosuppressive medication (with one exception), CD4⁺ and CD8⁺ T cell blood counts were not significantly different from those enumerated using donor samples that were cryopreserved at transplant. However, compared with donors, recipients had significantly fewer naive T cells, fewer CD4⁺ central memory cells, more effector CD8⁺ cells, and more regulatory T cells ¹⁶. No clinical correlates were reported.

T cell reconstitution has been shown to be affected by the combined effects of GVHD prophylaxis and treatment, and acute and cGVHD itself ^{75, 76}. CD4⁺ T cell reconstitution is impacted by the use of T-cell depletion and GVHD ⁷². At 2 years post-HCT, the number of CD4⁺ CD29⁺ T cells was higher in recipients with extensive cGVHD suggesting that cGVHD affected T cell immune reconstitution ⁴¹. In another study chronic GVHD did not influence CD8⁺ T cell recovery, while naive CD4⁺ subsets were strongly affected ⁷⁴.

T cell immune reconstitution following autologous HCT—The timing of T cell reconstitution differs between autologous and allogeneic HCT, with some studies showing more prolonged CD4 lymphopenia after autologous HCT.⁷⁵ Renewed thymopoiesis is possible in adults >30 years old, but decreases with increasing age. ⁷⁷ In a study of autologous HCT for breast cancer, TREC numbers correlated positively with naïve T cell recovery and TCR repertoire diversification. Naïve T cells were evident by 100 days after autologous HCT for myeloma; thymic function fully recovered by 2 years, was age dependent and positively correlated with naive T-cell recovery and TCR repertoire diversity ⁴³. In another study, prolonged total and naïve CD4⁺ T cell lymphopenia persisted until 2 years after autologous HCT ⁷⁵. After autologous HCT, T cell recovery predicted OS and PFS in patients with hematologic malignancies and breast cancer ⁷⁸.

In contrast to the allogeneic HCT setting, fewer detailed studies of functional immune reconstitution have been performed in autologous HCT recipients, likely due to the lower incidence of infections associated with deficits in cell mediated immunity (e.g., CMV). However, even for infections occurring relatively frequently in autologous HCT recipients (e.g., reactivations of HSV, VZV), little is known about the pace or quality of functional T cell recovery.

Regulatory T cell immune reconstitution—The development and maintenance of immune tolerance after HCT requires the balanced reconstitution of “conventional” effector CD4+ (Tcons), tolerizing CD4+ regulatory T cells (Tregs) and CD8+ T cells, which may also be important in the pathogenesis of cGVHD ⁷⁹. Very limited data exist about Treg recovery beyond one year after HCT. As noted in one study, at 10 years after HCT recipients had significantly more Tregs compared to donors ¹⁶. One study with 2 years of follow-up showed that Tregs and Tcons recover at similar pace and slower than CD8 T cells, and were predominantly of central and effector memory phenotype ⁸⁰. Thymic Treg production was very limited within the first two years in contrast to the production of naïve Tcons and CD8 T cells. Early recovery of naïve Tregs and Tcons correlated with the development of chronic GVHD particularly if there was an imbalance of Tcons over Tregs ⁸⁰. Low telomerase activity in Tregs has been associated with severe chronic GVHD after allo-HCT ⁸¹. Lastly, the use of autologous HCT to treat autoimmune diseases via tolerance induction is thought to depend on increased Treg TCR diversification ⁸². Another T cell population whose role is less understood in HCT, are the IL-10 producing TR1 cells ⁸³. Moreover, other potentially important regulatory populations include regulatory B cells, NK cells, and macrophages ^{83, 84, 85, 86, 87}. Their role in immune reconstitution and responses to exogenous stimuli late after HCT is poorly understood.

B cell immune reconstitution

Translational studies have led to a greater appreciation of post-HCT B-cell deficiencies and clinical determinants of B-cell recovery kinetics, including alloreactivity. Recovery of functional immune cells after autologous HCT has been likened to fetal ontogeny, requiring re-encounter of the new donor immune system to microbes over many years. Most patients after autologous HCT eventually regain functional immunity. By contrast, functional immune recovery in the presence of alloantigen is a lifelong process, especially when immune tolerance is not achieved (i.e., in cGVHD).

The paucity of IgD-negative post-GC cells and CD27+ B cells and a ‘fetal-like’ B cell compartment may persist for years. B cell dysregulation results in auto- and allo-antibody production, which is more profound in cGVHD patients.^{88–90} Despite the clinical importance of these phenomena, the recovery of late B-cell function remains underexplored.

Hypogammaglobulinemia after HCT is associated with dramatically increased infection risk, including encapsulated organisms and viruses.^{87, 91} Collectively, published data support the notion that patients with and without cGVHD achieve varying states of B-cell immune function and are variably immune tolerant, analogous to genetic immunodeficiency

patients.^{92, 93} However, what molecular mechanisms account for persistent B cell dysfunction late after HCT remain largely unexplored.

The inability to mount a proper B-cell response to microbial insult after HCT has been associated with a paucity of memory B cell responses in the first 2 years after HCT^{94, 95}. Flow cytometric analyses of blood in patients after allogeneic HCT and autologous HCT have afforded detailed enumeration of B-cell subsets with functional correlates in the healthy setting that can be defined by cell surface marker criteria in both autologous and allogeneic HCT patients⁹⁶. Persistently low numbers of CD27+ memory B cells^{97, 98}, IgDLo, post-germinal center (GC) B cells⁹⁹ and immaturity of the B Cell Receptor (BCR) repertoire¹⁰⁰ suggest a failure of B cells to undergo key maturation steps including somatic hypermutation. In murine GVHD splenic atrophy and destruction of secondary lymphoid organs is evident and likely immune-mediated although limitations of tissue access make this difficult to study directly in humans^{101–104}. Abnormal *ex vivo* B cell responses have been attributed to steroid therapy¹⁰⁵, mitogen defects^{106, 107}, T-dependent IgG defects¹⁰⁸, B-cell activation signaling¹⁰⁹ and Ig-switching defects¹¹⁰. Rare antigen-experienced B cell subsets are capable of constitutive IgG secretion but HCT patients are known to have poor recall responses to vaccination.^{97, 111} HCT patients, especially those with cGVHD are unable to produce functional high affinity antibodies.

Factors contributing to long-term B cell functional aberrations after HCT remain largely unknown because, with few exceptions, most studies examine antibody and B cell responses within the first 2 years after HCT. One early study showed that patients (followed for nearly 5 years after HCT) who had been in vivo challenged with phage and pneumococcal antigen recovered normal primary and secondary antibody responses recovered if they did not have cGVHD.¹¹² Another study of patients followed for a median of 6.5 years after allogeneic HCT showed progressive loss of antibodies to measles, mumps and rubella over time, with most previously vaccinated patients becoming seronegative by 5 years.¹¹³ A European study of patients receiving the pneumococcal vaccine after HCT revealed that even in the absence of GVHD, IgG responses several years after primary vaccination were not durable.¹¹⁴ Ongoing susceptibility of patients, especially those with cGVHD to encapsulated organisms suggests that splenic B cell dysfunction may persist for years. Long-term reconstitution deficits and plasma Ig levels were determined in a study in which HCT patients with and without cGVHD were examined together. Low B cell numbers and low functional response to tetanus toxin were associated with increased infections at 6 years post HCT.¹⁵

While *ex vivo* assays have shown that B cells are constitutively activated in cGVHD,^{115, 116} B lymphopenia and humoral immune deficiency are distinctive characteristics of cGVHD.^{117–120}. Studies have characterized the composition of the peripheral blood B cell compartment and have begun to characterize factors leading to altered B cell homeostasis after HCT.^{104, 121} While functional anti-microbial antibodies are often persistently absent and HCT patients are hypogammaglobulinemic, cGVHD, is paradoxically associated with high titers of allo- and autoantibody.^{88, 89} In this regard, cGVHD patients appear to be similar to patients with common variable immune deficiency (CVID) given their common propensity toward B cell autoreactivity in the face of profound humoral immune deficiency.^{89, 122} A preponderance of CD21LoCD27-B cells has been found to be associated

with infectious complications⁹⁷ suggesting that typical antimicrobial GC reactions do not occur. Muted B cell responses to microbial pattern recognition receptors like lipopolysaccharide (LPS) potentially contribute to this GVHD-associated immune deficiency.¹²³ Evidence suggests that similar immunodeficiency states are associated with variable levels of immune tolerance and autoimmunity.^{124–126}

Reconstitution of innate immunity

Almost all studies evaluating innate immune populations (natural killer (NK) cells, invariant NKT cells, dendritic cells, macrophages, neutrophils, eosinophils, platelets, and monocytes.) have focused on immune constitution under one year after transplantation with the assumption that these populations normalize not only in numbers but function as well. Only a single study has shown that low early counts (up to day 180) of innate populations such as basophils, eosinophils, macrophages, and monocytes, may be associated with post-HCT infection risk after 1 year but the study is limited by very small numbers.⁸⁷ The few remaining studies have focused only on NK cells and shown that factors such as ATG, alemtuzumab^{127, 128} can delay their immune reconstitution but the impact after 1 year, if any, is unknown. Interestingly, late EBV infection may impact innate immunity inducing hemophagocytic lymphohistiocytosis.¹²⁹ Studies of the late recovery of innate immune responses and their interaction with T and B cell populations are warranted.

Key research priorities and recommendations

The difficulties of conducting detailed immunologic studies late after HCT were already mentioned. Additionally, the quality of registry data, while typically reliable for survival and relapse, is less robust related to reporting of infectious events in the late post-transplant interval.

Immune cell intrinsic and extrinsic pathways responsible for prolonged immune deficiency after HCT remain unknown. Immune function assays that determine infection risk have not been validated. The ability to understand immune dysregulation that persists into the late post-HCT interval will depend on prospective correlative sample collection that continues beyond one year, and the correlation of phenotypic and functional data with clinical data regarding late infection events, the presence of ongoing GVHD and the incidence and competing risks of mortality. The development of patient immunologic profiles with calendar and event driven collection of samples, including serum and PBMC, and clinical data should be encouraged to facilitate a better understanding of the determinants of late immune recovery. We recommend the following research priorities:

1. Studies that identify late dysfunctional adaptive immunity and probe the molecular mechanisms underlying it in the presence and absence of cGVHD.
2. Studies that address adaptive immune system neogenesis, maturation and exhaustion. In particular, it is important for us to understand how these processes are influenced by persistent alloreactivity, inflammation and viral infection.
3. Studies to assess late functional pathogen-specific T and B cell responses (to bacterial vaccines as well as viral antigens) as well as to pathogens not historically assessed in published studies (e.g., VZV, HSV, HHV-6). These

studies should aim to identify what factors that are associated with poor responses.

The detailed prospective study suggested in section 1 should include assessments at 1, 2 and 5 years post-HCT that addresses adaptive immune system cell neogenesis, maturation and exhaustion in the context of alloantigen activation and viral infection. Consideration should be given to event-driven storage of samples for future analysis.

Section 3. Interventions to improve immune function

This section will focus on the active generation of immune responses by vaccination and the passive transference of immunity with immunoglobulin (IVIG). Adoptive cellular immunotherapy will be addressed only briefly. We discuss what is known and areas that need study. The potential for intervention on the microbiome and its effects on late immunity, an area of the utmost interest, as mentioned earlier, is not yet known and will not be discussed ¹³⁷.

Vaccination post HCT is accepted as a basic principle of improving the immune response. ¹¹² While international guidelines recommend the administration of killed organism vaccines as early as 3–6 months post-transplant, ^{3, 138} implementation of vaccination schedules remains variable. ¹³⁹

Passive transfer of immunity with IVIG provides short-term protection against infection. IVIG may be given to treat active infections or to prevent infection ^{140–142143–145146}. In addition, there are times when IVIG products are used as adjunctive therapy for infections that are out of control ^{140, 147, 148}. Hypogammaglobulinemia on its own is a potential indication for IVIG replacement, although the threshold for repletion differs from center to center, perhaps most commonly < 400 mg/dL ¹⁴⁹. The published evidence on the benefits of IVIG is non-conclusive. The lower rates of relevant CMV related endpoints identified by early controlled single center studies and supported by meta-analyses did not translate onto improved overall survival, maybe in part due to increase in sinusoidal obstruction syndrome (SOS) ^{150, 151152153, 154155}. Use of IVIG products is not without its problems, and overuse may impair long-term humoral recovery after BMT ¹⁵⁶. Moreover, studies of its use late after HCT have not been conducted.

Current knowledge gaps

Vaccinations—There are many gaps in our understanding of the development of post-HCT active immunity through vaccination due to a lack of standardization and/or adequate studies (see Table 4). The former originates from the fact that vaccination protocols vary among centers as well as from country to country. The latter has occurred because systematic vaccination studies in large enough cohorts that address different stem cell sources, variations in HLA-match, conditioning regimens and GVHD activity, have not been a recent area of funded research except for a few vaccines under development in which the sponsor desired to have data that included HCT recipients.

Passive Immunity with Immunoglobulin—There are several knowledge gaps in the use of immunoglobulin after HCT. In particular, the relevance of older randomized studies and meta-analyses data regarding the risks of SOS and benefits of IVIG therapy is an open question given the current practices of more universal liver prophylaxis with ursodeoxycholic acid and busulfan therapeutic dose monitoring. The most recent meta-analyses still focus on myeloablative conditioning and matched sibling HCT without any focus on unrelated or haploidentical donors, cord or peripheral blood graft sources, or reduced intensity conditioning^{155, 157}.

There is no strong evidence regarding the IgG level at which replacement IVIG should be administered,³ and practices vary. The experience from the patients who underwent allogeneic HCT for primary immunodeficiency disease (PID) may provide some guidance.¹⁵⁸ Serum trough IgG levels are higher among PID patients when higher IVIG replacement doses are given, and the risk for pneumonia is lower.¹⁵⁹ IVIG replacement may be stopped once GVHD has resolved, immunosuppressive therapy has been discontinued, trough IgG levels are >600 mg/dL, and there is evidence of Ig-class switched B cells. However, specific antibody responses are followed. Correlative studies have not been performed for adults. Whether or not such observations are applicable for patients transplanted for other diseases has not been studied.

IVIG half-life varies widely from 1 to 10 days among HCT recipients, versus 18 to 23 days among healthy controls.^{160–162} Active infections can accelerate immunoglobulin catabolism which necessitates dose adjustments to maintain target IgG levels¹⁶². So, knowledge gaps include defining the optimal frequency for measuring IgG levels after HCT, as well as the specific indications for its administration (severe hypogammaglobulinemia *versus* specific infections). In addition, if immunoglobulin products are used, which are the safest products and what is the most cost-effective way of administering them?

Adoptive cellular immunotherapy—Adoptive cellular immunotherapy has emerged as a promising strategy for the control of otherwise untreatable viral infections. Proof-of-concept trials show that this approach is safe and well tolerated¹⁶³. Remaining issues include determining the best source of obtaining virus-specific cells (donor related or third-party¹⁶⁴). This technique has the potential to revolutionize the management of refractory viral infections after HCT. Only a few transplant centers have the capability to prepare the cell products, a gap that is well-recognized, and these have rarely been studied in the late period post-HCT.

Key research priorities and recommendations

There are a number of recommendations that can be made with respect to these three categories of interventions and which could become more favorable with future contextual studies, cost-effective utilization and ease of implementation in the future:

1. A retrospective study from a small number of centers of basic numeric immune reconstitution markers correlated with vaccine responses might shed some light on standardizing thresholds for initiating vaccination for the current portfolio of transplant types.

2. Prospective multicenter clinical trials are needed to both define and address the knowledge gaps in achieving active immunity after vaccination in the aforementioned comprehensive range of posttransplant scenarios (Table 4). Key study variables will include: harmonized vaccine schedules (including start times), clinical variables, and harmonization of calendar driven vaccine specific titers. These data will provide the evidence to support development of a schedule of required and optional vaccines with guidelines for administration of and monitoring of success (and failure) in the prevention of infection.
3. Regarding passive transfer of immunity we would like to gain knowledge regarding the current practice of IVIG therapy, perhaps through the use of online surveys. The surveys could start with a relatively small number of centers, with all types of transplants, to determine the range of practice and rates of infections and use the data to guide development of a study to determine what dose schedules provide protection from infection.

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Table 1

Selected Factors that influence late infections after HCT

Factor		References
Age	Higher incidence of late fungal infections in older patients	9
Preparative regimen	Fewer early infections with non-myeloablative (NMA) vs myeloablative conditioning (MAC) Higher infection rate with total body irradiation (TBI)	5, 10
T cell depletion	More CMV and Aspergillus seen with T cell depletion in MUD	7
Peripheral blood (PBSC) vs bone marrow (BM)	Higher incidence of infection over 2 years with BM	8
Alternative donors	High incidence of infection in recipients of mismatched unrelated donor (MMUD) and umbilical cord blood (UCB)	11
Chronic graft versus host disease (cGVHD)	In many studies cGVHD turns out to be the only independent risk factor for severe infection	107
CMV infection	CMV seropositivity and reactivation has been associated with delayed immune reconstitution and increased infectious mortality	10, 12, 13
Post-HCT rituximab	Patients treated with pre-emptive rituximab for EBV reactivation had increased late infections	14

Table 2

Determinants of late immune recovery after HCT:

Factor	Study Characteristics, time period, subject numbers	Findings in Children	Findings in Adults	Reference
Age	18 months, n=71, T-cell depletion, related or URD	Majority had normal numbers by 6–12 months	Majority had CD4 < 200/ul for 12–18 months.	40
	5 years Chronic GVHD and age affect immune reconstitution		Low CD4+ CD45RA+ T cells up to 5 years. The number of CD4+ CD45RA+ cells in 10–19- year old patients > 40– 49-year-old patients	41
	18–36 months Majority T-cell depleted	No data	TRECSs recovered during the second year in adults.	42
Source of graft and TCD				
CD34+ selected vs unmanipulated	2 years, n=40, Autologous, Multiple Myeloma, unmanipulated or CD34+ selected		At 2 years, No difference in CD4 and CD8 numbers; in the CD34-selected group, TRECs > than both baseline TRECs and unselected group-TRECs	43
T cell depletion	18–36 months Majority T-cell depleted (127/158)	TREC from T cell depleted catch up at 9 months (all ages) as high as healthy controls	TREC from T cell depleted catch up at 9 months (all ages) as high as healthy controls; no later observations	42
PBMC vs. CD34+ vs BM	13–18 months n=32 <i>Higher numbers of Long-term survivors (>12 Months-4y) among patients with CD8+ above 5th and the 50th percentile of age-matched normal levels.</i>	No difference in CD4+CD45RA+ and CD +CD45RA+ between the 3 groups after 13–18 months		44
Cord Blood	Dual UCB vs MSD vs MUD n=95		No difference in T cell numbers at 1 year	45
Preparative regimen RIC vs MA, NMA, RIC	NMA vs MA-ASCT n=66		No difference in T cell numbers at 1 year	46

Table 3

Measurement of Immune Function Late after HCT

Test	What is evaluated	What we have learned about Outcomes from use of the test	References
ALC	Early lymphoid recovery ALC > .2 × 10(9) cells/L	Higher OS and PFS	130, 37
Donor:recipient chimerism (whole blood)	Availability of donor cells		
T cell phenotyping for CD3/CD4/CD8	Anti-viral and fungal immunity	Early reconstitution of CD4 ⁺ CD8 ⁺ T cells correlated with overall survival (OS), non- relapse mortality, and improved progression- free survival (PFS).	131
B cell phenotyping for CD19+ B cells	Ability to respond to vaccines		
NK phenotyping for CD56+ and CD16+ NK cells	Early viral immunity		
Immunoglobulin IgG, IgA, IgM	General B cell functional reconstitution		
Specific antibody evaluations Tetanus Diphtheria Pertussis Measles, Mumps Rubella	Memory T and B cell function post HCT		132
RBC pit counts	Splenic function	Decreased immunity of encapsulated organisms can result in rapid overwhelming sepsis	133–135
Specific response to neoantigens • HPV - peptide • Pneumococcus–polysaccharide	Ability of Naïve T cells and B cell to respond to an antigen		132, 136
Dendritic cell phenotyping	Overall evaluation of immune function	Higher numbers of myeloid DC associated with improved PFS	45, 87
Donor:recipient chimerism (T cell, B cells, myeloid, and dendritic cell)	More complete evaluation of donor immunity	If incomplete myeloid chimerism, can affect T and B cell repertoire. Incomplete B cell chimerism can affect immune responses to viral antigens	
Tregs and TR1 cells, Bregs, NKregs	Evaluation of regulatory function	Associated with immune tolerance and suppression of GvHD	83, 848586
Memory/CM/EM T cells			
Allergy testing if donor has known allergies	Evaluation of transfer of allergies from the donor	Significant allergies can be transferred to recipients from donors resulting and severe reactions	87

Table 4**Vaccination Strategy**

Goal	Current variables	Recommendation
Vaccination is universal:	What vaccines can be given when still receiving immunosuppression?	The effects of GVHD and use of corticosteroids and ATG remain unclear
	How do post-HCT therapies like anti-B cell monoclonal antibodies, CAR-T cells, tyrosine kinase inhibitors, proteasome inhibitors affect ability to respond to vaccines?	These variables need to be included in the clinically annotated data that will accompany proposed immune reconstitution studies.
	Do vaccine responses need to be measured routinely or can responses be assumed?	Future vaccine studies need to incorporate post-vaccination titers so that if responses are shown to be near universal then practice guidelines could assume protection without measuring responses or identify subgroups in which it is important.
	Can pediatric combination vaccines (e.g. Pediarix = DTaP/IPV/HepB) be used conveniently and cost-effectively in adults	Among centers with good research infrastructure that give Pediarix versus separate Tdap/Td/Td, IPV and Hep B, it could be beneficial to retrospectively compare the response rates.
	Functional (i.e chronic GVHD) and/or surgical asplenia	Try to move from a pragmatic schedule that starts with conjugate vaccines (Hib, PCV13, MCV4) by studying vaccine responses in this important high-risk subgroup.
Killed organism	How early post-HCT can a successful immune response be obtained from vaccination? Data supporting early vaccination is strongest for the conjugate vaccines in matched sibling BMT without significant hypogammaglobulinemia or severe chronic GVHD	Studies have not adequately addressed different stem cell sources, variations in HLA-match, conditioning regimens and GVHD activity, hypogammaglobulinemia and this needs to be studied prospectively.
	Should seasonal influenza vaccination differ from other vaccination policy; specifically how early after HCT can flu shots be given? One study found 2 doses of flu vaccine (vs. standard 1 dose) did not enhance response and, response rates were double among recipients > 1 y vs <1 y post-HCT ¹⁶⁵ .	Consensus guidelines advise giving the flu shot from 6 months post-HCT and to giving earlier during influenza outbreaks but evidence to support these recommendations is lacking. Additional studies are needed to confirm that post-HCT recipients do not benefit from 2 shots or higher dose influenza vaccine. Quadrivalent versus high-dose trivalent needs to be studied.
	Do CD4, CD19 cell counts, IgG, IgA and IgM levels or IViG therapy influence administration of vaccines? Receiving IViG for hypogammaglobulinemia might be a surrogate for delayed immune reconstitution.	We need to study how levels of basic numeric immune reconstitution (CD4, CD19, IgG, IgA, IgM) influence when to vaccinate because existing guidelines don't adequately address this for the wide range of HCT scenarios.
Select live organism vaccines	When should MMR, or Varicella vaccine be given?	There is only scant evidence to support consensus guidelines that advise at least 2 years post-HCT and long enough off immunosuppressive therapy that resumption of immunosuppression is unlikely.