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Impaired pulmonary immunity post-bone marrow transplant

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Abstract

Infectious complications are a serious cause of morbidity and mortality following hematopoietic stem cell transplantation (HSCT), and the lung is a particular target organ post-transplant. Our laboratory has used a murine bone marrow transplant model to study alterations in immunity that occur as a result of transplantation. Our studies focus on immune responses that occur following immune cell reconstitution in the absence of immunosuppressive drug therapy or graft-versus-host disease. We have found that impaired clearance of both bacterial and viral pulmonary infections is related to specific alterations in immune cell function and cytokine production. Our data offer insight into mechanisms that contribute to opportunistic infections in HSCT recipients.

Keywords

Hematopoietic stem cell transplant; Pneumonia; *Pseudomonas aeruginosa*; Murine gammaherpesvirus-68; Opportunistic infections; Innate immunity; Adaptive immunity

Overview of HSCT

Since the first transplants in human patients were performed in the late 1950s [1, 2], hematopoietic stem cell transplantation (HSCT) has become an important therapy in the treatment of several malignant, inherited, and autoimmune disorders [3]. During HSCT, patients are treated with a conditioning regimen and are then infused intravenously with their own hematopoietic stem cells (autologous) or HSCs from a donor (allogeneic). Autologous transplants are performed more frequently than allogeneic [4]. Various preparative regimens for HSCT have been used, but conditioning generally includes chemotherapy, such as cyclophosphamide and/or total body irradiation [5]. These regimens have several consequences, including eradication of malignant or self-reactive cells and providing an immunosuppressed environment for the transplantation of allogeneic cells [5, 6]. Stem cells for transplantation are derived from a variety of sources. Cells may be

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collected by aspiration of bone marrow, harvested from umbilical cord blood [7], or donors may be treated with growth factors such as granulocyte colony-stimulating factor (G-CSF) to mobilize stem cells to peripheral blood [8]. Additionally, cell infusions may or may not include specific subsets of cells. For instance, the inclusion of donor T cells in allogeneic grafts can be useful for promoting an immune response of grafted cells against malignant cells [9].

Post-transplant complications

Despite promising success in the treatment of many diseases, the efficacy of HSCT is limited due to significant transplant-related morbidity and mortality. There are a wide variety of complications that can occur following HSCT, both non-infectious and infectious. Specifically, pulmonary complications comprise a large group of post-transplant problems and are reported to occur in up to 60% of HSCT recipients [10]; one study of deceased HSCT recipients found pulmonary complications in 89% of the cohort [11].

Noninfectious complications may include graft-versus-host disease (GVHD) in the allogeneic setting [12], second malignancy [13], and late non-infectious lung complications, such as bronchiolitis obliterans and idiopathic pneumonia syndrome [14].

Infectious complications can occur in both allogeneic [15] and autologous recipients [16, 17], though they are more common in allogeneic transplants [10], presumably due to GVHD and immunosuppressive drug therapy. Infections tend to occur in the lung, skin, and genitourinary tract and are associated with the presence of indwelling catheters [18]. Historically, cytomegalovirus (CMV) was the most significant viral complication of HSCT; mortality rates of CMV pneumonia in HSCT recipients were near 85% prior to the development of anti-viral drug therapy [19]. Despite advances in prophylaxis and antiviral therapy, CMV disease remains a significant complication of HSCT [20]. HSCT recipients are additionally at-risk for infections by other herpesviruses, such as human herpes-virus-6, herpes simplex virus, and varicella zoster [10, 21, 22], as well as community-acquired respiratory viruses, such as influenza [21]. *Pseuodomonas aeruginosa* and *Klebsiella pneumonia* represent important gram-negative bacterial infections in HSCT recipients [23], and common gram-positive bacterial infections in HSCT populations are Staphylococcus epidermidis, Staphylococcus aureus, and viridians streptococci [18]. Fungal infections caused by Candida and Aspergillus species are also significant threats post-HSCT [24].

Infectious complications can be characterized by the time point during which they occur post-HSCT, including pre-engraftment (during neutropenia, 1 month post-HSCT), early post-engraftment, and late post-engraftment (approximately 3 months post-transplant) [18, 23, 25]. Table 1 summarizes infections reported to occur during these post-transplant time periods. Opportunistic infections, though rare, have been reported to occur late post-transplant in autologous patients [26, 27]. This fact suggests that transplantation, even in the absence of immunosuppressive therapy and GVHD, can lead to long-term immune dysfunction.

Immune defects post-HSCT

Beginning with the emergence of the innate immune system, reconstitution of donor-derived immune cells spans over the course of several months to a year following HSCT [28–30]. However, reconstitution of various immune cell compartments does not typically coincide with restoration of immune function. Within the first few months of transplant, marked reduction in neutrophil chemotaxis, phagocytosis, and bacterial killing is observed, contributing to patient susceptibility to a number of infections [31]. Similar defects are seen in tissue macrophage function [32]. Impaired mitogen proliferation and cytokine production

also remain a common feature among both B- and T-cell subsets [28, 29, 33]. Although these defects have been broadly reported, mechanisms behind reduced cellular-mediated immunity following immune reconstitution are poorly understood. Our work has employed a murine model of HSCT to determine potential causes for impaired immune function following donor-cell reconstitution, with particular focus on the impact of reduced immunity on host defense in the lung.

Animal modeling

Our laboratory has developed two murine models of bone marrow transplantation (BMT) to examine the effect of HSCT on pulmonary immune function and host defense. This includes syngeneic and allogeneic BMT, where bone marrow is harvested from C57BL/6 or Balb/c mice, respectively, and infused by tail vein injection into lethally irradiated C57BL/6 recipients. Ablation of host-derived HSC in our mice has involved either TBI or cyclophosphamide/busulfan chemotherapy preparative regimens. However, we chose TBI as the primary means to ablate host-derived HSC, given that TBI eradicates host-derived HSC more efficiently than chemotherapy regimens and maximizes reconstitution of donor-derived cells [34]. Using this method, recipient mice are given a fractionated dose of 13 Gy TBI from either a ^{137}Cs or x-ray orthovoltage source. Complete immune reconstitution is achieved five weeks following infusion of 5 9 10^6 whole bone marrow cells into TBI recipients [34, 35]. The percentage of donor-derived cells is approximately 94.9 ± 1.1% in the spleen at this time point, as assessed by transplanting CD45.1$^+$ bone marrow into C57Bl/6 CD45.2$^+$ mice [34]. However, addition of 1×10^6 purified splenic T cells at the time of whole bone marrow infusion increases the rate of donor-cell reconstitution from 5–3 weeks [34, 36, 37].

Given that HSCT patients are increasingly susceptible to bacterial and viral infections of the lung throughout preand post-engraftment phases (Table 1), our research has focused primarily on pulmonary host defense against model opportunistic pathogens post-HSCT. To date, we have used the gram-negative bacteria, *Pseudomonas aeruginosa*, and the murine herpesvirus, gammaherpesvirus (*γ*HV)-68, as our model opportunistic pathogens. Five weeks following syngeneic BMT in mice, we find that lung phagocytes display reduced host defense against *P. aeruginosa* both in vivo and in vitro compared to non-transplant controls. Furthermore, T-cell function in the lung is defective in BMT mice, contributing to reduced clearance of murine *γ*HV-68 following intranasal challenge.

This impairment in pulmonary immunity is directly related to the highly immunosuppressive environment of the lung post-BMT. Following BMT, increased production of immunosuppressive mediators, such as prostaglandin E_2 (PGE_2) and TGF-*β*, is detected [35, 36]. In subsequent sections, we describe our models of bacterial and viral pneumonia post-BMT as well as the mechanisms that we have identified which contribute to impaired immunity in the lung following BMT.

Model of bacterial pneumonia post-BMT

P. aeruginosa infection is prevalent within the first 100 days following transplant [23, 25, 38]. *P. aeruginosa* is an ubiquitous pathogen, and ordinarily, exposure to *P. aeruginosa* through the lung airway is cleared by resident phagocytes [39]. In immunocompromised patients, however, *P. aeruginosa* is especially virulent and poses an increased risk in these individuals for pneumonia, bacteremia, and sepsis [40, 41]. To assess the ability of the immune reconstituted host to clear an opportunistic bacterial infection, our laboratory set up a model of acute *P. aeruginosa* lung infection in syngeneic BMT mice. Using syngeneic BMT, we have been able to explore how the transplant procedure alone (without GVHD or immunosuppressive drug therapy) impacts ongoing pulmonary immunity.

Impaired pulmonary host defense against *P. aeruginosa* post-BMT

Defective innate immunity

Following intratracheal challenge with a sublethal dose of *P. aeruginosa*, BMT mice display increased bacterial burden in the lung and dissemination of *P. aeruginosa* to the blood at 24 h relative to non-transplant controls [37]. This impairment in bacterial clearance coincides with reduced function of lung phagocytes, where BMT alveolar macrophages (AMs) show significant reduction in phagocytosis of both serum- and non-serum-opsonized bacteria [36, 37]. Furthermore, increased survival of ingested *P. aeruginosa* is observed in BMT AMs, suggesting defects in bacterial killing mechanisms in these phagocytes [36]. Neutrophils recruited to the lung following intratracheal injection of *Pseudomonas*-derived LPS also display similar defects in bacterial killing [36]. These results are consistent with experiments showing impaired bacterial killing mechanisms in AMs and neutrophils from HSCT patients [31, 32].

Cytokine and eicosanoid dysregulation

In addition to defective phagocytosis and microbial killing, BMT AMs have dysregulated production of proinflammatory cytokines and eicosanoids [42]. Post-BMT AMs display diminished TNF-α production relative to non-transplant controls. Furthermore, whole lung homogenates have reduced TNF-α levels, 24 h following *P. aeruginosa* lung infection. This defect in TNF-α production may explain the reduction we observe in pulmonary host-defense post-BMT, given that TNF-α can activate bacterial killing mechanisms in both macrophages and neutrophils [43–46]. Decreased production of cysteinyl leukotrienes (cys-LTs) is also observed in BMT AMs. Cys-LTs are a group of eicosanoids that can enhance proinflammatory cytokine production, phagocytosis, and microbial killing [47, 48]. Interestingly, exogenous treatment with the cys-LT, leukotriene D_4, restores phagocytosis of serum- and non-serum-opsonized bacteria in BMT AMs.

Diminished production of proinflammatory mediators in the lung post-BMT may be related to elevated production of the immunosuppressive eicosanoid, prostaglandin E_2 (PGE_2), by alveolar epithelial cells, AMs, and recruited lung neutrophils [36]. PGE_2 is known to inhibit phagocytosis, bacterial killing, chemotaxis, and proinflammatory cytokine production in phagocytes primarily via signaling through the E prostanoid 2 (EP2) receptor [49–51]. It is interesting to note that AMs from BMT mice express increased levels of the EP2 receptor making them more susceptible to the inhibitory actions of PGE_2 [36]. In our model, we have also shown that pharmacologic inhibition of PGE_2 production in vivo can restore host defense against *P. aeruginosa* pneumonia in BMT mice [36]. Furthermore, inhibition of PGE_2 production can restore BMT AM and neutrophil function in vitro [36].

Granulocyte macrophage colony-stimulating factor (GM-CSF) production is also dysregulated in the lung post-BMT. GM-CSF can induce terminal differentiation of myeloid cells, including AMs, and is a critical factor in pulmonary host defense against *P. aeruginosa* [52–54]. In our model, BMT AMs produce higher levels of GM-CSF under LPS-stimulated and unstimulated conditions compared to control AMs [55]. However, overall production of GM-CSF is decreased in BMT lung homogenates [55], suggesting that lung parenchymal GM-CSF production is reduced post-transplant. To determine a role for GM-CSF in modulating host defense against *P. aeruginosa* post-BMT, WT mice were transplanted with GM-CSF-deficient bone marrow. Bacterial clearance and phagocyte host defense function were improved in GM-CSF -/- BMT mice compared to WT BMTs [55]. In these transplants, the predominant source of GM-CSF in the lung was from structural cells, and this scenario showed improved host-defense post-BMT. Interestingly, creating the opposite chimera by transplanting WT bone marrow into GM-CSF -/- mice resulted in

severe susceptibility to *P. aeruginosa* infection [55]. In these transplants, the lack of GM-CSF production by structural cells was clearly harmful even if the hematopoietic cells were producers. These results highlight the complexity of cytokine compartmentalization in the setting of BMT, as well as the importance of cross talk between lung parenchymal cells and AMs in the regulation of bacterial clearance following BMT.

Restored function in GM-CSF -/- BMT AMs was surprisingly associated with increased PGE_2 production; however, activation of the PGE_2 cyclic AMP (cAMP) signaling cascade was diminished [55]. This is likely due to the surprising reduction in expression of the EP2 receptor on BMT AMs in the absence bone marrow-derived GM-CSF [55]. How GM-CSF may regulate EP2 receptor expression and activation of the cAMP signaling cascade has not yet been determined.

Taken together, our research suggests a critical role for PGE_2 and GM-CSF in the ongoing impairment of pulmonary host-defense post-BMT. Much of our current work has been focused on uncovering mechanisms for PGE_2-mediated inhibition of alveolar macrophage function and identifying novel effectors in the PGE_2 signaling pathway that may be targeted for potential therapeutic benefit.

Mechanisms for impaired pulmonary innate immunity in BMT mice

Post-BMT, elevated PGE_2 levels in the lung inhibit a number of host defense mechanisms in phagocytes that help mediate proper uptake and clearance of bacterial pathogens. However, the cause of increased PGE_2 production in the lung post-BMT remains unknown. In AMs, we have observed upregulation in expression of prostaglandin synthetic enzymes post-BMT, including cyclooxygenase (COX)-2 and PGE synthases 1 and 2 [56]. This may also explain the concomitant increases we observe in BMT AM production of prostacyclin, PGI_2 [34]. Although it is not known why prostaglandin synthesis is upregulated post-BMT, our laboratory has identified two effectors in the PGE_2 signaling pathway that may mediate inhibition of BMT AM function. These effectors are interleukin receptor-associated kinase (IRAK)- M and phosphatase and tensin homolog deleted on chromosome ten (PTEN).

Downstream targets of the PGE_2 signaling cascade: PTEN and IRAK-M

Via the second messenger cAMP, the PGE_2 signaling pathway is known to activate various downstream effectors for inhibition of phagocytosis, microbial killing, and TNF-α production in leukocytes [57]. PGE_2 can signal through four G-protein-coupled receptors, EP receptors 1–4 [58]. PGE_2 engagement of EP2 and EP4 is known to induce cAMP production by stimulating G-protein-adenylyl cyclase activity [59]. cAMP can then act on two effectors, exchange protein activated by cAMP (Epac)-1 and protein kinase A (PKA) to inhibit phagocytosis, microbial killing, and proinflammatory cytokine production [57]. cAMP binding to Epac-1 has been shown to increase activation of the lipid phosphatase PTEN in a Src homology phosphatase-1-dependent manner [60]. This elevation of PTEN lipid phosphatase activity inhibits phagocytosis in AMs by negatively regulating pathways that mediate phagocytic uptake of IgG-opsonized particles [60–63]. Similarly, our current research has shown that PTEN activity is increased in BMT AMs, as a result of AM overproduction of PGE_2, and pharmacologic inhibition of PTEN improves innate immunity in the lung post-BMT (unpublished observation).

IRAK-M was also identified as an effector in the PGE_2 signaling pathway that may mediate inhibition of host defense mechanisms in BMT AMs [56]. IRAK-M is a member of the IRAK family of serine/threonine kinases [64]. IRAK-1 and IRAK-4 interact with the MyD88 adaptor complex to mediate IL-1R/TLR signaling [64–66]. IRAK-M negatively regulates IL-1R/TLR signaling by binding to the adaptor complex and inhibiting activation

of downstream signaling pathways that induce NF-κB-mediated proinflammatory cytokine production [67].

Given that BMT AMs have decreased production of the proinflammatory cytokine TNF-α, our laboratory sought to determine whether PGE_2 was inducing expression of IRAK-M in BMT AMs. As predicted, BMT AMs have an approximately 3.5-fold increase in IRAK-M protein expression, which is related to increased PGE_2 signaling [56]. Transplanting WT mice with IRAK-M-deficient bone marrow restores pulmonary host defense against *P. aeruginosa* post-BMT and improves AM bacterial killing, phagocytosis, and production of cysLTs and TNF-α [56]. No differences are observed in recruitment of neutrophils or other leukocyte effector populations to the lungs of WT and IRAK-M -/- BMT mice following *P. aeruginosa* infection [56]. This restoration in host defense occurs despite overproduction of PGE_2 by BMT AMs [56], suggesting that the absence of IRAK-M mitigates the inhibitory effect of PGE_2 signaling in AMs.

Currently, we are investigating whether PTEN, in addition to other known effectors of the PGE_2 signaling pathway, is required for PGE_2-mediated elevation of IRAK-M. It is plausible, however, that PTEN and IRAK-M may be downstream targets in the PGE_2 signaling pathway that act independently of one another to regulate similar AM host defense mechanisms.

Model of viral pneumonia post-HSCT

Herpesvirus infections are particularly common opportunistic infections at all time points post-HSCT (see Table 1) [10, 21, 68]. Furthermore, the emergence of viral strains resistant to antiviral therapy [69, 70] warrants a better understanding of post-HSCT immune responses to viral infections. To understand how transplantation of HSCs leads to alterations in anti-viral immune responses post-transplant, despite complete immune cell reconstitution, we use the well-characterized murine herpesvirus, γHV-68, as a model pathogen. γHV-68, when administered intranasally, will establish a lytic infection principally in lung epithelial cells which lasts 7–10 days and will subsequently persist latently in epithelial cells, B cells, and macrophages [71, 72]. The immune response to this virus is complex (reviewed in [73]), but it is established that IFNγ production by CD4 + T cells is critical for control of lytic viral infection [74]. Studies of anti-viral immunity in our model have been performed in syngeneic and/or allogeneic (non-severe GVHD) transplant settings, and all experiments have been performed 5–6 weeks post-BMT, at a time when reconstitution of immune cell numbers has occurred [35].

Reduced viral clearance and enhanced pneumonitis in BMT mice

When infected intranasally with γHV-68, BMT mice are found to have increased lytic virus in the lung at day 7 post-infection [35]. This is shown by plaque assay, real time RT–PCR detection of two viral genes (DNA polymerase and gB, a viral capsid glycoprotein), and viral immunohistochemistry. At 21 days post-infection, however, there is no difference in viral load between BMT and control mice, yet there are considerable histological changes in BMT lungs at this time point, including significant alveolar airspace inflammation [75]. This latter observation supports previous data showing that herpes simplex type 1-induced pneumonitis in a murine GVHD model is independent of viral load [76]. Together, these data suggest that the anti-viral immune response in BMT mice is altered compared to non-transplanted control mice.

Mechanisms for impaired anti-viral immunity

Unlike our bacterial pneumonia model, upregulated PGE_2 does not appear to be responsible for increased viral loads in BMT mice. Pharmacologic inhibition of PGE_2 concurrent with viral infection does not abrogate increased viral loads, suggesting that PGE_2 is not directly impairing the anti-viral immune response. Rather, impaired anti-viral immunity seems to relate to the overproduction of TGFβ in the lung, particularly from alveolar epithelial cells. The overproduction of TGFβ occurs concurrently with an expanded population of Foxp3-expressing regulatory T cells (Tregs) in lungs post-BMT. Although we initially hypothesized that Tregs might be suppressing the effector T-cell responses in this model, BMT mice treated with anti-CD25 prior to infection still had increased viral loads [35]. These data are in accordance with another study showing that adoptive transfer of Tregs in a BMT model can reduce GVHD but not alter anti-viral immunity [77]. Rather, our data suggest that TGFβ signaling may directly alter the effector T cells. Transplanting mice with bone marrow from a donor mouse that expresses a dominant negative TGFβ receptor II in T cells (T-cell DN-TGFβRII) creates a BMT mouse in which donor-derived CD4 and CD8 T cells are unresponsive to TGFβ signaling and leads to a restoration in anti-viral immunity [35].

Altered T-cell skewing in BMT mice

In order to assess the effects of TGFβ on T cells in our BMT mice, we analyzed cytokine production by T cells at day 7 post-infection. TGFβ is known to limit Th1 [78] and promote Th17 differentiation [79]. Indeed, at day 7 post-infection, BMT mice have decreased IFNγ-expressing CD4 cells and increased IL-17a-expressing CD4 cells in the lung, compared to non-transplant controls [35]. This alteration is correlated with a decrease in RNA expression of the Th1-promoting cytokine IL-12p35 in lung-derived CD11c + cells and IL-12p70 protein from bone marrow-derived dendritic cells (BMDCs) at baseline, prior to infection (unpublished observations). Accordingly, splenic T cells from BMT mice express decreased levels of Eomesodermin and Tbet, transcription factors associated with IFNγ production. Expression of these transcription factors in BMT splenic T cells decreases with increased irradiation dose, highlighting again the importance of TBI-conditioned structural cells in regulating the phenotype of donor-derived hematopoietic cells (unpublished observations). BMT mice transplanted with T-cell DN-TGFbRII bone marrow have restored T-cell IFNγ production [35]. Taken together, these data support a model wherein BMT mice are skewed away from a Th1 environment in both the lung and periphery at baseline, perhaps through actions of TGFβ and altered antigen presenting cell function, leading to a diminished anti-viral immune response.

Conclusion

Data from our laboratory suggest that both bacterial and viral pulmonary infections that occur following HSCT may be related to specific alterations in immune parameters that occur as a result of transplantation. Specific cellular alterations that we have found in our BMT models are summarized in Table 2. In our models, the lung environment is immunosuppressive post-BMT, even following immune cell reconstitution. Differential mechanisms appear to be responsible for impaired immunity to bacterial and viral infections, but both are due to the procedure of transplantation. Our work provides insight into understanding how the transplantation procedure, outside of immunosuppressive drug therapy and severe GVHD, leads to immunodeficiency post-transplant. Our next steps are to determine whether alterations that characterize these murine models are also present in humans post-HSCT. It is our hope that targeted therapies such as cyclooxygenase inhibitors or treatments that alter TGFβ signaling might offer novel treatments to prevent the devastating consequences of infection in HSCT patients.

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Table 1

Timeline of infectious complications post-HSCT (Adapted from [18, 23, 25])

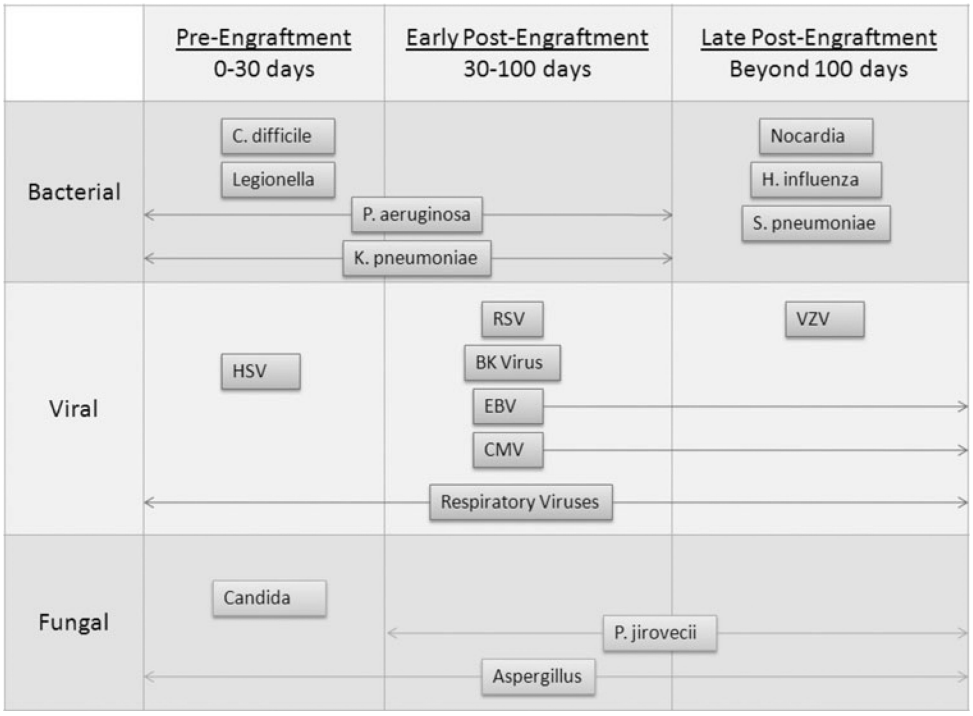

Table 2

Cellular alterations post-BMT

Cell Type	Baseline alterations post-HSCT	Further alterations observed post-infection
Alveolar macrophages	↑PGE_2, IRAK-M, GM-CSF, PTEN activity ↓TNFα, CysLTs	In response to P. aeruginosa: ↓ Phagocytosis& killing
Lung neutrophils	↑PGE_2	↓Bacterial killing
CD4T cells	↓Responsein MLR ↑Lung Tregs	In response to γHV-68: ↓Th1 ↑Th17
Alveolarepithelial cell:	↑TGFβ ↑PGE_2, GM-CSF	In response to P. aeruginosa: ↓ GM-CSF, CCL2
Bone marrow-derived dendritic cells	↑ IL-10 ↓ IL-12p70 ↑Responsein MLR	

This table summarizes alterations in specific cell types that we have observed in our BMT model both pre- and post-infection