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Pleiotropic Effects of Transforming Growth Factor- β in Hematopoietic Stem Cell Transplantation

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Abstract

Transforming growth factor β (TGF β) is a pleiotropic cytokine with beneficial and detrimental effects post-hematopoietic stem cell transplantation (HSCT). TGF β is increased in specific sites post-engraftment and can suppress immune responses and maintain peripheral tolerance. Thus, TGF β may promote allograft acceptance. However, TGF β is also the central pathogenic cytokine in fibrotic disease and likely promotes pneumonitis. Although TGF β can enhance leukocyte recruitment and IgA production, it inhibits both innate and adaptive immune cell function and inhibits anti-viral host defense post-HSCT. This review will focus on the current understanding of TGF β biology and the numerous ways it can impact outcomes post-transplant.

Keywords

acute graft versus host disease (GVHD); chronic GVHD; infection; T regulatory cells

Hematopoietic stem cell transplantation (indications and complications)

Hematopoietic stem cell transplantation (HSCT) is a therapy commonly used for the treatment of various cancers, autoimmune diseases, and inherited disorders. The source of stem cells can either be from the patient (autologous) or from a matched related or non-related donor (allogeneic). In each case, patients are subjected to a conditioning regimen (total body irradiation (TBI), chemotherapy or a combination of both), with the aim of ablating the recipient's hematopoietic cells. Patients are then infused with donor cells which can be derived from a variety of sources including bone marrow, mobilized peripheral blood stem cells, or umbilical cord blood (1).

Various complications may arise post-HSCT, and these account for significant morbidity and mortality among recipients. Pulmonary complications are frequent post-HSCT, occurring in 30 to 60% of patients (2). Viral, bacterial, and fungal pulmonary infections as well as noninfectious and fibrotic complications including idiopathic pneumonia syndrome

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and bronchiolitis obliterans have been reported (2,3). Graft-Versus-Host Disease (GVHD) occurs in the allogeneic transplant setting, where donor T cells mount an immune response against host proteins, leading to severe problems often with fibrotic manifestations involving the skin, gastrointestinal tract, liver, kidneys, and lungs (4). Unfortunately, HSCT patients are at risk for various complications both pre- and post-engraftment highlighting the need to better understand the root causes of susceptibility. Because of its ability to regulate immune responses and its association with fibrotic disease, TGF β isoforms have been studied as potential contributors to many of these post-HSCT complications.

Regulation of TGF β post-HSCT

The production of TGF β is affected by the conditioning regimens used to prepare HSCT recipients for transplant. During the conditioning period, an initial drastic reduction in serum TGF β 1 levels was shown to occur in both autologous and allogeneic HSCT recipients (5), and TGF β 1 levels in the plasma of allogeneic HSCT recipients underwent a 4-fold decrease (6). This pattern was also noted in a mouse model of allogeneic transplant, where total serum TGF β was greatly reduced 7 days post-transplant (7). Serum TGF β levels in both allogeneic and autologous patients were shown to return to normal levels between 20 and 50 days post-transplant and this recovery correlated with the return of white blood cell and platelet counts, major sources of TGF β 1 in the serum (5). Leukocyte sources of TGF β include lymphocytes, antigen presenting cells and NK cells (8).

Structural cells also produce TGF β (9) and contribute to organ-specific increases in this cytokine post-HSCT. TGF β mRNA transcripts were found to be elevated in skin biopsies of patients experiencing cutaneous GVHD (10). Additionally, high dose irradiation can stimulate overproduction of TGF β 1 in the human lung (11). In a murine model of allogeneic HSCT, TGF β transcript levels in the lung were shown by in situ hybridization to be increased relative to that of non-transplanted control mice at day 7 post-transplant; including cyclophosphamide in the pre-HSCT conditioning regimen potentiated this increase (7). TGF β 1 has also been shown to be elevated in lungs post-engraftment using murine models of allogeneic or syngeneic bone marrow transplant (BMT) at 5 weeks post-transplant. In this study, alveolar epithelial cells were shown to be one source of elevated TGF β 1 production (12).

TGF β biology

While TGF β 1 is the prototypical TGF β family member, there are three isoforms of TGF β expressed in mammals (TGF β 1, - β 2, and - β 3) (13). Each of these is secreted in the latent form, non-covalently associated with a homodimer of the latency-associated peptide (LAP) (14), which is capable of inhibiting TGF β activity (15). LAP, in turn, can be linked by disulfide bonds to the latent-TGF β -associated protein which allows the whole latent TGF β complex to be associated with the extracellular matrix (14). TGF β must be released from its latent form, disassociating from LAP, in order to function. There are several ways in which TGF β can become activated including proteases (16), integrins (17), or change in pH (18).

TGF β mediates its functions by binding to the transmembrane TGF β type II receptor (TGF β RII), which then phosphorylates the TGF β type I receptor (TGF β RI). TGF β RI then will phosphorylate and activate SMAD (S-Mothers Against Decapentaplegic Homolog) proteins, which translocate to the nucleus to arbitrate changes in gene expression (19).

Immune effects of TGF β

TGF β has been reported to have both immunosuppressive and immunostimulatory functions, even on the same cell types (20). With respect to T cells, TGF β is a known chemoattractant

(21). TGF β can also act to limit their proliferation (22) and their differentiation into Th1 and Th2 phenotypes, subsets of helper T cells characterized by production of the cytokines interferon- γ and interleukin-4, respectively (23,24). In contrast, TGF β 1 in conjunction with IL-6 can induce the differentiation of naïve CD4 T cells into the interleukin-17a- producing Th17 phenotype (25). Finally, TGF β 1 can induce the expansion of regulatory T cells (Tregs), a subset of immunoregulatory T cells which can be identified by expression of the transcription factor Foxp3 and are critical for maintenance of peripheral tolerance (26). More recently it has been appreciated that the Treg phenotype may not be stable in all circumstances and that Tregs may undergo conversion to Th17 cells with appropriate inflammatory stimuli [reviewed in (27)].

In terms of B cells, TGF β signaling has been shown to inhibit B cell proliferation and inhibit IgG serum immunoglobulin levels (28). In contrast TGF β promotes IgA production which is essential for protection of mucosal sites (28). Indeed, conditional inactivation of the TGF β receptor in B cells leads to a reduction in antigen-specific antibody responses following immunization at mucosal sites (29).

With respect to innate immune cells, TGF β acts as a chemoattractant for neutrophils (30) and monocytes (31). Additionally, TGF β can induce these cells to produce the proinflammatory cytokine IL-1 β (31). In contrast, macrophage activation can be limited by the presence of TGF β 1 and - β 2, and NK cell function is also limited by TGF β (32,33). Table 1 summarizes the known immune effects of TGF β , and it is clear that this cytokine may act to either limit or enhance host defense and alloresponses in the transplant setting. Since acute GVHD (aGVHD) is mediated by T cells, TGF β may provide a protective role in limiting the donor leukocyte alloresponses. This may be via direct inhibition of effector T cell function or via the induction of Tregs (34). Additionally, TGF β 1 may promote production of IgA which could serve to protect mucosal barriers which are weakened due to chemotherapy and irradiation conditioning. The downside of this protection however, is that host defense, in particular host defense against viruses and fungi, may be compromised. As discussed below, persistent TGF β also promotes the pathological outcomes of chronic GVHD (cGVHD), fibrosis and pneumonitis.

TGF β and aGVHD

aGVHD occurs following allogeneic transplantation and involves the activation of donor lymphocytes against host antigens (4). Presentation of major and minor histocompatibility antigens by both donor and host antigen presenting cells can lead to the activation of donor T cells, resulting in damage to the skin, gut, and liver epithelia (35). Much of the experimental evidence describes a beneficial role for the presence of TGF β in the context of aGVHD. In a study of allogeneic HLA-identical HSCT recipients, kinetics of TGF β 1 levels following transplant differed between patients that developed aGVHD and those that did not; serum cytokine levels in aGVHD patients initially increased following engraftment, but fell again around 9 weeks after transplant (36). Accordingly, plasma levels of TGF β 1 were shown to fall at a rate of 43% per week in the two weeks prior to onset of aGVHD in HSCT recipients, while remaining constant in patients that did not develop aGVHD (6). Finally, skin biopsies show reduced levels of TGF β mRNA transcripts in biopsies from patients with aGVHD when compared to biopsies taken from patients with lichenoid or sclerodermoid forms of cGVHD (10).

The way in which TGF β may limit aGVHD has been explored in murine models. In one study, neutralization of TGF β using antibody treatment which neutralizes all three isoforms led to a dramatic increase in the severity of aGVHD, with a corresponding increase in donor cell proliferation and splenocyte alloresponses (37). In another study, inclusion of IL-2

activated NK cells in the donor inoculum led to protection from aGVHD onset; this effect was abrogated by neutralization of TGF β , suggesting that NK cells can act in a TGF β -dependent manner to reduce GVHD (38). aGVHD was also shown to be prevented in mice that were given recipient-type dendritic cells that were pre-treated with TGF β 1 (39). Thus, the presence of TGF β may be beneficial during the development of aGVHD.

TGF β and cGVHD

The development of cGVHD occurs at later time points following HSCT; the pathogenesis and mechanisms of cGVHD are distinct from and are less well-defined than those involved in aGVHD (4,40). While aGVHD involves epithelial damage, the development of cGVHD often presents as organ fibrosis (35). Mechanistically, allogeneic T cells are known to be required for cGVHD, but the target antigens and factors that initiate cGVHD remain elusive (35).

There is some evidence that TGF β propagates cGVHD, opposing its beneficial role in aGVHD. For instance, in the same murine model where neutralization of TGF β led to increased aGVHD, late neutralization of TGF β (days 14–42 post-transplant) led to decreased cGVHD scores and reduced pathophysiology in both the GI tract and the skin, but not the liver (37). Additionally, cGVHD is often associated with the development of cutaneous and organ fibrosis, and blocking SMAD3 phosphorylation has been proposed as one way to alleviate cGVHD symptoms (41). As mentioned earlier, levels of TGF β mRNA were found to be highest in skin biopsies of cGVHD patients when compared to patients with aGVHD (10). Furthermore, in this same study, the levels of TGF β transcripts negatively correlated with the length of survival (10). In contrast to the idea that TGF β promotes cGVHD, murine studies have demonstrated that recipient Tregs that survived irradiation were shown to suppress development of cGVHD in a murine model (42), and while the mechanism of suppression was not shown in that study, production of TGF β is a major mechanism of Treg-mediated T cell suppression (43). Thus, it is possible that TGF β may limit cGVHD if delivered in the context of Tregs. Our ability to translate these murine findings to human disease is somewhat limited due to the lack of an established cGVHD model in mice that closely resembles human cGVHD (35).

In human patients, the evidence implicating TGF β in cGVHD is mainly correlative. One study has shown that the development of cGVHD is associated with increased levels of serum TGF β 1 (5). However, gene array analyses have shown that decreased expression of components of the TGF β signaling pathway in donor CD4 $^{+}$ and CD8 $^{+}$ cells is correlated with increased incidence of cGVHD in recipients (44). Thus, the precise role that TGF β has in propagating cGVHD is not well-defined. Furthermore, it is not yet clear how Tregs correlate with cGVHD. In the study looking at skin fibrosis, there was no correlation between the grade of GVHD and the levels of FoxP3 transcripts (10). However, two previous studies, (one in humans and one using a murine model), have shown a reduction in the severity of GVHD with increased numbers of FoxP3 $^{+}$ T cells in the blood, suggesting that Tregs may inhibit development of GVHD (45,46). This is consistent with a study by Chen et al. who suggested that a loss of Tregs followed by increases in Th1 and Th17 cells ultimately lead to the development of cGVHD (47). Because development of aGVHD is a strong risk factor for development of cGVHD (35), it may be the timing of TGF β expression and Treg accumulation that is important.

TGF β and fibrotic complications

Organ injury and fibrosis are detrimental complications of HSCT. Fibrosis is characterized by dysregulated deposition of extracellular matrix proteins such as collagen, ultimately leading to organ scarring and failure. TGF β 1 can stimulate collagen deposition by

fibroblasts and myofibroblasts leading to fibrosis (9). Furthermore, the ability of TGF β 1 to induce alpha smooth muscle actin expression in lung myofibroblasts also leads to alveolar contraction with further limits gas exchange (48).

Presumably because of chronic injury related to conditioning regimens and GVHD, fibrosis is a serious complication in HSCT patients. Hepatic veno-occlusive disease, for instance, can occur in around 10% of allogeneic HSCT recipients (49). Pulmonary fibrosis can also occur as a complication of HSCT (2). TGF β , as a central molecule in the pathogenesis of fibrotic disease (50), is a potential biomarker for development of these complications. Patients who developed either hepatic veno-occlusive disease or pulmonary fibrosis as a result of autologous transplant for treatment of breast cancer had increased levels of plasma TGF β prior to transplant conditioning when compared to patients who did not develop disease and healthy controls (51). Additionally, in a murine model of sclerodermatous GVHD, the development of skin thickening and lung fibrosis was completely abrogated following transplant in mice that were treated with a neutralizing antibody toward TGF β (52). The role of transplant-induced TGF β in the induction of fibrotic disease following HSCT in humans has not been evaluated; however, irradiation injury has been shown to induce TGF β 1 in both human and animal lungs (11,12,53). Taken together, these data strongly support a role for TGF β in mediating the fibrotic complications that occur late post-HSCT.

TGF β and graft rejection

Although graft rejection is a rare complication among HSCT recipients, it does occur, especially in patients receiving treatment for severe aplastic anemia (54). The risk for graft rejection increases in patients that have received multiple blood transfusions prior to HSCT, and this may be related to priming with minor histocompatibility antigens (55). There is limited data on the role of TGF β in graft rejection. However, one study indicates that TGF β may be protective against graft rejection in that neutralization of TGF β prior to allogeneic bone marrow transplant in mice led to a significant increase in graft rejection (56). Thus, it is likely that the ability of TGF β to limit alloresponses may protect against both aGVHD and immune-mediated graft failure.

TGF β and infectious complications

Because TGF β can act as an immunosuppressive cytokine, the presence of TGF β following HSCT could complicate host defense, particularly for pathogens that depend on T cells for clearance. As discussed previously, this could occur either by direct effects of TGF β on leukocytes or via the induction of Tregs. Data from our laboratory have demonstrated that mice which have received either an allogeneic or a syngeneic bone marrow transplant (BMT) are more susceptible to lytic infection on day 7 following pulmonary challenge with murine gammaherpesvirus, γ HV68, at 5 weeks post-transplant despite full hematopoietic reconstitution and normal immune cell accumulation. The impaired host defense is associated with elevated production of TGF β 1 in the lung and increased numbers of Tregs. Interestingly, when mice are transplanted with bone marrow from a mouse which expresses a dominant negative TGF β receptor in both CD4 and CD8 cells (TGF β RDN BMT), the resulting chimeras have restored antiviral immunity, when compared to mice transplanted with wild type bone marrow despite persistence of elevated numbers of Tregs (12). Further experiments confirmed that increased numbers of Tregs were not limiting host defense in this model. Rather, the TGF β RDN BMT mice generate protective Th1 responses whereas the WT BMT mice generate a non-protective Th17 response (12). Thus, in these studies, TGF β seemed to alter the effector responses of the donor T cells directly in a Treg-independent manner post-BMT.

It is also common for HSCT patients to develop severe pneumonitis following lung infections even when the infectious organism can not be isolated (57). This can also be modeled in mice. Figure 1 demonstrates the lung pathology that is seen in control (untransplanted) and BMT mice on day 21 post- γ HV68 infection, a time point when levels of latent viral genome are not different between control and BMT mice. It is evident that the BMT mice show enhanced pneumonitis which includes areas of inflammation and diffuse alveolar damage. Interestingly, TGF β has been implicated in the development of pneumonitis independent of viral load. Twelve weeks following allogeneic transplant, mice with GVHD developed more severe pneumonitis on day 7 following herpes simplex virus infection than did control mice, or mice with allogeneic transplants without GVHD. Surprisingly, the degree of pneumonitis in this model did not correlate with lytic viral loads measured at day 4 (58). Despite this, neutralization of TGF β significantly reduced the occurrence of pneumonitis in the mice with GVHD (58). Thus, depending on the stage of infection, TGF β may inhibit host defense against viruses and promote the development of pneumonitis.

Therapeutic Implications

The pleiotropic nature of TGF β complicates its use as a therapeutic target post-HSCT (Figure 2). Identifying hematopoietic or structural sources of TGF β and their kinetics post-HSCT may offer intervention insights. Tregs can act via both TGF β -dependent and -independent mechanisms (43,59). Murine models demonstrate inclusion of Tregs in bone marrow inoculums limits GVHD (60,61). Thus, there is interest in using Tregs to control GVHD, even though it is not clear how Tregs correlate with human disease (62). Our data have demonstrated that TGF β can directly inhibit host defense against the persistent pathogen γ HV68 in a Treg-independent manner (12). However, there is data to suggest that Treg therapy may not carry the same risk for host defense. Using a murine model of allogeneic GVHD, it was demonstrated that Treg infusion protected mice from severe GVHD, but did not impair immune responses to acute lethal cytomegalovirus infection (63).

In reality, downstream effectors of TGF β signaling may make better targets for therapeutic intervention. There has been interest in using Halofuginone, an inhibitor of SMAD phosphorylation, or Tranilast, an inhibitor of TGF β -induced matrix production to treat fibrotic symptoms of cGVHD (41,64). Similarly, blockade of connective tissue growth factor (65) or plasminogen activator inhibitor-1(66), both molecules induced by TGF β , can limit fibrotic outcomes; these therapies may translate to treat fibrosis associated with HSCT.

Summary

The complications of HSCT, both infectious and non-infectious, severely limit the usefulness of this procedure to cure or treat many malignancies and inherited disorders. It is likely that TGF β is the single most important cytokine in determining most of the severe complications associated with HSCT (37). TGF β immune effects are both complex and contradictory. Although TGF β may limit aGVHD (6,37), its therapeutic potential is limited by the risk of developing cGVHD and fibrosis (37,50). Early post allogeneic-HSCT, the presence of TGF β may prevent graft rejection and thus promote graft acceptance (56). The role of TGF β in host defense is also complex. While there are beneficial effects in terms of leukocyte recruitment (21,30,31,67) and IgA production (29), there is also evidence that TGF β can directly inhibit antiviral host-defense and can promote pneumonitis (12,58). The ability of TGF β to promote Treg differentiation may also influence outcomes in HSCT. While most evidence suggests that Tregs may limit cGVHD (45–47), there are also studies that show no correlation (10). In these studies with Tregs, it is not yet known whether the beneficial effects are TGF β -dependent or independent. Taken together, is likely that the

timing of TGF β expression is important in determining post-transplant complications (37). Given the complexity of TGF β responses, it is likely that therapies directed at particular downstream effectors may offer a better therapeutic approach for patients experiencing complications from HSCT.

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Figure 1. Pneumonitis is increased following γ HV-68 infection in BMT mice

Untransplanted mice (panels A, C) or mice at 5 weeks post-syngeneic BMT (panels B, D) were infected with γ HV-68 and lungs were analyzed for histology 21 days post-infection. At this time point, latent viral loads are similar in control and BMT mice, yet pneumonitis is severe in the BMT mice. As seen in the 100 $\times$ magnifications (panels A, B), inflammation in the control mice is centered around airways (bronchitis) whereas in BMT mice, the inflammation is more extensive and peripheral (pneumonitis). On higher power (400 $\times$, panels C, D) evidence of diffuse alveolar damage and destruction of alveolar architecture is noted in the BMT mice.

Figure 2. Pleiotropic Effects of TGFβ in HSCT

Schematic representation showing both the beneficial and detrimental effects that TGFβ can have in the setting of HSCT.

Table 1Effects of TGF β on Immune Cells

Stimulatory	Inhibitory
With IL-6 induces Th17 (25)	Decreases T cell proliferation (22)
Increases Tregs (26)	Decreases Th1 and Th2 (23,24)
Increases IgA production (29)	Decreases B cell proliferation and IgG production (28)
Increases monocyte, neutrophil, lymphocyte and mast cell chemotaxis (21,30,31,67)	Decreases macrophage activation and NK function (32,33)