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Novel insights into GVHD and immune reconstitution after allogeneic hematopoietic cell transplantation

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Abstract

Effective control of the graft-versus-host disease (GVHD) and immune reconstitution are crucial in improving the outcome of allogeneic hematopoietic stem cell transplantation (HSCT) as well as the quality of life of the transplant survivors. Recent basic and clinical studies have deepened our understanding of the mechanisms of the immunological sequelae of HSCT, GVHD, and compromised immune systems. Based on the findings, various novel approaches have also been developed and tested clinically. However, further studies are necessary to develop therapeutic strategies with significant clinical benefits.

Key words acute GVHD, chronic GVHD, immune reconstitution, allogeneic hematopoietic stem cell transplantation

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Introduction

Graft-versus-host disease (GVHD) is a major devastating complication of allogeneic hematopoietic stem cell transplantation (HSCT). Acute GVHD develops early after HSCT, typically within 100 d post-transplantation. Chronic GVHD is also a major cause of late non-relapse mortality (NRM) after HSCT and significantly impairs the quality of life of the transplant survivors[1]. Acute GVHD is an alloreactive T cell-mediated systemic inflammatory disorder, typically involving the skin, gut, and liver[2]. Chronic GVHD is a more complex wasting syndrome involving various immune effector mechanisms and clinical manifestations[1,3]. However, effective prevention and treatment of acute GVHD remain challenging.

Allogeneic HSCT is associated with severe immune compromise, with defects in both innate and adaptive immunity. In particular, T-cell recapitulation takes a long time after HSCT. GVHD targets the immune system, and its prophylaxis and treatment with immunosuppressants prevents immune reconstitution (IR). Therefore, a better understanding of transplant immunology is required to develop effective strategies to prevent GVHD and facilitate IR.

Novel Insights into the Biology and Treatment of Acute GVHD

Acute GVHD is a major barrier to successful allogeneic HSCT; it is a leading cause of morbidity and NRM after HSCT. Unfortunately, current therapies for GVHD lack precision and rely on the induction of global immunosuppression, which increases the rates of NRM secondary to infection and the risk of post-transplant relapse. Furthermore, the prevalence of treatment resistance in patients with GVHD and GVHD progression due to severe immune deficiency suggest the importance of mechanisms other than those involved in the alloimmune response in influencing

GVHD severity. Morbidity and mortality associated with GVHD are mainly driven by gastrointestinal tract injuries. Therefore, immunosuppression alone may be insufficient to control GVHD after HSCT.

Studies on GVHD pathophysiology have primarily focused on the induction phase of GVHD, particularly the mechanisms of donor T-cell activation by professional and non-professional antigen-presenting cells. However, several recent studies on the mechanisms of target tissue injury have revealed tissue stem cells as targets of GVHD, leading to impaired tissue homeostasis and regeneration. Hence, GVHD is recognized as a disorder of tissue regeneration and repair[4]. Recent insights into intestinal homeostasis after HSCT have greatly reconciled our understanding of GVHD pathophysiology and helped reshape contemporary GVHD prophylaxis and treatment strategies. Gut GVHD is a major cause of GVHD-associated mortality. Emerging data indicate that intestinal stem cells (ISCs) and their niche Paneth cells are targeted in acute GVHD, leading to the dysregulation of intestinal homeostasis and microbiota[5-7]. The microbiota and their metabolites shape the immune system and maintain intestinal homeostasis; therefore, intestinal dysbiosis may alter the host susceptibility to GVHD. In the skin, Lgr5+ hair follicle stem cells (HFSCs) exhibit a multipotent capacity to regenerate the epidermis. HFSCs are targeted in GVHD, which impairs the healing process[8]. These novel findings reconcile GVHD as a disorder of tissue regeneration and repair[4]. Therefore, protection of tissue stem cells and target tissue cell components may be a novel approach to prevent GVHD and associated infections. R-Spondin is one of the three essential factors involved in the generation of organoids from a single Lgr5+ ISC. R-Spondin is produced by intestinal lymphatic endothelial cells (LECs); the number of LECs is markedly reduced in GVHD, which impairs the production of R-Spondin[9], suggesting that the administration of R-Spondin can potentially improve the transplant outcomes. Brief administration of R-Spondin has been reported to protect ISCs and ameliorate systemic GVHD in murine models[5,10]. Similarly, interleukin (IL)-22 has been reported to protect ISCs and ameliorate systemic GVHD[6,11], and various clinical trials on IL-22 are ongoing. Epidermal growth factor (EGF) restores epithelial and mucosal integrity after intestinal injury and EGF plasma levels are low in patients with acute GVHD[12]. EGF is an inexpensive commercially available drug (urinary-derived human chorionic gonadotropin) that has shown promising results in clinical trials of patients with steroid-refractory acute GVHD[13]. Glucagon-like peptide 2 (GLP-2) is produced by intestinal L-cells and stimulates intestinal growth and function. Colon biopsies of patients with acute GVHD revealed a low number of L cells as a risk factor for steroid-refractory GVHD[13]. Administration of the GLP-2 analog, teduglutide, has been reported to protect ISCs and ameliorate GVHD in mice[14].

Intestinal secretory, Paneth, and goblet cells protect the host from pathogens. Paneth cells in the small intestine secrete antimicrobial peptides that regulate the intestinal ecology. Paneth cell injury in GVHD decreases antimicrobial peptide production, leading to intestinal dysbiosis[7,15]. Goblet cell injury in GVHD reduces the inner mucus layer, which contains the host defense molecules such as Lypd8, to protect the epithelium from bacterial invasion and facilitate bacterial translocation[16]. Administration of carbapenems after HSCT facilitates the growth of mucus-degrading *Bacteroides* and aggravates GVHD[17]. Thus, both GVHD and carbapenems disturb intestinal homeostasis and exaggerate the vicious cycle of GVHD and infection. IL-25 induces goblet cell hyperplasia. Administration of IL-25 has been reported to protect the goblet cells, prevent infections, and ameliorate GVHD in mice[16]. Low numbers of Paneth cells in upper gastrointestinal biopsy and goblet cells in colon biopsy are associated with clinical GVHD severity and a high incidence of NRM[16,18]. Microbial diversity is reduced with *Enterococcus* dominance in stool samples of patients with GVHD after allo-stem cell transplantation, which is associated with high GVHD-related mortality and low survival rate[19,20].

High dose and long duration of systemic corticosteroid treatment for both acute and chronic GVHD are associated with poor transplant outcomes due to increased risks of infection and leukemia relapse[21]. Recent studies have uncovered previously unrecognized adverse effects of corticosteroids, and long-term corticosteroid treatment has been reported to damage skin stem cells[8]. Interestingly, the use of carbapenem facilitates the growth of *Bacteroides*, which degrades mucus in the colon and aggravates GVHD. Therefore, antibiotics disrupt the repair of GVHD-mediated tissue injury.

In summary, GVHD impairs tissue regeneration by inducing ISC injury. Tissue injury caused by a pretransplant conditioning regimen makes tissues more vulnerable to GVHD by impairing various tissue intrinsic properties, such as repair, homeostasis, and microbial ecology[22]. Hence, strategies to increase the tissue recovery capacity in response to immune insults can facilitate effective tissue repair and restoration of tissue homeostasis. Deeper understanding of GVHD biology can further aid in the development of novel treatment strategies with significant clinical benefits.

Novel Insights into the Biology and Treatment of Chronic GVHD

Allogeneic HSCT is a curative treatment for otherwise incurable hematologic diseases, including malignancies and bone marrow failure syndromes. With improvements in immunosuppressive therapy and supportive care, only a few patients develop acute GVHD, as more patients survive beyond the first year of transplantation. However, chronic GVHD, resulting in clinical manifestations resembling those of autoimmune diseases, remains a major complication after allogeneic HSCT that can cause morbidity and NRM in long-term survivors after HSCT. Similar to autoimmune diseases, both T and B cell responses appear to play important roles in the pathogenesis of chronic GVHD, suggesting a general loss of immune tolerance in affected patients. Recent studies have elucidated T-cell- and B-cell-based pathogenesis of chronic GVHD and proposed a mutual relationship between the two[23].

CD4+CD25+Foxp3+ regulatory T cells (Tregs) are known to play a crucial role in maintaining immune tolerance after allogeneic HSCT. We previously demonstrated that altered Treg homeostasis under prolonged lymphopenia after HSCT leads to increased susceptibility to apoptosis in this subset, which results in a relative deficiency of Tregs based on the prolonged imbalance of Treg homeostasis that is associated with a high incidence of extensive chronic GVHD[24]. To preserve Treg homeostasis, we conducted a clinical trial using low-dose IL-2. IL-2 therapy resulted in the selective increase and decrease in the levels of phosphorylated signal transducer and activator of transcription 5 (STAT5) in Tregs and conventional T cells, respectively, which induced several changes in Treg homeostasis, including increased cell proliferation, thymic export, and resistance to apoptosis. With the restoration of Treg homeostasis after IL-2 therapy, the clinical symptoms of chronic GVHD were reportedly improved in approximately 60% of all patients[25,26]. These results clearly indicate that alteration in Treg homeostasis is a major pathogenic process in chronic GVHD that can potentially be used as a therapeutic target.

Recent studies have shown that altered B cell homeostasis plays an important role in the pathogenesis of chronic GVHD. Delayed reconstitution of naïve B-cell subsets, including IL-10-producing regulatory B cells, and the compensatory increase in soluble B-cell-activating factor promote the responsiveness of B cells to antigens and increase the survival of activated B cells[27]. Clinical studies have shown that pathological B-cell depletion by rituximab can suppress the incidence and severity of chronic GVHD[28]. Murine studies have shown that excessive differentiation of naïve B cells into germinal center B cells results in the deposition of IgG alloantibodies, leading to tissue damage in chronic GVHD target organs[29]. Based on these findings, inhibitors of Bruton's and spleen tyrosine kinases, which signal downstream of the B-cell receptor, have been developed as treatments for steroid-resistant chronic GVHD[29,30].

In addition to the individual mechanisms of T- and B-cells, their interrelationships are also important. In particular, altered T-B interactions in lymph nodes have been shown to be an important aspect of the pathogenesis of chronic GVHD. Studies have demonstrated a significant decrease in T-follicular helper (Tfh) cells, which support B cell activation in lymph nodes, in peripheral blood and increase in the plasma concentration of C-X-C motif chemokine ligand 13, a ligand of C-X-C motif chemokine receptor 5, in patients with active chronic GVHD, suggesting that the migration of Tfh cells from peripheral blood to lymph nodes is an important process in chronic GVHD. Tregs and T-follicular regulatory cells regulate the interaction between B and Tfh cells at the germinal center[31,32].

Above-mentioned studies have mainly investigated samples from patients with chronic GVHD after HLA-matched transplantation. However, IR and chronic GVHD after HLA-mismatched transplantation have not yet been properly studied. Clinical studies have suggested that PTCy-based HLA-haploidentical transplantation (PTCY-haplo) and umbilical cord blood transplantation (CBT) are associated with a low incidence of chronic GVHD[33,34]. Recently, we examined early IR, especially early B-cell lymphogenesis and differentiation, in three different types of alternative donor transplants, including PTCy-haplo, CBT, and T-cell-replete haploidentical peripheral blood with low-dose anti-thymocyte globulin (ATG-haplo), in comparison with HLA-matched peripheral blood transplants[35]. Our data demonstrated that early IR was very different among different transplant types. Notably, B cell reconstitution within eight weeks post-transplantation was substantially affected by the donor source and presence of acute GVHD. Impaired early recovery of the naïve B cell pool is associated with future development of chronic GVHD. PTCy-haplo restored favorable B-cell homeostasis, leading to the enhanced emergence of naive fractions from the bone marrow and suppression of excessive early activation in peripheral lymph nodes. These findings indicate that abnormal B-cell lymphopoiesis in the very early post-transplant period is a major trigger for the basal pathogenesis of chronic GVHD, and PTCy-haplo is a promising strategy to prevent initial abnormalities and establish long-term immune tolerance in patients with GVHD.

Detailed mechanisms by which PTCy-haplo can re-

store B-cell lymphogenesis remain unclear but may involve the immune environment early after transplantation. In general, PTCy-haplo may enable an immunologically calm environment by efficiently depleting allo-aggressive effector T cells and sparing Tregs. This may be much less immunogenic than ATG-haplo, in which a haploidentical T-cell replete graft is infused with only low-dose ATG. Our results showing the negative impact of acute GVHD on B-cell lymphogenesis also suggest that aggressive inflammation should be controlled sufficiently to avoid initial B-cell abnormalities. Further studies on animal models will provide insights on the etiology of abnormal B-cell homeostasis and aid in the development of therapeutic and prophylactic approaches for chronic GVHD.

Based on our understanding of its pathogenesis, various novel therapies are being developed to treat chronic GVHD. Although there is an increase in the treatment options for chronic GVHD, there are no available biomarkers to support the treatment choices. Therefore, future studies should focus on the identification of clinically relevant biomarkers for GVHD. With the continuous development of treatment strategies for chronic GVHD in both basic and clinical research, the quality of life of long-term survivors after allo-HSCT is expected to improve.

Towards Predictable IR after Transplantation

Severe immune compromise with defects in both innate and adaptive immunity is observed after allogeneic HSCT. While restoration of innate immunity typically occurs in the first month after transplantation, defects in adaptive immunity persist for a long period[36-40]. These defects arise from the need to eradicate the recipient immune response to prevent the rejection of non-self HSCs and suppress the donor immune system to prevent overwhelming hyperacute GVHD. Successful HSCT involves the re-establishment of T cell immunity, especially CD4+ T cell immunity, in the post-transplant period. Reconstitution of T cell immunity occurs by both homeostatic expansion of infused populations at the time of transplant (early IR) and de novo thymic-dependent T cell production over a long period (months to years). Research over the last two decades has revealed that the pace of this process is affected by the recipient age, donor/host human leukocyte antigen (HLA) disparity, intensity of the conditioning regimen, method of GVHD prophylaxis, incidence of GVHD, and graft composition[41]. IR generally takes up to 1-2 years, but a significant number of patients require an even longer recovery period[36-40].

Some of the factors controlling IR, such as the recipient age, are non-modifiable. Approaches to minimize the incidence of GVHD have profound effects on the pace of post-transplant IR. Standard approaches for the prevention of GVHD include serotherapy (rabbit-ATG and alemtuzumab) to eliminate T cells via antibody-dependent cellular cytotoxicity, use of calcineurin inhibitors to limit T-cell activation, and use of methotrexate and mycophenolate mofetil to limit the proliferation of T cells. These approaches are effective in limiting the incidence of GVHD in HLA-matched HSCT and CBT patients; however, they are insufficient to control GVHD in HLA-mismatched transplant patients. In addition to serotherapy, approaches to limit the incidence of GVHD by eliminating donor T cells either before or after infusion include in vivo and ex vivo depletion of T cells. Currently, three methods are available for T cell depletion: positive selection of CD34+ HSCs, negative selection of a/b T cells, and in vivo depletion of T cells with post-transplant treatment with cyclophosphamide or anti-T-cell antibodies, such as ATG and alemtuzumab. Currently, all these approaches are in clinical use. However, any technique to limit the number and function of T cells in the post-transplant period poses the risk of delaying IR[42-46] and increasing the risk of relapse in cases of hematological malignancies. Several other factors, including the type of cells depleted (T cells only vs. other populations, such as B and natural killer cells), also influence the incidence of GVHD and rate of IR in these approaches. Better IR in terms of the number of T cells and time period (early after HSCT) is critical for improving the non-relapse- and relapse-related transplant outcomes.

We previously found that early CD4+ T-cell reconstitution can predict the survival of patients after HSCT[47]. We found that a threshold of CD4+ IR, 50 CD4+ T-cells/μL, within 100 d post-transplantation is associated with decreased NRM and improved event-free and overall survival. A retrospective analysis of a large cohort of adult and pediatric recipients of CD34+ HSCT also reported similar findings[43]. Moroever, this measure of early CD4+ T-cell reconstitution can overcome the risk associated with GVHD and virus reactivation[42,46,47]. A prospective individualized ATG dosing trial showed that the IR of CD4+ T cells is better predicted by individualizing the ATG dose, as it decreases NRM and improves the overall survival[40]. Variable exposure to other agents, such as fludarabine, has also been reported to influence the transplant outcomes, such as overall survival[48]. Developing a simple and easily replicable milestone that is informative regardless of the recipient age, indication for transplant, and transplant platform will help in optimizing the design of new predictable transplant platforms that can be feasibly used in small centers as well as those with limited resources[49].

In the future, strategies should be developed to effec-

tively predict IR (CD4+ IR), reduce toxicity (virus reactivation) and NRM (after transplantation), and achieve better disease control in GVHD[49]. Pharmacokinetics and dynamics are important in achieving this goal.

Author Contributions

TT wrote the abstract and introduction as well as the section on "Novel insights in biology and treatment of acute GVHD". KM and JJB wrote the section on "Novel insights in biology and treatment of chronic GVHD" and "Towards Predictable Immune Reconstitution after Transplantation", respectively.

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Conflicts of Interest

KM declare no conflict of interest. Disclosure forms provided by the authors are available on the website.

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