12 Pathophysiology of Lung Injury After Hematopoietic Stem Cell Transplantation

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CONTENTS

1. INTRODUCTION

Over the last several decades, hematopoietic stem cell transplantation (SCT) has emerged as an important therapeutic option for a number of malignant and nonmalignant conditions. Unfortunately, the utility of this treatment strategy is limited by several side effects, the most serious of which include the development of graft-vs-host disease (GVHD) and pulmonary toxicity. Pulmonary dysfunction, specifically diffuse lung injury, is a major complication of SCT; it occurs in 25–55% of SCT recipients and can account for approximately 50% of transplant-related mortality *(1–6)*. Diffuse lung injury is described as either acute or chronic with respect to both the time of onset after SCT and the tempo of disease progression once the diagnosis has been established. Approximately 50% of the time, an infectious etiology is uncovered, whereas in the remaining 50% of cases, no microbial organisms are identified in the lungs of affected patients *(7)*. In recent years, the judicious use of broad-spectrum antimicrobial prophylaxis has tipped the balance of pulmonary complications after SCT from infectious to noninfectious. In this context, two types of pulmonary dysfunction have been recognized: acute noninfectious lung injury (termed idiopathic pneumonia syndrome [IPS]) and subacute or chronic noninfectious lung injury. Two forms of subacute/chronic lung injury are common in patients over 100 d posttransplant: airflow obstruction and restrictive lung injury *(8–16)*. Each form of noninfectious lung injury is associated with significant morbidity and mortality and, unfortunately, clinical responses to standard therapeutic approaches are limited. This chapter will be devoted to noninfectious lung injury occurring both early and late

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after allogeneic SCT (alloSCT), with the goal of providing a better understanding of the definition, risk factors, and pathogenesis of these important transplant-related complications.

2. ACUTE LUNG INJURY: IDIOPATHIC PNEUMONIA SYNDROME

2.1. Overview

Idiopathic pneumonia syndrome refers to diffuse, noninfectious lung injury that occurs early in the time-course of SCT. In 1993, a panel convened by the National Institutes of Health (NIH) proposed a broad working definition of IPS to include widespread alveolar injury in the absence of active lower-respiratory-tract infection following SCT *(7)*. The NIH panel was careful to stress that they considered this definition to be that of a clinical *syndrome*, with variable histopathologic correlates and several potential etiologies *(7)*. Diagnostic criteria of IPS include signs and symptoms of pneumonia, evidence for nonlobar radiographic infiltrates, abnormal pulmonary function, and the absence of infectious organisms in the lower respiratory tract as determined by broncho-alveolar lavage (BAL) or lung biopsy *(2,7)*. A variety of histopathologic findings have been associated with IPS, including hyaline membranes, bronchiolitis obliterans organizing pneumonia (BOOP), and lymphocytic bronchitis; however, the most frequently reported pattern is interstitial pneumonitis, a term historically used interchangeably with IPS *(17)*. The median time of onset for IPS was initially described to be 6–7 wk after SCT, with a range from 14 to 90 d after the infusion of donor stem cells *(7)*. Perhaps the most striking feature of IPS is its impact on overall survival; mortality rates of 50–80% have been reported, with survival being less than 5% for patients requiring mechanical ventilation *(2,3,5–7,18,19)*. Although a more recent retrospective study from the Seattle group showed a lower incidence and earlier onset of IPS than previously reported, the typical clinical course involving the rapid onset of respiratory failure leading to death remained unchanged *(6)*. A retrospective review performed at the University of Michigan Medical Center demonstrated that the frequency of IPS after alloSCT ranged from 5% to 25% depending on donor source and the degree of antigenic mismatch. Consistent with the Seattle report, the median time for development of IPS was 18 days after transplant in unrelated donor (URD) recipients, and 13 d in the allogeneic peripheral blood stem cell (PBSC) group. Strikingly, the overall d 100 mortality in patients with IPS was 90% and the median time to death from onset of IPS was 13 days, despite high-dose steroids and broad-spectrum antimicrobial therapy *(20)*. As noted, these findings are consistent with published reports and underscore the critical nature of this transplant-related problem.

Potential risk factors for IPS are several and include SCT conditioning with total-body irradiation (TBI), acute graph-vs-host disease (GVHD), older recipient age, SCT for malignancies other than leukemia, and methotrexate (MTX) for GVHD prophylaxis *(5,21–23)*. Furthermore, the likelihood of developing IPS increases with the number of identified risk factors *(3)*. Whereas the effects of MTX and recipient age on IPS have been disputed, the correlation of TBI use or the development of acute GVHD with IPS has been observed in several reports *(2,5,6,23–25)*. The definition of IPS encompasses numerous descriptive forms of pulmonary toxicity as well, including diffuse alveolar hemorrhage (DAH), peri-engraftment respiratory distress syndrome (PERDS), and delayed pulmonary toxicity syndrome (DPTS) *(19)*. DAH generally develops in the immediate post-SCT period and is characterized by progressive shortness of breath, cough, and hypoxemia with or without fever *(19,26–28)*. Although hemoptysis is rare, BAL showing progressively bloodier aliquots of lavage return has traditionally

diagnosed DAH *(26)*. Mortality has been reported in up to three-quarters of affected patients despite high-dose (250 mg/kg to 2 g/kg) steroids, with death occurring within 3 wk of diagnosis *(27)*. Peri-engraftment syndrome and DPTS typically occur after autologous SCT (autoSCT) *(19)*. Each is characterized by fever, dyspnea, and hypoxemia and tends to have a more favorable response to corticosteroids and overall prognosis *(29–31)*. By definition, PERDS occurs within 5 d of engraftment, whereas the onset of DPTS may be delayed for months and commonly occurs following high-dose chemotherapy (HDC) containing cyclophosphamide, cisplatin, and bischloroethylinitrosurea (BCNU) and stem cell rescue for breast cancer *(31)*.

2.2. Pathogenesis of Idiopathic Pneumonia Syndrome: The Lung As a Potential Target of the GVH Response

Potential etiologies for IPS are several and include direct toxic effects of SCT conditioning regimens, occult pulmonary infections, and inflammatory cytokines that have been implicated in other forms of pulmonary injury *(32–36)*. In addition, immunologic factors may be important. Support for the latter can be found in several large series in which IPS was associated with allogeneic (vs autologous or syngeneic) SCT and severe GVHD (vs mild or absent) *(2,3,5,6,18,19)*. In many instances, acute GVHD often precedes IPS, suggesting a possible causal relationship between the two entities *(5,21,37,38)*. Although the lung is not recognized as a classic target organ of GVHD, the clinical association between lung injury and GVHD and the demonstration of pathologic lung changes in rodents with acute GVHD make this possibility intriguing *(2,3,5,6,39–43)*. The pathophysiology of GVHD is complex and is now known to involve donor T-cell responses to host antigens, inflammatory cytokine effectors such as tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1), and endotoxin *(16,44–48)*. Endotoxin or lipopolysaccharide (LPS) is a component of endogenous bowel flora and is a potent enhancer of inflammatory cytokine release. Translocation of LPS across a gut mucosa damaged early in the posttransplant period by the effects of conditioning regimens and GVHD has been demonstrated after both experimental and clinical SCT *(49–52)*. When LPS reaches the systemic circulation, it induces the release of inflammatory cytokines, which, together with cellular effectors, contribute to GVHD target organ damage and dysfunction *(44,53,54)*.

The role of GVHD and specifically alloreactive donor lymphocytes in the pathogenesis of IPS remains a topic of considerable debate. Although acute pulmonary dysfunction has been associated with the development of systemic GVHD, IPS has also been reported after allogeneic T-cell-depleted SCT and when signs and symptoms of GVHD are limited or absent *(55–58)*, making a causal relationship between the two entities difficult to establish. The principal objection to the identification of the lung as a target of the GVH reaction is that epithelial apoptosis, a finding classically attributed to selective T-cell-mediated injury and considered pathognomonic for acute GVHD in other target tissue, has not been consistently identified in the lungs of patients with IPS *(38,59–61)*. In 1978, Beschorner and colleagues reported an association between the severity of clinical GVHD and a histologic pattern consistent with lymphocytic bronchitis found on postmortem exams. This finding was not seen in patients who received auto SCT or in untransplanted controls *(38)*. Although initially considered a potential histopathologic correlate for GVHD of the lung, the association between lymphocytic bronchitis and the development of systemic GVHD was not consistently identified in subsequent reports *(59–61)*.

The heterogeneity of pulmonary histopathology after clinical SCT is complicated further by the nonspecific changes that occur after mechanical ventilation and by the risks associated with

lung biopsy procedures that can significantly limit the quality and quantity of pathology specimens obtained. Despite the lack of classic GVHD histopathology, it is not unreasonable to suggest that pulmonary epithelial and endothelial cells can be potential targets for activated donor T cells after allo SCT. First, the lung is a rich source of major and minor histocompatibility (HC) antigens and professional antigen-presenting cells *(62,63)* and is the site of complex immunologic networks, the proper balance of which allows for infectious surveillance and maintenance of structural integrity, whereas dysregulation of such networks can result in tissue injury and scarring *(64)*. Furthermore, the inflammatory mediators TNF-α and LPS, which are believed to play a part in GVHD *(52,54,65)*, have also been implicated as contributors to pulmonary dysfunction in several experimental systems and clinical syndromes, including adult respiratory distress syndrome (ARDS), lung allograft rejection, and pneumonitis after toxin exposure *(32–36,39,66–69)*. The role of T lymphocytes in immune-mediated pulmonary inflammation has recently been confirmed by several groups and is thought to involve dendritic cells, macrophages, and the secretion of cytokines *(70,71)*. Enhanced lymphocyte activation has been reported in the lungs of patients after BMT and during lung allograft rejection as well *(55,56,72)*.

Second, as discussed in detail later in this chapter, the association of chronic GVHD with obstructive lung disease after alloSCT is well accepted *(8,9,73–76)*. Although a causal link between these two entities has yet to be definitively established, the striking similarities between the consistent histopathologic features of bronchiolitis obliterans seen after SCT and that observed during lung transplant rejection, along with reports of improvement in lung function with immunosuppressive agents, strongly suggest an immunologic component to this pulmonary process *(9,74–76)*. Third, epithelial cell apoptosis is not a requirement of GVHD pathology; the thymus is a known target of GVHD and displays extensive cytolytic damage early in the course of this process, but epithelial cell apoptosis is not a prominent histologic feature *(77)*. Finally, recent studies have demonstrated that GVHD target organs vary with respect to their susceptibility to injury by inflammatory effectors such as cytotoxic T lymphocytes (CTLs), TNF-α, and FasL *(48,78)*. If the mechanisms of GVHD related tissue injury can differ between individual target organs, it is possible that the histopathologic manifestation of this injury may also vary.

2.3. Murine Models of IPS After Allogeneic BMT

2.3.1. Overview

Using well-established rodent SCT models, several investigators have recently explored the relationship between alloreactivity and IPS and have consistently shown that animals with systemic GVHD develop lung injury *(39,42,79,80)*. Importantly, these studies have uncovered potential roles for both inflammatory mediators and cellular effectors in the evolution of IPS and support the hypothesis that the lung may, indeed, be vulnerable to a "two-pronged" immunologic attack after allo SCT. Advantages of these systems include the unlimited availability of tissue for pathologic analysis, tight control over SCT parameters (including HC differences between donor and host, SCT conditioning regimens, and T-cell dose) and the ability to analyze the development of tissue injury without the confounding influences of immunosuppressive chemoprophylaxis, underlying disease, or prior treatment. Surprisingly, even under controlled experimental conditions, several patterns of lung injury have been identified. For example, using a B10 → (CBA × B10)F1 murine SCT model, Piguet and co-workers observed both an acute hemorrhagic alveolitis and a late-onset interstitial pneumonitis (IP) after infusion of B10 parental lymphocytes, whereas induction of GVHD with T cells from CBA donors led to IP

only *(39)*. In addition, the development of interstitial pneumonitis along with a lymphocytic bronchiolitis/bronchitis comparable to the histopathology seen in lung allograft rejection was noted in an unirradiated rat GVHD model *(79)*. Similar pulmonary pathology has been reported in several mouse SCT systems that model a variety of HC antigenic mismatches between donor and host *(40–43,80,81)*.

In studies completed by Cooke and colleagues, B10.BR donor stem cells and T lymphocytes were transplanted into CBA recipients. This donor/recipient strain combination is matched at the loci but differs at multiple minor HC antigens and therefore most closely models a SCT from a matched unrelated donor. At 6 wk after SCT, lungs of mice receiving syngeneic transplants maintained virtually normal histology. By contrast, two major abnormalities were apparent in the allogeneic group: a dense mononuclear cell infiltrate around both pulmonary vessels and bronchioles and an acute pneumonitis involving the interstitium and alveolar spaces *(42)*. The alveolar infiltrate was composed of macrophages, lymphocytes, epithelial cells, and scattered polymorphonuclear cells within a fibrin matrix *(42)*. Both of these histopathologic patterns closely resemble the microscopic features of the nonspecific, diffuse interstitial pneumonias seen in allo SCT recipients *(7,17,38,59)*. As noted earlier, similar histopathology has been observed using other strain combinations where the GVH reaction is induced across (1) other minor antigens, (2) class I or class II antigens only, and (3) major and minor HC antigenic differences, whereas findings of diffuse alveolar injury, including alveolar hemorrhage, edema, or hyaline membranes, were not seen *(82–84)*. Pulmonary function has been measured in live transplanted mice in order to assess the physiologic consequences of lung pathology present after SCT *(43,80)*. Mice with GVHD showed significant reductions in both dynamic compliance and airway conductance compared with syngeneic controls consistent with both the interstitial and peribronchial infiltrates seen microscopically *(43)*. Of note, no differences in pulmonary function or lung histopathology were observed between animals with mild and moderate GVHD. Thus, initial studies suggested that the development of IPS after allo SCT correlated with the presence, but not the severity, of systemic GVHD. The nonlinear relationship between lung injury and the severity of acute GVHD was consistent with clinical reports of IPS in patients whose signs and symptoms of GVHD were mild or absent *(8,9,57,73,74)*. Physiologically significant lung injury has also been reported in a fully major HC mismatched system within the first 2 wk of SCT *(80)*, suggesting that increasing antigenic disparity between donor and host may directly correlate with the time of onset of IPS in these mouse BMT systems.

2.3.2. Inflammatory Effectors TNF-α and LPS and the Development of IPS

Experimental models have also provided insight into the possible pathophysiologic mechanisms responsible for acute noninfectious lung injury occurring after SCT. Consistent with the mixed inflammatory alveolar infiltrates observed on histopathology, lung injury in recipients of allo SCT has been shown to be associated with a significant increase in the number of BAL lymphocytes, macrophages, and neutrophils *(42)*. Furthermore, increased expression of TNF-α mRNA and protein has been detected in the lungs and BAL fluid of animals with GVHD *(40–42,45,81)*. The correlation between increased BAL fluid TNF-α levels, neutrophil content, and pulmonary pathology in the absence of infection suggests that endotoxin (LPS) might also play an important role in the observed damage. Not only are increased levels of LPS noted in the BAL fluid of mice with IPS, but LPS may also be a "trigger" for the release of inflammatory cytokines that directly contribute to lung damage; LPS injection 6 wk after SCT increased the total number

of neutrophils in the BAL fluid and significantly amplified the severity of lung injury in animals with advanced GVHD *(42)*. These pathologic changes were associated with large increases in BAL fluid levels of TNF-α and LPS and with the development of alveolar hemorrhage *(42,81)*. The role of TNF-α in the development of experimental IPS has been examined further by using strategies that neutralize the effects of this inflammatory cytokine *(45,81,82)*. Recently, the effects of a soluble, dimeric, TNF-binding protein (rhTNFR:Fc; Immunex Corp. Seattle, WA) on lung injury were studied after allo SCT. Administration of rhTNFR:Fc around the time of LPS challenge effectively reduced mortality and prevented increases in pulmonary pathology, BAL fluid cellularity and endotoxin content, confirming that TNF-α is central to LPS-mediated systemic and pulmonary toxicity in this setting *(81)*. Furthermore, TNF-α neutralization from wk 4 to wk 6 after SCT significantly reduced the severity of lung injury and prevented the progression of systemic and hepatic GVHD seen in the control group during the treatment period *(81)*.

TNF-α is likely to contribute to the development of IPS through both direct and indirect mechanisms. TNF-α increases MHC expression, modulates leukocyte migration, facilitates cell-mediated cytotoxicity, and is itself cytotoxic *(45,85)*. It is also possible that the protective effects seen in the lung are secondary to a systemic anti-inflammatory response *(86)* because TNF-α blockade also attenuates the progression systemic and hepatic GVHD *(81)*. The partial reduction in lung injury provided by TNF-α neutralization is consistent with reports from many groups *(39,45,51,52,78,87,88)* and suggests that other inflammatory mediators and cellular mechanisms that are involved in acute GVHD may also contribute to the development of IPS *(47,48,78)*. Specifically, interleukin (IL)-1β, transforming growth factor-β (TGF-β), and nitrating species including nitric oxide and peroxynitrite have been implicated in the generation of early lung toxicity after allo SCT, particularly when cyclophosphamide is included in the conditioning regimen *(80,89,90)*.

The results of endotoxin challenge experiments confirm that TNF-α mediates systemic and pulmonary toxicity caused by LPS *(91–93)*. The reduction in BAL fluid LPS after TNF-α neutralization was intriguing however and strongly suggested that in addition to directly neutralizing TNF-α in the alveolar space, treatment with rhTNFR:Fc altered the systemic inflammatory response to LPS "upstream" from the lung *(86)*. From this perspective, the structural and functional integrity of the liver is likely to be critical. The liver is pivotally located between the intestinal reservoir of Gram-negative bacteria and their toxic byproducts and the rich capillary network in the lung. Kupffer cells in the liver detoxify and subsequently clear endotoxin from the systemic circulation *(94)* and protect the lung in experimental models of sepsis and ARDS *(95,96)*. Inflammation engendered during the normal clearance of endotoxin remains contained within the reticulo-endothelial system of the liver *(94)*. If, however, the capacity of the liver to clear an endotoxin challenge is exceeded, both inflammatory cytokines and unprocessed LPS can traverse into the systemic circulation and cause acute end-organ damage. Several experimental studies have shown that pre-existing injury decreases the ability of the liver to neutralize endotoxin effectively *(97–100)*. In the setting of acute GVHD, an endotoxin surge can arise from increased leakage of LPS across damaged intestinal mucosa. In this scenario, underlying hepatic damage as a consequence of direct target organ injury could then serve to decrease the liver's capacity for LPS uptake and clearance. Animals with mild or no GVHD effectively detoxify exogenous endotoxin and protect their lungs from further damage, whereas mice with extensive disease are unable to do so and ultimately develop severe pulmonary toxicity, including alveolar hemorrhage *(42)*. In the studies noted earlier using

rhTNFR:Fc, all animals had advanced GVHD at the time of analysis. As expected, administration of LPS to animals treated with control IgG overwhelmed the liver's capacity to clear circulating endotoxin and caused enhanced hepatic injury and the propagation of systemic and pulmonary disease. By contrast, systemic neutralization of TNF-α protected the liver from endotoxin-induced inflammation and resulted in decreased mortality and a reduction of BAL fluid LPS levels and pulmonary inflammation *(81)*.

These data demonstrate that the inflammatory mediators TNF-α and LPS both contribute to experimental IPS. Moreover, they support the hypothesis that a "gut–liver–lung" axis of inflammation may play a role in IPS pathophysiology and suggest that any process or combination of events that eventually results in large amounts of endotoxin and/or TNF-α into the pulmonary circulation could contribute to the development of lung injury. This hypothesis is supported by the clinical observation of increased levels of TNF-α in the serum of patients that develop IPS *(101)*. A role for hepatic dysfunction in pulmonary toxicity after SCT is also consistent with clinical reports of acute noninfectious pulmonary toxicity associated with severe GVHD and veno-occlusive disease (VOD) *(2,102)*. Furthermore, evidence for cytokine activation and LPS amplification in the broncho-alveolar compartment, which has been noted during ARDS *(103)*, has recently been demonstrated in patients with IPS after SCT as well *(1)*. Clark and colleagues found increased pulmonary vascular permeability and BAL fluid levels of IL-1, IL-12, IL-6, and TNF-α and components of the LPS amplification system (LPB and CD14) in patients with IPS *(1)*. The investigators conclude that pro-inflammatory cytokine activation contributes to IPS and suggest that patients with this complication may be at increased risk for LPS-mediated lung injury.

2.3.3. Cellular Effectors and the Development of IPS

2.3.3.1. The Role of Neutrophil/Polymorphonuclear Cells

As demonstrated earlier, the presence of neutrophils, in the absence of infection, is a major component of the inflammatory infiltrate seen in animals with IPS *(42)*. A role for neutrophils in noninfectious lung injury has been observed in both the acute and chronic setting; neutrophilia is a prominent finding in acute respiratory distress syndrome (ARDS) and in the early and late stages of bronchiolitis obliterans (BO) that develops during lung allograft rejection *(104–109)*. Polymorphonuclear (PMN) products are abundant in the BAL fluid of patients with ARDS and are believed to significantly contribute to endothelial and epithelial damage that occurs in this setting *(104)*, whereas similar increases in PMN activation markers may be early indicators of BO after lung transplant *(106)*. Neutrophils are likely to play a role in lung injury after SCT as well; more that 60% of patients diagnosed with IPS at the University of Michigan developed signs and symptoms of pulmonary dysfunction within 7 d of neutrophil engraftment *(20)*. Furthermore, a significant neutrophilic influx has also been observed in the BAL fluid and biopsy specimens of SCT recipients with BO *(110,111)*. In mouse IPS models, the influx of neutrophils is most prominent between wk 4 and 6 after SCT and is associated with the presence of TNF-α and LPS in the BAL fluid *(42,81)*. The relationship among neutrophils, TNF-α, and LPS is underscored by the outcome of LPS challenge and TNF-α neutralization experiments; administration of rhTNFR:Fc completely abrogated the robust influx of PMN cells resulting from LPS administration *(81)*. Importantly, this finding directly correlated with protection from enhanced pulmonary histopathology (including hemorrhage) and the preservation of pulmonary function *(81)*. Furthermore, reduction in lung injury resulting from neutralizing TNF from wk 4 to 6 was also accompanied by a significant decrease in neutrophils in BAL

fluid. Taken together, these data support a role for neutrophils in the injury incurred during IPS and suggest that aspects of the innate immune response may also contribute to this process.

2.3.3.2. Role of Donor Accessory Cells in the Development of IPS

The relationship among LPS, TNF-α, and donor leukocytes in the pathophysiology IPS has been examined further by determining whether the responsiveness of donor cells to LPS stimulation would influence the development of lung injury after allo SCT. To test this hypothesis, two related substrains of mice, C3H/Hej and C3Heb/Fej, that differ in their response to the lethal effects of LPS *(112)* were used as SCT donors. C3Heb/Fej animals exhibit normal murine sensitivity to LPS challenge (LPS-s), whereas a genetic mutation in the Toll-like receptor 4 (Tlr 4) gene of C3H/Hej mice has made this strain resistant to LPS (LPS-r) *(112–115)*. Initial experiments demonstrated that transplantation of cells from LPS-r donors resulted in a significant decrease in systemic GVHD. Specifically, LPS-r SCT reduced early intestinal injury mediated by TNF-α, a finding that was independent of donor T-cell response to host antigens *(52)*. In subsequent experiments, recipients of LPS-r SCT were also found to develop significantly less lung toxicity as measured by pathology, function, and BAL fluid cellularity *(82)*. This protective effect was associated with decreased TNF-α secretion in vivo and in vitro; BAL fluid TNF-α levels were lower after LPS-r SCT and BAL cells harvested from LPS-r recipients produced approx 30-fold less TNF-α to LPS stimulation compared to cells collected from recipients of LPS-s SCT *(82)*. This finding correlated with the naïve phenotype of C3H/Hej and C3Heb/Fej BAL cells, respectively, and was consistent with the observation that more than 98% of BAL cells are of donor origin by wk 4 after transplant. BAL LPS concentrations were also decreased after LPS-r SCT and correlated with a reduction in intestinal toxicity and serum LPS levels at wk 1 and with decreased intestinal and hepatic injury at wk 5 *(52,82)*. Similar reductions in systemic GVHD and lung injury have also been observed when animals deficient in CD14, a cell surface receptor critical to the innate immune response and an important receptor for LPS, were used as SCT donors in a second P → F1 SCT model *(116)*. These data demonstrate that resistance of donor accessory cells to LPS stimulation reduces the severity of lung injury after allo SCT. Importantly, these findings also reveal a significant role for donor-derived macrophages in IPS and support an etiologic link between gut and lung damage that occurs after alloSCT.

2.3.3.3. Role of Donor-Derived T-Cell Effectors

Although the induction of GVHD fundamentally depends on interactions between donor T cells and host antigen-presenting cells *(117)*, the role of alloreactive donor T cells in the pathogenesis of IPS has been a topic of considerable debate. The importance of lymphocytes to lung injury after experimental SCT has, however, been suggested by several groups *(40,80,118,119)*. Donor T cells are critical to the early pro-inflammatory events associated with lung toxicity that develops within the first week of SCT across MHC antigens, whereas in a minor HC antigen mismatch system, donor lymphocytes have been shown to persistently respond to host antigens and contribute to physiologically significant lung histopathology at later time-points after SCT *(43,80)*. Furthermore, donor T-cell clones that recognize CD45 polymorphisms result in a rapidly progressive pulmonary vasculitis within the first 3 d after their injection into nonirradiated recipients *(40,119)*. Finally, Gartner and colleagues showed that pulmonary natural killer (NK)-cell activity remained increased over an extended period of time during GVHD in contrast to the transient and mild increase in splenic NK activity that occurred during the same interval *(120)*. These experimental data support clinical observations

suggesting that alveolar lymphocytosis associated with interstitial pneumonitis after allo BMT could represent a pulmonary manifestation of chronic GVHD *(121)*.

Additional experiments have been completed to determine whether donor cytotoxic T lymphocyte (CTL) effectors contribute to lung injury via cell–cell-mediated killing. Two primary cytolytic pathways have been identified: the perforin–granzyme pathway and the Fas–Fas ligand (FasL) pathway. Both perforin and Fas pathways contribute to cytolysis mediated by CTLs and lymphokine-activated killer (LAK) cells *(122–125)*. The Fas pathway is primarily used by CD4+ cells *(126)*, whereas perforin-mediated killing has been shown to involve both CD4+ and CD8+ T-cell populations *(48,127)*. Furthermore, each cytolytic pathway has been shown to play a role in the development of GVHD and lung injury in non-SCT settings *(128–131)*. Using a parent → F1 model, significant CTL activity has been observed in the lungs of allo SCT recipients; alloantigen-specific killing using both perforin and Fas/FasL pathways was present as early as wk 2 after BMT and persisted over time as lung injury developed *(83)*. The relative contribution of each cytolytic pathway to the development of IPS was determined by using wild-type mice or animals deficient in either perforin (*pfp*) or FasL (*gld*) as SCT donors. Recipients of *gld*, but not *pfp–/–* SCT developed significantly less lung injury compared to allogeneic controls, a finding that was associated with reductions in BAL fluid cellularity, donor CD4+ and CD8+ T cells, and TNF-α levels *(83)*.

As mentioned earlier however, noninfectious lung injury has been reported in patients in whom systemic GVHD is mild or absent, making a causal relationship between alloreactive T cells and IPS difficult to establish *(8,9,57,73,74)*. Of interest, T-cell depletion (TCD) at the time of SCT using the B10.BR → CBA system reduced, but did not abrogate, lymphocyte responses in the lungs even though the number of T cells in the donor stem cell inoculum was insufficient to cause clinical or histologic GVHD. The observation that host reactive donor lymphocytes were present in the BAL fluid but not the spleens of animals after TCD SCT was intriguing and suggested that the lung may be particularly sensitive to the effects of these cells even when systemic tolerance has been established. Clinically, BAL fluid lymphocytosis has been described after TCD SCT in association with pneumonitis that resulted from a local immune response; pulmonary T cells appeared to be activated despite systemic immune suppression *(55)*. Collectively, these data support a role for cellular effector mechanisms in IPS pathophysiology. Donor-derived T cells can contribute to lung injury after SCT, even when systemic GVHD is mild or absent. In addition, CTL activity is present in the lungs of mice with IPS, and Fas–FasL but not perforin-mediated killing significantly contributes to the development of lung injury in an experimental system.

2.3.4. Role of Host Antigen-Presenting Cells in the Development of IPS

Although several groups have generated data to support a role for alloreactive donor T cells in the evolution of lung injury after SCT, the precise mechanisms by which these cells interact with host antigens and cause injury remain unresolved. This process is likely to be complex and to ultimately involve the interaction of donor lymphocytes with pulmonary antigen-presenting cells (APCs). It is conceivable that pulmonary dendritic cells, which are potent stimulators of primary T-cell responses, are intimately involved with this process *(132,133)*. These cells are thought to play a critical role in the initiation and regulation of immune responses in the lung, and recent data suggest that they are important to both acute and chronic rejection after lung transplantation *(134–137)*. Furthermore, the Th1 cytokines IL-2, and interferon-γ (IFN-γ), which are critical to the development of GVHD *(138)* are felt to be involved in the activation

and recruitment of dendritic cells to sites of inflammation *(139,140)*. The specific requirement of host APCs for the generation of acute GVHD was recently reported in a CD8+ T-cell-driven GVHD model in which chimeric animals that did not express alloantigen (MHC class I) on their APCs were used as SCT recipients *(117)*. These results were recently extended by the work of Teshima and colleagues, who showed that alloantigen expression on host epithelial cells is not required for the development of acute GVHD; rather, recognition of alloantigen on host APCs is necessary and sufficient to induce a GVH reaction in which early cytotoxic damage to GVHD target organs is driven by inflammatory cytokines *(65)*. It is possible that radio-resistant, pulmonary APCs in the host persist longer than those in other organs, thus allowing sustained presentation of host antigens in the lung (but not in other visceral sites) to small numbers of donor T cells trapped within the pulmonary microvascular circulation. This hypothesis could account for the apparent "sanctuary" status of the lung with respect to donor T cells and may have important implications with regard to the evaluation and treatment of pulmonary dysfunction after SCT even when clinical GVHD is absent.

2.3.5. Mechanisms of Leukocyte Recruitment to the Lung After Allo SCT

Although cellular effectors likely play a significant role in development of IPS, the mechanisms by which white blood cells (WBCs) traffic to the lung and cause inflammation have yet to be determined. WBC trafficking to sites of inflammation is a complex process involving interactions between leukocytes and endothelial cells that are facilitated by adhesion molecules, chemokines, and their receptors *(141)*. Chemokines are a large family of 8- to 10-kDa polypeptide molecules that have well-defined roles in directing cell movements of lymphocytes, monocytes, and neutrophils during immune responses and do so both directly via their chemoattractant properties (i.e., by providing "directional clues") and indirectly via integrin activation *(142,143)*. The 50+ chemokines that have been identified to date are classified structurally into 4 main groups according to the configuration of cysteine residues near the NH2-terminus (CC, CXC, C, and CX_3C) *(143)*. Actions of chemokines are mediated through a large family of seven-transmembrane-spanning, serpentine, G_i-protein-coupled receptors that have ligand specificity and a restricted expression on subclasses of leukocytes. However, ligand specificities can overlap; some chemokines bind to several receptors and some receptors bind multiple ligands *(144)*. Chemokines and their receptors can be functionally divided into two broad categories: "inducible" or "inflammatory" chemokines that are regulated by pro-inflammatory stimuli, help orchestrate innate and adaptive immunity, and recruit leukocytes to sites of inflammation in response to physiologic stress and "constitutive" or "homeostatic" chemokines responsible for basal leukocyte migration during immune surveillance and formation of the architectural framework of secondary lymphoid organs. "Inducible" or "inflammatory" chemokines are produced by a variety of cell types and are induced to high levels of expression by inflammatory stimuli such as LPS, IL-1 and TNF-α *(141)*. The corresponding "inflammatory" chemokine receptors tend to have more promiscuous or redundant ligand-binding interactions compared to "homeostatic" receptors and tend to be expressed on cells with an "effector" phenotype *(145)*.

Although chemokines have been shown to facilitate the recruitment of leukocytes to the lung in a variety of inflammatory states, including asthma, ARDS, infectious pneumonia, pulmonary fibrosis, and lung allograft rejection *(145,146)*, investigators have just begun to explore their role in IPS. In each scenario, the composition of the accompanying leukocytic infiltrate is determined by the pattern of chemokine expression in the inflamed lung. The mixed

pulmonary infiltrate observed in mice after allo SCT suggests, therefore, that chemokines responsible for the recruitment of monocytes, lymphocytes, and neutrophils may be upregulated during the development of IPS. This hypothesis is supported by the work of Panoskaltsis-Mortari and co-workers, who noted that enhanced expression of monocyte- and T-cell-attracting chemokines in the lungs correlated with lung injury that developed within the first 2 wk after SCT *(147)*. Work by the same group specifically demonstrated that T-lymphocyte production of MIP-1α is critical to the recruitment of CD8+ T cells to GVHD target organs, including the lung at later time-points after SCT *(148)*. These findings are supported by the observation that specific interactions between MIP-1α and CCR5+ CD8+ T cells also contribute to the pathogenesis of liver GVHD *(149)*. Studies are ongoing to more specifically determine the role of inflammatory chemokines in leukocyte recruitment during the development of IPS.

2.3.6. Summary

Extensive preclinical and clinical data suggest that both inflammatory and cellular effectors participate in the development of IPS after alloSCT; TNF-α and LPS appear to be significant, albeit not exclusive, contributors to IPS, and cells of both myeloid and lymphoid origin also play a direct role in lung injury that occurs in this setting. In particular, the contribution of donor accessory cells appears to be tightly linked to the relationship between LPS and TNF-α as it exists along a "gut–liver–lung" axis of inflammation, whereas donor-derived T-cell effectors can home to the lung and cause damage even when systemic GVHD is mild or absent. These findings have led to the development of a schema of IPS pathophysiology wherein it is hypothesized that the lung is susceptible to two distinct but interrelated pathways of injury involving aspects of both the adaptive and innate immune response (*see* Fig. 1). These studies are significant because they support a paradigm shift away from the current understanding of acute lung injury after SCT as an idiopathic clinical syndrome to a process in which the lung is the target of an alloantigen-specific, immune-mediated attack. It is anticipated that mechanistic insights gained using experimental models will form the basis for translational research protocols with the specific intent of treating or preventing IPS after SCT.

2.4. Treatment Strategies for IPS After AlloSCT

Currently, standard treatment regimens for IPS include supportive care measures in conjunction with broad-spectrum antimicrobial agents with or without intravenous corticosteroids *(6,20)*. Although reports of anecdotal responses to standard therapy are available, these responses are limited; despite such measures, the mortality of patients diagnosed with IPS remains unacceptably high *(19)*. Furthermore, prospective studies addressing the treatment of IPS and specifically the use of steroids are lacking in the literature. In the light of the poor response rate to standard treatment and preclinical and clinical data that suggest a potential role for TNF-α in the development of IPS, etanercept (Enbrel, Immunex, Seattle, WA) a soluble, dimeric TNF-α binding protein, was administered to three consecutive pediatric patients at the University of Michigan SCT program who met criteria for IPS *(20)*. All three patients underwent bronchoscopy with BAL 24–48 h prior to etanercept administration, and in each case, BAL fluid analysis was negative for infection. Pulmonary edema from fluid overload and cardiogenic etiologic factors were also ruled out in all cases. Each patient received empiric broad-spectrum antimicrobial therapy and methylprednisolone (2 mg/kg/d) prior to and during etanercept therapy. The administration of etanercept in combination with standard immunosuppressive therapy was well tolerated and associated with significant improvements in pulmonary function within the

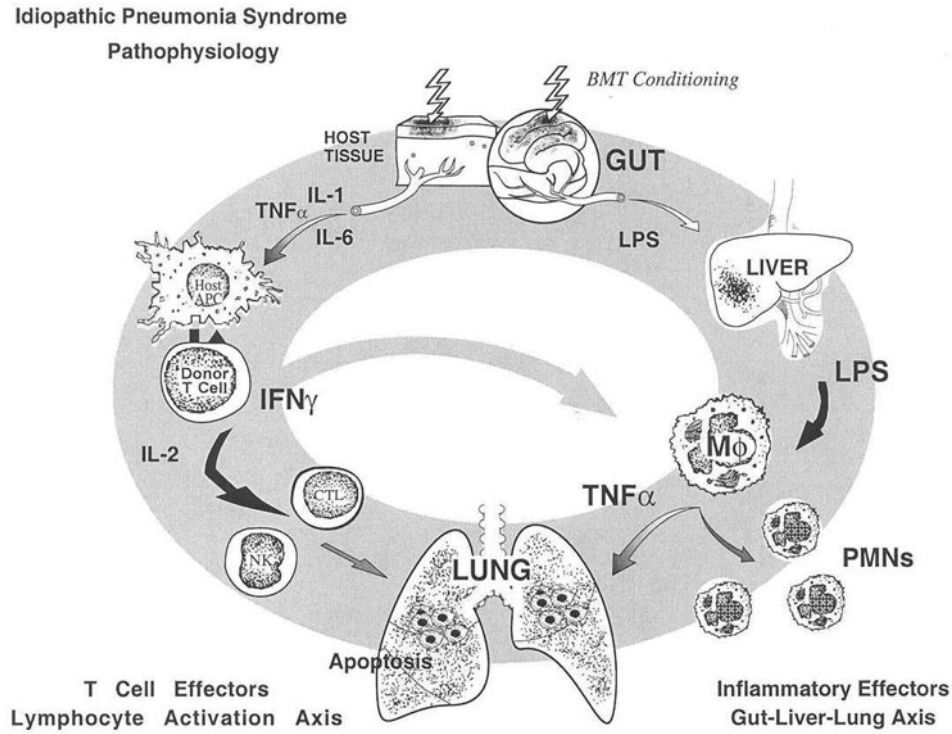

Fig. 1. Pathophysiology of noninfectious lung injury. Data generated using murine SCT models have been incorporated into a working hypothesis of IPS physiology. This schema postulates that the lung is susceptible to two distinct but interrelated pathways of immune-mediated injury that occur along a T-lymphocyte activation axis and a "gut–liver–lung" axis of inflammation. The lymphocyte-activation axis fundamentally depends on interactions between donor T cells and host APCs. Chemo-radiotherapy of SCT conditioning causes TNF-α and IL-1 release that enhances the ability of host APC to present alloantigens to mature donor T cells present in the BM inoculum *(16,182)*. Once engaged, donor T cells become activated and secrete a number of cytokines, including IFN-γ, which is a critical cytokine for the priming of pulmonary macrophages (Mϕ) and monocytes *(183–185)*, and IL-2, which facilitates T-cell activation, and proliferation and generation of both CTL and NK cells. Donor-derived, host reactive T cells and CTLs are present in the lung after alloSCT and contribute to pulmonary toxicity via Fas–FasL-mediated cell killing. The inflammatory axis focuses on the relationship between the cellular activating effects of LPS and the downstream production of TNF-α as it occurs along a gut–liver–lung axis of inflammation. During GVHD, the production of IFN-γ by allogeneic donor T cells is both necessary and sufficient to prime macrophages in those animals to secrete lethal amounts of TNF-α *(44)*. These primed macrophages are triggered to secrete inflammatory cytokines by doses of exogenous endotoxin too small to stimulate normal cells. Endotoxin enters the systemic circulation through gaps in the intestinal mucosa *(44,49–52)*. The ability of systemic endotoxin to reach the alveolar space is related to the consequences of GVHD in other target organs, particularly the liver, which is pivotally located immediately downstream (via the splanchnic circulation) of the intestinal reservoir of Gram-negative bacteria and their toxic byproducts. When confronted with a sudden endotoxin surge from increased LPS crossing a damaged intestinal mucosa, liver macrophages secrete inflammatory cytokines. If the endotoxin load surpasses the hepatic capacity for its clearance, both inflammatory cytokines (TNF-α) and unprocessed LPS spill over into the systemic circulation *(96,97,100)*. Underlying liver damage from hepatic GVHD decreases the liver's capacity for LPS uptake and clearance; thus, LPS remains in the systemic circulation for prolonged periods. Once in the alveolar space, LPS triggers pulmonary macrophage populations to secrete additional TNF-α, which results in the recruitment of neutrophils to the lung and enhances tissue damage.

first week of therapy. These data suggest that etanercept may represent a safe, noncrossreactive, therapeutic option for patients with IPS, and clinical trials studying etanercept for this indication are ongoing *(20)*.

3. SUBACUTE PULMONARY TOXICITY AFTER SCT: OBSTRUCTIVE LUNG DISEASE AND RESTRICTIVE LUNG DISEASE

3.1. Overview

Two forms of subacute pulmonary toxicity are common in patients over 100 d posttransplant: obstructive lung disease and restrictive lung injury *(8,9,14,74,150,151)*. Obstructive lung disease involves enhanced resistance to airflow on expiration and reflects conditions in the smaller airways and bronchioles. Obstructive defects are demonstrated by decreases in forced expiratory volumes at 1 s (FEV_1) and specifically by reductions in the forced expiratory ratio FEV_1/FVC (defined below) as measured by standard pulmonary function testing (PFT) *(150,152)*. By contrast, restrictive lung disease is classically associated with reductions in forced vital capacity (FVC), total lung capacity (TLC) and diffusion capacity of the lung for carbon monoxide (DLCO) with the FEV_1/FVC ratio maintained near 100% *(10,14,19,74,150)*. The reported incidence of both airflow obstruction and restrictive lung disease in alloSCT survivors ranges from 20% to 50% depending on donor source and time interval post SCT *(8–15,57,150)*.

3.2. Obstructive Lung Disease After SCT

Obstructive lung disease (OLD) is a well-recognized cause of morbidity following alloSCT *(8,74,151–154)*. Obstructive defects as defined by a FEV1.0/FVC < 70% on pulmonary function testing have been observed in approx 15–25% of allogeneic transplant recipients by d 100 and can persist for years after SCT *(10,57,74,150)*. Airflow obstruction may be a sequelae of extensive restrictive changes in small airways or may be related to small-airway destruction *(155)*. Lung biopsies from patients with OLD have shown a variety of histologic patterns, including lymphocytic bronchitis, chronic and acute interstitial pneumonitis, and varying degrees of bronchiolar inflammation, including BO *(8,57,76,111,153)*. This variation in histopathology is complicated further by the methods used to procure lung tissue; specifically, transbronchial lung biopsies rarely include an adequate sampling of distal bronchiolar structures and, therefore, are frequently considered nondiagnostic.

Despite these limitations, BO remains the most common form of histopathology associated with OLD and has been used historically to describe "GVHD of the lung" and interchangeably with OLD after SCT *(8,57,76,111,153)*. As the name implies, BO describes the histopathologic pattern of small-airway inflammation with fibrinous obliteration of the bronchiolar lumen that is classically associated with a fixed obstructive defect on PFT *(8,9,12,57,155)*. Airflow obstruction may, however, exist without BO, and BO may be present on biopsy without evidence for significant pulmonary dysfunction *(156)*. Furthermore, OLD is diagnosed by the appropriate clinical and PFT findings without histopathologic confirmation in the majority of cases. In this context, two phrases have been used to identify affected patients. The term "obstructive bronchiolitis" has been used to describe patients with airflow obstruction noted on PFT that have signs and symptoms consistent with bronchiolar inflammation *(151)*. Second, the phrase "bronchiolitis obliterates syndrome" or "BOS" has been developed to define the constellation of clinical, functional, and pathologic findings that accompany rejection after

lung transplantation *(157)*. BOS is specifically defined as an irreversible decline in FEV1 of at least 20% from baseline and is graded using the international heart and lung transplantation criteria: BOS stage 0 = FEV1 80% baseline; stage 1 = FEV1 from 66% to 79%; stage 2 = FEV1 from 51% to 65%; stage 3 = FEV1 50% of baseline value *(157)*.

The lack of consistent terminology and variability in diagnostic criteria used to define OLD has contributed to the wide variation in the reported incidence of this form of lung injury after SCT. A review by Afessa and colleagues found that OLD was reported in 8.3% of 2152 allo SCT patients included in 9 studies and that the incidence varied between 6% and 20% in long-term survivors with chronic GVHD *(19)*. When compared to IPS, the onset of airflow obstruction tends to be later (ranging from 3 to 18 mo after SCT) and more insidious. However, the rate of progression of disease once symptoms are established is variable, with rapid deterioration in FEV1 being associated with a poor outcome *(8,9,73,152)*. Symptoms may include cough, dyspnea, and wheezing; however, many patients remain asymptomatic despite having evidence of moderate to severe airway obstruction on PFTs *(8,74)*. Chest radiographs may show patchy, diffuse infiltrates but are frequently unrevealing except for hyperinflation and flattening of the diaphragm *(8,57,73)*. Likewise, findings on chest computed tomography (CT) can range from essentially normal early in the course of disease to demonstrating extensive peribronchial inflammation, bronchiectasis, significant air trapping, and diffuse parenchymal hypoattenuation *(9,158,159)*.

The clinical course of OLD varies from mild, with slow deterioration, to diffuse, necrotizing, fatal bronchiolitis of the small airways. Mortality rates of 25–50% have been reported in association with the latter form of lung injury *(11–13,152)*. Response to bronchodilator therapy is usually marginal because airflow obstruction tends to be "fixed" rather than "reversible." Furthermore, response to immunosuppressive therapy, including steroids alone or in combination with cyclosporine, or azathioprine, is limited and typically results in preservation (rather than significant improvement) of existing lung function, suggesting that early detection of disease is important *(8,9,13,152)*. In this light, two studies have suggested that analysis of maximum mid-expiratory flow rates (MMFR) may be used as an earlier indicator of impending airflow obstruction than FEV_1 *(13,73)*. Because enhanced immunosuppression significantly increases the risk of infection, the utility of such therapy is questionable when a clinical response is not seen within the first months of treatment or when pulmonary dysfunction is long standing.

As with IPS, the etiology of airflow obstruction after SCT is likely to be multifactorial and may include the effects of pretransplant conditioning regimens, concomitant infections, chronic aspiration, and the occurrence of GVHD targeting the lung. Significant airflow obstruction has been reported in association with older donor age, use of methotrexate for GVHD prophylaxis, lower levels of serum immunoglobulins, the presence of esophageal dysfunction (with aspiration), mismatched stem cell grafts, and busulfan (rather than TBI)-containing SCT conditioning regimens *(8,9,13,74,151,152,160)*. From an infectious disease perspective, donor and recipient baseline cytomegalovirus (CMV) status have not been shown to impact on the development of OLD. However, a history of both respiratory syncytial virus (RSV) and adenoviral infections has been suggested as possible etiologies for the higher incidence of OLD in the pediatric population *(9)*. From an immunologic standpoint, the development of OLD is strongly associated with cGVHD, particularly in patients with low serum IgG levels *(8,152)* and chronic hepatic GVHD *(9)*. Furthermore, recipients of mismatched related donor or matched unrelated donor grafts have a much higher incidence of OLD than patients receiving matched related

donor transplants (40% vs 13%) *(9)*. Collectively, these data suggest that immunologic mechanisms that are responsible for systemic GVHD may also contribute to OLD after alloSCT.

3.3. Restrictive Lung Disease After SCT

Reductions in lung volume (FVC, TLC) and diffusion capacity (DLCO) are common during the posttransplant period *(10,14,74,150)*. By 100 d posttransplant, significant decreases in FVC or TLC have been reported in as many as 25–45% of allogeneic transplant recipients and occur with greater frequency than obstructive abnormalities at this time *(10,14,15,150)*. An increase in nonrelapse mortality has been associated with the presence of a decline in TLC or FVC at 100 d posttransplant, even if the absolute values for each were within the normal range *(10)*. The presence of restrictive lung disease (RLD) at 1 yr or more posttransplant has likewise correlated with increased nonrelapse mortality *(14)*. Increasing recipient age, underlying diagnosis, total-body irradation (TBI) containing conditioning regimens and the presence of acute GVHD have been associated with lower lung capacities and higher mortality rates *(10,14,15,161–163)*. In contrast to airflow obstruction, RLD posttransplant has not been consistently associated with chronic GVHD *(10)*. In one pediatric study, the incidence of RLD was less common than in adult patients, but the incidence of these defects increased with increasing patient age *(15)*. A more recent report revealed that a large proportion of children receiving SCT in the 1990s were at risk for significant pulmonary dysfunction despite the absence of symptoms *(150)*. This risk was greatest for patients with more advanced disease at the time of SCT.

3.4. Pathogenesis of Subacute Pulmonary Toxicity After SCT

3.4.1. Obstructive Lung Disease/Bronchiolitis Obliterans Syndrome

In comparison to IPS, the pathophysiology of subacute lung injury after SCT is less well defined. This limitation stems from the lack of correlative data obtained from afflicted SCT recipients and the paucity of suitable SCT animal models for either form of injury. The development of OLD is characterized by bronchiolar leukocyte recruitment leading to fibro-obliteration of the airway. The mechanism of injury likely involves an initial insult to the small airway epithelium followed by an ongoing inflammatory response. The duration of the inciting stimulus determines the ultimate outcome of the ensuing inflammatory response: A static insult may result in wound healing with resolution and repair, whereas a persistent stimulus can lead to an overexuberant reparative response resulting in a more destructive and less reversible state characterized by airway obliteration and airflow obstruction. The normal repair mechanism is predicated on the proper balance between pro-inflammatory and anti-inflammatory "mediators" and changes in this balance can significantly influence the ultimate outcome of the immune response.

Most of what is known about the pathogenesis of OLD has been formulated from clinical investigation of lung allograft recipients and from murine heterotopic tracheal transplant models. The absence of an initial inflammatory response from BMT conditioning regimens and the presence of a "host vs graft" rather than "graft vs host" reaction are just two of the issues that limit the extrapolation of data obtained from these systems to that which occurs after SCT. However, clinical and experimental pulmonary allograft rejection are characterized by exuberant alloantigen-driven, immune-mediated injury to the bronchial structures of the lung. This response most certainly involves antigen presentation, T-cell activation, leukocyte recruitment, and enhanced expression of various mediators of inflammation, suggesting therefore

that mechanisms of lung injury may be similar in each scenario. Data generated from humans and mice support the hypothesis that the development of OLD or BO involves the interactions among cytokine, chemokine, and cellular effectors. Compared to healthy transplant recipients, analysis of BAL fluid obtained from patients with BO has revealed elevations in IL-1ra, TGF-β, IL-8, and MCP-1, all of which have been implicated in other fibro-proliferative processes *(106,164–166)*. Elevations of TGF-β and MCP-1 have also been observed in murine models of BO *(164,167)*. TNF-α is also known to play a critical role in the development of interstitial lung disease and fibrosis *(168,169)*. Although marked elevations of TNF-α have been reported during the development of murine BO, similar increases have not been observed in the BAL fluid of lung allograft recipients with BO *(165)*.

Because IL-8 is a potent chemoattractant for neutrophils, elevations of this chemokine during the development of BO is consistent with the reproducible finding of BAL neutrophilia that accompanies this process *(109,170)*. Clinical data also support a role for the interaction between pulmonary APCs and lymphocytes in the development of BO because effector T cells and dendritic cells expressing the costimulatory molecules CD80 and CD86 are present in the lungs of patients with BOS *(171,172)*. These observations have been extended by animal models; BO developing in heterotopic tracheal allografts requires donor-type rather than host-type APCs and can occur in the absence of either MHC class I or II antigens on donor tissue. These findings suggest that direct allorecognition by either CD8+ or CD4+ cells is important to this form of airway injury *(173)*. Additional studies have shown that CD28–B7 interactions are critical to this response because blocking this pathway using CTLA4IgG abrogates the development of BO *(174)*.

3.4.2. Restrictive Lung Injury

Similar to that which occurs during the development of BO, the pathogenesis of restrictive lung injury involves a chronic inflammatory process and the interplay between immune effector cells (that have been recruited to the lung) with the resident cellular constituents of the pulmonary vascular endothelium and interstitial space. In the setting of BMT, expression of class II MHC antigens on pulmonary epithelium, APCs, and vascular endothelium could promote alloantigen recognition and immune activation. The resulting inflammatory response would likely include secretion of pro-inflammatory cytokines, including TNF-α, IL-1β, IL-6, and TGF-β along with chemokines like IL-8, MCP-1, and MIP-1-α, all of which are known to stimulate fibroblast proliferation and promote collagen synthesis and deposition or leukocyte recruitment to inflamed tissue *(175,176)*. As chronic inflammation proceeds, fibroblasts increase dramatically in number within the lung leading to the loss of type I epithelial cells, proliferation of type II cells, the recruitment and proliferation of endothelial cells, and enhanced collagen deposition *(176)*. Ultimately, this process would result in interstitial thickening, loss of alveolar architecture, and end-stage fibrosis leading to significant loss of lung volume and severely impaired gas exchange.

Within this conceptual framework, Shankar and colleagues have suggested a biphasic model of noninfectious lung injury involving the interplay of ionizing irradiation and alloreactive donor T cells. This irradiated murine SCT model is characterized by early pro-inflammatory cytokine release and the promotion of lymphocyte influx, followed by a shift to a pro-fibrotic environment and the persistent secretion of TNF-α and IL-12 *(175)*. As noted earlier, TNF-α is one of several mediators that is known to promote chemotaxis, activation and proliferation of fibroblasts, and stimulation of collagen synthesis in vitro *(176)*. TNF-α gene expression has

been shown to rise after administration of agents that cause pulmonary fibrosis in rats *(168)*. A causal role for TNF-α in the development of interstitial lung disease and fibrosis has been shown using various methods to block the effects of TNF-α. Neutralization of TNF-α results in reduction of lung fibrosis in murine models by decreasing the cellularity of the lung parenchyma, attenuating destruction of the alveolar architecture, and reducing total lung hydroxyproline content *(168,169)*. In addition, mutant mice deficient in both TNF receptors (p55 and p75 knockout mice) are protected from injury after silica and bleomycin exposure *(177)*. Perhaps the most compelling evidence for a role of TNF-α in interstitial lung injury stems from a study in which targeted overexpression of TNF-α in the lungs of transgenic mice resulted in the development of lymphocytic and fibrosing alveolitis *(178)*. Although early lung histopathology observed in the TNF transgenic mice was not dissimilar to that seen in experimental IPS models *(42)*, the histologic changes associated with more chronic exposure to TNF-α resembled those seen in interstitial lung disease *(175)*.

3.5 Treatment of Subacute Lung Injury After SCT

Evaluation of treatment strategies for subacute lung injury after SCT is again hampered by the absence of controlled clinical trials addressing this problem. Furthermore, most reports focus on patients treated for OLD rather than RLD. Although the etiology of airflow obstruction after SCT is likely to be multifactorial, the undoubted association of OLD and chronic GVHD has resulted in a general acceptance that immunologic damage contributes to this process. Thus, "standard" therapy has historically employed enhanced immunosuppression in conjunction with supportive care, including supplemental oxygen therapy and broad-spectrum antimicrobial prophylaxis. Unfortunately, the response to agents, including steroids, cyclosporine, tacrolimus, and azathioprine, is limited, and when present, it tends to occur early in the course of treatment *(8,13,57,73,152)*. Patients with more severe disease at the start of treatment have a poor prognosis and high mortality rates, suggesting that recognition of OLD at a more reversible stage may be important *(13,73,152)*. Although no agent or combination of agents has been proven superior with respect to treating OLD, a study by Payne and colleagues showed that when compared to historical controls receiving prednisone and methotrexate for GVHD prophylaxis, the use of cyclosporine and methotrexate was protective against the development of OLD *(179)*. Unfortunately, results of prospective, randomized trials studying the impact of current GVHD prophylaxis regimens on the incidence and severity of OLD, have yet to be reported. However, a recent clinical trial examining the effectiveness of inhaled steroids in addition to standard systemic immunosuppression to prevent BOS after lung transplant was completed and found no benefit to such treatment when compared to placebo controls *(180)*. The poor response to standard therapy and the unacceptable morbidity and mortality associated with subacute lung injury after SCT is underscored by the recent report of a successful lung transplant in a SCT recipient with BO *(181)*. Collectively, these findings necessitate the development of prospective trials that will 1) enhance our understanding of the immunologic mechanisms responsible for OLD and RLD after SCT, 2) determine the most appropriate therapeutic approach, and 3) test new agents in this clinical setting.

4. SUMMARY

Diffuse noninfectious lung injury remains a significant problem following allo SCT both in the immediate posttransplant period and in the months to years that follow. Along with the development of GVHD, pulmonary toxicity limits the broader application of SCT and can have

significant implications with respect to the quality of life of SCT survivors. Although it is plausible that noninfectious lung injury and GVHD are connected mechanistically, a causal relationship between these two entities has yet to be definitively determined. Our understanding of the immunologic mechanisms involved with lung injury is limited by the absence of controlled clinical and the resultant paucity of clinical data, but these limitations have been overcome in part by observations made using animal SCT models; significant preclinical data suggest that the lung may be vulnerable to a two-pronged immunologic attack involving both inflammatory cytokine/chemokine and cellular effectors. As animal models for acute lung injury after SCT are explored further and those for subacute lung injury are developed, it is hoped that insights gained from each will improve our understanding of these disease processes and ultimately lead to the development of successful therapeutic strategies designed to diagnose, treat, or prevent noninfectious pulmonary toxicity in our SCT recipients.

REFERENCES

1. Clark J, Madtes D, Martin T, et al. Idiopathic pneumonia after bone marrow transplantation: cytokine activation and lipopolysaccharide amplification in the bronchoalveolar compartment. *Crit Care Med* 1999;27:1800.
2. Crawford S, Hackman R. Clinical course of idiopathic pneumonia after bone marrow transplantation. *Am Rev Respir Dis* 1993;147:1393.
3. Weiner RS, Mortimer MB, Gale RP, et al. Interstitial pneumonitis after bone marrow transplantation. *Ann Intern Med* 1986;104:168–175.
4. Quabeck K. The lung as a critical organ in marrow transplantation. *Bone Marrow Transplant* 1994;14:S19–S28.
5. Crawford S, Longton G, Storb R. Acute graft versus host disease and the risks for idiopathic pneumonia after marrow transplantation for severe aplastic anemia. *Bone Marrow Transplant* 1993;12:225.
6. Kantrow SP, Hackman RC, Boeckh M, et al. Idiopathic pneumonia syndrome: changing spectrum of lung injury after marrow transplantation. *Transplantation* 1997;63:1079–1086.
7. Clark J, Hansen J, Hertz M, et al. Idiopathic pneumonia syndrome after bone marrow transplantation. *Am Rev Respir Dis* 1993;147:1601–1606.
8. Holland HK, Wingard JR, Beschorner WE, et al. Bronchiolitis obliterans in bone marrow transplantation and its relationship to chronic graft-versus-host disease and low serum IgG. *Blood* 1988;72:621–627.
9. Schultz KR, Green GJ, Wensley D, et al. Obstructive lung disease in children after allogeneic bone marrow transplantation. *Blood* 1994;84:3212–3220.
10. Crawford SW, Pepe M, Lin D, et al. Abnormalities of pulmonary function tests after marrow transplantation predict nonrelapse mortality. *Am J Respir Crit Care Med* 1995;152:690–695.
11. Sullivan K, Mori M, Sanders J, et al. Late complications of allogeneic and autologous bone marrow transplantation. *Bone Marrow Transplant* 1992;10:127–134.
12. Wiesendanger P, Archimbaud E, Mornex J, et al. Post transplant obstructive lung disease ("broncliolitis obliterans"). *Eur Respir J* 1995;8:551–558.
13. Sanchez J, Torres A, Serrano J, et al. Long term follow up of immunosuppressive treatment for obstructive airway disease after allogeneic bone marrow transplantation. *Bone Marrow Transplant* 1997;20:403–408.
14. Badier M, Guillot C, Delpierre S, et al. Pulmonary function changes 100 days and one year after bone marrow transplantation. *Bone Marrow Transplant* 1993;12:457–461.
15. Quigley P, Yeager A, Loughlin G. The effects of bone marrow transplantation on pulmonary function in children. *Pediatr Pulmonary* 1994;18:361–367.
16. Abhyankar S, Gilliland DG, Ferrara JLM. Interleukin 1 is a critical effector molecule during cytokine dysregulation in graft-versus-host disease to minor histocompatibility antigens. *Transplantation* 1993;56:1518–1523.
17. Yousem SA. The histological spectrum of pulmonary graft-versus-host disease in bone marrow transplant recipients. *Hum Pathol* 1995;26:668–675.
18. Neiman P, Wasserman PB, Wentworth BB, et al. Interstitial pneumonia and cytomegalovirus infection as complications of human marrow transplantation. *Transplantation* 1973;15:478–485.
19. Afessa B, Litzow MR, Tefferi A. Bronchiolitis obliterans and other late onset non-infectious pulmonary complications in hematopoietic stem cell transplantation. *Bone Marrow Transplant* 2001;28:425–434.

20. Yanik G, Hellerstedt B, Custer J, et al. Etanercept as a novel therapy for idiopathic pneumonia syndrome after allogenic bone marrow transplantation. *Biol Blood Marrow Transplant* 2002; 8:395–400.
21. Wingard JR, Mellits ED, Sostrin MB, et al. Interstitial pneumonitis after allogeneic bone marrow transplantation. Nine-year experience at a single institution. *Medicine* 1988;67:175–186.
22. Weiner RS, Horowitz MM, Gale RP, et al. Risk factors for interstitial pneumonitis following bone marrow transplantation for severe aplastic anemia. *Br J Haematol* 1989;71:535.
23. Meyers JD, Flournoy N, Thomas ED. Nonbacterial pneumonia after allogeneic marrow transplantation: a review of ten years' experience. *Rev Infect Dis* 1982;4:1119–1132.
24. Atkinson K, Turner J, Biggs JC, et al. An acute pulmonary syndrome possibly representing acute graft-versus-host disease involving the lung interstitium. *Bone Marrow Transplant* 1991;8:231.
25. Della Volpe A, Ferreri AJ, Annaloro C, et al. Lethal pulmonary complications significantly correlate with individually assessed mean lung dose in patients with hematologic malignancies treated with total body irradiation. *Int J Radiat Oncol Biol Phys* 2002;52:483–488.
26. Robbins RA, Linder J, Stahl MG, et al. Diffuse alveolar hemorrhage in autologous bone marrow transplant recipients. *Am J Med* 1989;87:511–518.
27. Lewis ID, DeFor T, Weisdorf DJ. Increasing incidence of diffuse alveolar hemorrhage following allogeneic bone marrow transplantation: cryptic etiology and uncertain therapy. *Bone Marrow Transplant* 2000;26:539–543.
28. Metcalf JP, Rennard SI, Reed EC, et al. Corticosteroids as adjunctive therapy for diffuse alveolar hemorrhage associated with bone marrow transplantation. University of Nebraska Medical Center Bone Marrow Transplant Group. *Am J Med* 1994;96:327–334.
29. Capizzi SA, Kumar S, Huneke NE, et al. Peri-engraftment respiratory distress syndrome during autologous hematopoietic stem cell transplantation. *Bone Marrow Transplant* 2001;27:1299–1303.
30. Wilczynski SW, Erasmus JJ, Petros WP, et al. Delayed pulmonary toxicity syndrome following high-dose chemotherapy and bone marrow transplantation for breast cancer. *Am J Respir Crit Care Med* 1998;157:565–573.
31. Bhalla KS, Wilczynski SW, Abushamaa AM, et al. Pulmonary toxicity of induction chemotherapy prior to standard or high-dose chemotherapy with autologous hematopoietic support. *Am J Respir Crit Care Med* 2000;161:17–25.
32. Kelley J. Cytokines of the lung. *Am Rev Respir Dis* 1990;141:765–788.
33. Piguet P, Collart M, Grau G, et al. Requirement of tumour necrosis factor for development of silica-induced pulmonary fibrosis. *Nature* 1990;344:245–247.
34. Schmidt J, Pliver CN, Lepe-Zuniga JL, et al. Silica-stimulated monocytes release fibroblast proliferation factors identical to interleukin-1. A potential role for interleukin-1 in the pathogenesis of silicosis. *J Clin Invest* 1984;73:1462–1472.
35. Suter P, Suter S, Girardin E, et al. High bronchoalveolar levels of tumor necrosis factor and its inhibitors, interleukin-1, interferon, and elastase, in patients with adult respiratory distress syndrome after trauma, shock or sepsis. *Am Rev Resp Dis* 1992;145:1016.
36. Hyers T, Tricomi S, Dettenmier P, et al. Tumor necrosis factor levels in serum and bronchoalveolar lavage fluid of patients with the adult respiratory distress syndrome. *Am Rev Respir Dis* 1991;144:268.
37. Bortin M, Ringden O, Horowitz M, et al. Temporal relationships between the major complications of bone marrow transplantation for leukemia. *Bone Marrow Transplant* 1989;4:339.
38. Beschorner W, Saral R, Hutchins G, et al. Lymphocytic bronchitis associated with graft versus host disease in recipients of bone marrow transplants. *N Engl J Med* 1978;299:1030–1036.
39. Piguet PF, Grau GE, Collart MA, et al. Pneumopathies of the graft-versus-host reaction. Alveolitis associated with an increased level of tumor necrosis factor MRNA and chronic interstitial pneumonitis. *Lab Invest* 1989;61:37–45.
40. Clark JG, Madtes DK, Hackman RC, et al. Lung injury induced by alloreactive Th1 cells is characterized by host-derived mononuclear cell inflammation and activation of alveolar macrophages. *J Immunol* 1998;161:1913–1920.
41. Shankar G, Bryson J, Jennings C, et al. Idiopathic pneumonia syndrome in mice after allogeneic bone marrow transplantation. *Am J Respir Cell Mol Biol* 1998;18:235–242.
42. Cooke KR, Kobzik L, Martin TR, et al. An experimental model of idiopathic pneumonia syndrome after bone marrow transplantation. I. The roles of minor H antigens and endotoxin. *Blood* 1996;8:3230–3239.
43. Cooke KR, Krenger W, Hill GR, et al. Host reactive donor T cells are associated with lung injury after experimental allogeneic bone marrow transplantation. *Blood* 1998;92:2571–2580.

44. Nestel FP, Price KS, Seemayer TA, et al. Macrophage priming and lipopolysaccharide-triggered release of tumor necrosis factor alpha during graft-versus-host disease. *J Exp Med* 1992;175:405–413.
45. Piguet PF, Grau GE, Allet B, et al. Tumor necrosis factor/cachectin is an effector of skin and gut lesions of the acute phase of graft-versus-host disease. *J Exp Med* 1987;166:1280–1289.
46. Antin JH, Ferrara JLM. Cytokine dysregulation and acute graft-versus-host disease. *Blood* 1992;80:2964–2968.
47. Braun YM, Lowin B, French L, et al. Cytotoxic T cells deficient in both functional Fas ligand and perforin show residual cytolytic activity yet lose their capacity to induce lethal acute graft-versus-host disease. *J Exp Med* 1996;183:657–661.
48. Baker MB, Altman NH, Podack ER, et al. The role of cell-mediated cytotoxicity in acute GVHD after MHC-matched allogeneic bone marrow transplantation in mice. *J Exp Med* 1996;183:2645–2656.
49. Fegan C, Poynton C, Whittaker J. The gut mucosal barrier in bone marrow transplantation. *Bone Marrow Transplant* 1990;5:373–377.
50. Jackson SK, Parton J, Barnes RA, et al. Effect of IgM-enriched intravenous immunoglobulin (Pentaglobulin) on endotoxaemia and anti-endotoxin antibodies in bone marrow transplantation. *Eur J Clin Invest* 1993;23:540–545.
51. Hill GR, Crawford JM, Cooke KJ, et al. Total body irradiation effects on acute graft versus host disease. The role of gastrointestinal damage and inflammatory cytokines. *Blood* 1997;90 pp. 3204–3213.
52. Cooke K, Hill G, Crawford J, et al. Tumor necrosis factor-α production to lipopolysaccharide stimulation by donor cells predicts the severity of experimental acute graft versus host disease. *J Clin Invest* 1998;102:1882–1891.
53. Hill GR, Teshima T, Rebel VI, et al. The p55 TNF-alpha receptor plays a critical role in T cell alloreactivity. *J Immunol* 2000;164:656–663.
54. Cooke K, Gerbitz A, Hill G, et al. LPS Antagonism reduces graft-versus-host disease and preserves graft-versus-leukemia activity after experimental bone marrow transplantation. *J Clin Invest* 2001;7:1581–1589.
55. Milburn HJ, Poulter LW, Prentice HG, et al. Pulmonary cell populations in recipients of bone marrow transplants with interstitial pneumonitis. *Thorax* 1989;44:570.
56. Milburn HJ, Du Bois RM, Prentice HG, et al. Pneumonitis in bone marrow transplant recipients results from a local immune response. *Clin Exp Immunol* 1990;81:232.
57. Schwarer AP, Hughes JMB, Trotman-Dickenson B, et al. A chronic pulmonary syndrome associated with graft-versus-host disease after allogeneic marrow transplantation. *Transplantation* 1992;54:1002–1008.
58. Sutedja TG, Apperley JF, Hughes JMB, et al. Pulmonary function after bone marrow transplantation for chronic myeloid leukemia. *Thorax* 1988;43:163–169.
59. Sloane J, Depledge M, Powles R, et al. Histopathology of the lung after bone marrow transplantation. *J Clin Pathol* 1983;36:546–554.
60. Hackman RC, Sale GE. Large airway inflammation as a possible manifestation of a pulmonary graft-versus-host reaction in bone marrow allograft recipients. *Lab Invest* 1981;44:26A.
61. Connor R, Ramsay N, McGlave P, et al. Pulmonary pathology in bone marrow transplant recipients. *Lab Invest* 1982;46:3.
62. Madtes DK, Crawford SW. Lung injuries associated with graft-versus-host reactions. In: Ferrara JLM, Deeg HJ, Burakoff SJ, eds. *Graft-vs.-Host Disease*. New York: Marcel Dekker, 1997:425.
63. Beaumont F, Schilizzi BM, Kallenberg CG, et al. Expression of class II-MHC antigens on alveolar and bronchiolar epithelial cells in fibrosing alveolitis. *Chest* 1986;89:136.
64. Kunkel SL, Strieter RM. Cytokine networking in lung inflammation. *Hosp Prac* 1990:63–76 Vol 10 pp. 63–66, 69 73–76..
65. Teshima T, Ordemann R, Reddy P, et al. Acute graft-versus-host disease does not require alloantigen expression on host epithelium. *Nature Med* 2002;8:575–581.
66. Jordana M, Richards C, Irving LB, et al. Spontaneous in vitro release of alveolar-macrophage cytokines after the intratracheal instillation of bleomycin in rats. *Am Rev Respir Dis* 1988;137:1135–1140.
67. Fattal-German M, Le Roy Ladurie F, et al. Expression of ICAM-1 and TNF alpha in human alveolar macrophages from lung-transplant recipients. *Ann NY Acad Sci* 1996;796:138–148.
68. Sumitomo M, Sakiyama S, Tanida N, et al. Difference in cytokine production in acute and chronic rejection of rat lung allografts. *Transplant Int* 1996;9:S223–S225.
69. Stephens KE, Ishizaka A, Larrick JW, et al. Tumor necrosis factor causes increased pulmonary permeability and edema. Comparison to septic acute lung injury. *Am Rev Respir Dis* 1988;137:1364–1370.

70. Curtis JL, Byrd PK, Warnock ML, et al. Requirement of CD4-positive T cells for cellular recruitment to the lungs of mice in response to a particulate intratracheal antigen. *J Clin Invest* 1991;88:1244.
71. Toews GB. Pulmonary dendritic cells: sentinels of lung associated lymphoid tissues. *Am J Respir Cell Mol Biol* 1991;4:204.
72. Rabinowich H, Zeevi A, Paradis IL, et al. Proliferative responses of bronchoalveolar lavage lymphocytes from heart-lung transplant patients. *Transplantation* 1990;49:115.
73. Curtis DJ, Smale A, THien F, et al. Chronic airflow obstruction in long-term survivors of allogeneic bone marrow transplantation. *Bone Marrow Transplant* 1995;16:169–173.
74. Clark JG, Schwartz DA, Flournoy N, et al. Risk factors for air-flow obstruction in recipients of bone marrow transplants. *Ann Intern Med* 1987;107:648–656.
75. Urbanski SJ, Kossakowska AE, Curtis J, et al. Idiopathic small airways pathology in patients with graft-versus-host disease following allogeneic bone marrow transplantation. *Am J Surg Pathol* 1987;11:965.
76. Wyatt SE, Nunn P, Hows JM, et al. Airways obstruction associated with graft-versus-host disease after bone marrow transplantation. *Thorax* 1984;39:887.
77. Hakim FT, Mackall CL. The immune system: effector and target of graft-versus-host disease. In: Ferrara JLM, Deeg HJ, Burakoff SJ, eds. *Graft-vs.-Host Disease*. New York: Marcel Dekker, 1997:257.
78. Hattori K, Hirano T, Miyajima H, et al. Differential effects of anti-fas ligand and anti-tumor necrosis factor a antibodies on acute graft-versus-host disease pathologies. *Blood* 1998;91:4051–4055.
79. Workman D, Clancy JJ. Interstitial pneumonitis and lymphocytic bronchiolitis/bronchitis as a direct result of acute lethal graft-versus-host disease duplicate the histopathology of lung allograft rejection. *Transplantation* 1994;58:207.
80. Panoskaltsis-Mortari A, Taylor PA, Yaegar TM, et al. The critical early proinflammatory events associated with idiopathic pneumonia syndrome in irradiated murine allogenic recipients are due to donor T cell infusion and potentiated by cyclophoshamide. *J Clin Invest* 1997;100:1015–1027.
81. Cooke K, Hill G, Gerbitz A, et al. TNFα neutralization reduces lung injury after experimental allogeneic bone marrow transplantation. *Transplantation* 2000;70:272–279.
82. Cooke K, Hill G, Gerbitz A, et al. Hyporesponsiveness of donor cells to LPS stimulation reduces the severity of experimental idiopathic pneumonia syndrome: potential role for a gut–lung axis of inflammation. *J Immunol* 2000;165:6612–6619.
83. Cooke K, Kobzik L, Teshima T, et al. A role for Fas–Fas ligand but not perforin mediated cytolysis in the development of experimental idiopathic pneumonia syndrome. *Blood* 2000;96:768a.
84. Gerbitz A, Wilke A, Eissner G, et al. Critical role for CD54 (ICAM-1) in the development of experimental indiopathic pneumonia syndrome. *Blood* 2000;96:768a.
85. Jasinski M, Wieckiewicz J, Ruggiero I, et al. Isotype-specific regulation of MHC Class II gene expression in human monocytes by exogenous and endogenous tumor necrosis factor. *J Clin Immunol* 1995;15:185.
86. Smith S, Skerrett S, Chi E, et al. The locus of tumor necrosis factor-α action in lung inflammation. *Am J Respir Cell Mol Biol* 1998;19:881.
87. Vallera DA, Taylor PA, Vannice JL, et al. Interleukin-1 or tumor necrosis factor-alpha antagonists do not inhibit graft-versus-host disease induced across the major histocompatibility barrier in mice. *Transplantation* 1995;60:1371–1374.
88. Clark JG, Mandac JB, Dixon AE, et al. Neutralization of tumor necrosis factor-alpha action delays but does not prevent lung injury induced by alloreactive T helper 1 cells. *Transplantation* 2000;70:39–43.
89. Haddad I, Ingbar D, Panoskaltsis-Mortari A, et al. Activated alveolar macrophage-derived nitric oxide predicts the development of lung damage after marrow transplantation in mice. *Chest* 1999;116:37S.
90. Haddad I, Panoskaltsis-Mortari A, Ingbar D, et al. High levels of peroxynitrite are generated in the lungs of irradiated mice given cyclophosphamide and allogeneic T cells: a potential mechanism of injury after marrow transplantation. *Am J Respir Cell Mol Biol* 1999;20:1125.
91. Christ W, Asano O, Robidoux A, et al. E5531, a pure endotoxin antagonist of high potency. *Science* 1995;268:80–83.
92. Garbrecht B, Di Silvio M, Demetris A, et al. Tumor necrosis factor-a regulates in vivo nitric oxide synthesis and induces liver injury during endotoxemia. *Hepatology* 1994;20:1055.
93. Freudenberg M, Galanos C. Tumor necrosis factor alpha mediates lethal activity of killed gram-negative and gram-positive bacteria in D-galactosamine treated mice. *Infect Immun* 1991;59:2110.

94. Crawford J. Cellular and molecular biology of the inflamed liver. *Curr Opin Gastroenterol* 1997;13:175.
95. Matuschak G, Pinksy M, Klein E, et al. Effects of D-galactosamine induced acute liver injury on mortality and pulmonary responses to Escherichia coli lipopolysaccharide. *Am Rev Respir Dis* 1990;141:1296.
96. Matuschak GM, Mattingly ME, Tredway TL, et al. Liver–lung interactions during *E. coli* endotoxemia. *Am J Respir Crit Care Med* 1994;149:41–49.
97. Nakao A, Taki S, Yasui M, et al. The fate of intravenously injected endotoxin in normal rats and in rats with liver failure. *Hepatology* 1994;19:1251.
98. Lehmann V, Freudenberg M, Galanos C. Lethal toxicity of lipopolysaccharide and tumor necrosis factor in normal and D-galactosamine treated mice. *J Exp Med* 1987;165:657.
99. Galanos C, Freudenber M, Reutter W. Galactosamine induced sensitization to the lethal effects of endotoxin. *Proc Natl Acad Sci USA* 1987;76:5939.
100. Katz M, Grosfeld J, Gross K. Impaired bacterial clearance and trapping in obstructive jaundice. *Am J Surg* 1984;199 Vol 199 pp. 14–20.
101. Holler E, Kolb HJ, Moller A, et al. Increased serum levels of tumor necrosis factor alpha precede major complications of bone marrow transplantation. *Blood* 1990;75:1011–1016.
102. Borin M, Ringden O, Horowitz M, et al. Temporal relationship between the major complications of bone marrow transplantation for leukemia. *Bone Marrow Transplant* 1989;4:339.
103. Martin T, Rubenfeld G, Ruzinski J. Relationship between soluble CD14, lipopolysaccharide binding protein, and the alveolar inflammatory response in patients with acute respiratory distress syndrome. *Am J Respir Crit Care Med* 1997;155:937.
104. Martin T, Goodman R. The role of chemokines in the pathophysiology of the acute respiratory distress syndrome (ARDS). In: Hebert C, ed. *Chemokines in Disease*. Totowa, NJ: Humana, 1999:81–110.
105. DiGiovine B, Lynch J, Martinez F, et al. Bronchoalveolar lavage neutrophilia is associated with obliterative bronchiolitis after lung transplantation: role of IL-8. *J Immunol* 1996;157:4194–5202.
106. Riise GC, Andersson BA, Kjellstrom C, et al. Persistent high BAL fluid granulocyte activation marker levels as early indicators of bronchiolitis obliterans after lung transplant. *Eur Respir J* 1999;14:1123–1130.
107. Zheng L, Walters EH, Ward C, et al. Airway neutrophilia in stable and bronchiolitis obliterans syndrome patients following lung transplantation. *Thorax* 2000;55:53–59.
108. Reynaud-Gaubert M, Thomas P, Badier M, et al. Early detection of airway involvement in obliterative bronchiolitis after lung transplantation. Functional and bronchoalveolar lavage cell findings. *Am J Respir Crit Care Med* 2000;161:1924–1929.
109. Elssner A, Vogelmeier C. The role of neutrophils in the pathogenesis of obliterative bronchiolitis after lung transplantation. *Transplant Infect Dis* 2001;3:168–176.
110. St John RC, Gadek JE, Tutschka PJ, et al. Analysis of airflow obstruction by bronchoalveolar lavage following bone marrow transplantation. Implications for pathogenesis and treatment. *Chest* 1990;98:600–607.
111. Urbanski SJ, Kossakowska AE, Curtis J, et al. Idiopathic small airways pathology in patients with graft-versus-host disease following allogeneic bone marrow transplantation. *Am J Surg Pathol* 1987;11:965–971.
112. Glode LM, Rosenstreich DL. Genetic control of B cell activation by bacterial lipopolysaccaride is mediated by multiple distinct genes or alleles. *J Immunol* 1976;117:2061–2066.
113. Poltorak A, Ziaolong H, Smirnova I, et al. Defective LPS signaling in C3H/HeJ and C57BL/20ScCr mice: mutations in Tlr4 gene. *Science* 1998;282:2085.
114. Watson J, Kelly K, Largen M, et al. The genetic mapping of a defective LPS response gene in C3H/Hej mice. *J Immunol* 1978;120:422–424.
115. Sultzer BM, Castagna R, Bandeakar J, et al. Lipopolysaccharide nonresponder cells: the C3H/HeJ defect. *Immunobiology* 1993;187:257–271.
116. Cooke K, Olkiewicz K, Clouthier S, et al. Critical role for CD14 and the innate immune response in the induction of experimental acute graft-versus-host disease. *Blood* 2001;98:776a.
117. Shlomchik W, Couzens M, Tang C, et al. Prevention of graft versus host disease by inactivation of host antigen-presenting cells. *Science* 1999;285:412–415.
118. Watanabe T, Kawamura T, Kawamura H, et al. Intermediate TCR cells in mouse lung. Their effector function to induce pneumonitis in mice with autoimmune-like graft-versus-host disease. *J Immunol* 1997;158:5805.
119. Chen W, Chatta K, Rubin W, et al. Polymorphic segments of CD45 can serve as targets for GVHD and GVL responses. *Blood* 1995;86(suppl):158a.
120. Gartner JG, Merry AC, Smith CI. An analysis of pulmonary natural killer cell activity in F1-hybrid mice with acute graft-versus-host reactions. *Transplantation* 1988;46:879–886.

121. Leblond V, Zouabi H, Sutton L, et al. Late CD8+ lymphocytic alveolitis after allogeneic bone marrow transplantation and chronic graft-versus-host disease. *Am J Crit Care Med* 1994;150:1056.
122. Trauth BC, Klas C, Peters AM, et al. Monoclonal antibody-mediated tumor regression by induction of apoptosis. *Science* 1989;245:301–305.
123. Itoh N, Yonehara S, Ishii A, et al. The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis. *Cell* 1991;66:233–243.
124. Lowin B, Hahne M, Mattmann C, et al. Cytolytic T-cell cytotoxicity is mediated through perforin and Fas lytic pathways. *Nature (London)* 1994;370:650–652.
125. Lee RK, Spielman J, Zhao DY. Perforin fas ligand and tumor necrosis factor are the major cytotoxic molecules used by lymphokine-activated killer cells. *J Immunol* 1996;157:1919–1925.
126. Teshima T, Hill G, Pan L, et al. IL-11 separates graft-versus-leukemia effects from graft-versus-host disease after bone marrow transplantation. *J Clin Invest* 1999;104:317–325.
127. Blazar B, Taylor P, Vallera D. CD4+ and CD8+ T cells each can utilize a perforin-dependent pathway to mediate lethal graft versus host disease in major histocompatibility complex-disparate recipients. *Transplantation* 1997;64:571–576.
128. Rafi AQ, Zeytun A, Bradley M, et al. Evidence for the Involvement of Fas ligand and perforin in the induction of vascular leak syndrome. *J Immunol* 1998;161:3077–3086.
129. Matute-Bello G, Liles WC, Steinberg KP, et al. Soluble Fas ligand induces epithelial cell apoptosis in humans with acute lung injury (ARDS). *J Immunol* 1999;163:2217–2225.
130. Hiroyasu S, Shiraishi M, Koji T, et al. Analysis of Fas system in pulmonary injury of graft-versus-host disease after rat intestinal transplantation. *Transplantation* 1999;68:933–938.
131. Hashimoto S, Kobayashi A, Kooguchi K, et al. Upregulation of two death pathways of perforin/granzyme and FasL/Fas in septic acute respiratory distress syndrome. *Am J Respir Crit Care Med* 2000;161:237–243.
132. Massard G, Tongiio MW, Wihlm JM, et al. The dendritic cell lineage: an ubiquitous antigen-presenting organization. *Ann Thorac Surg* 1996;61:252.
133. Armstrong LR, Christensen PJ, Paine R, et al. Regulation of the immunostimulatory activity of rat pulmonary interstitial dendritic cells by cell–cell interactions and cytokines. *Am J Repir Cell Mol Biol* 1994;11:682.
134. Dupuis M, McDonald DM. Dendritic-cell regulation of lung immunity. *Am J Respir Cell Mol Biol* 1997;17:284.
135. Christensen PJ, Armstrong LR, Fak JJ, et al. Regulation of rat pulmonary dendritic cell immunostimulatory activity by alveolar epithelial cell-derived granulocyte macrophage colony-stimulating factor. *Am J Respir Cell Mol Biol* 1995;13:426.
136. van Haarst JM, de Wit HJ, Drexhage HA, et al. Distribution and immunophenotype of mononuclear and dendritic cells in the human lung. *Am J Respir Cell Mol Biol* 1994;10:487.
137. Yousem SA, Ray L, Paradis IL, et al. Potential role of dendritic cells in bronchiolitis obliterans in heart-lung transplantation. *Ann Thorac Surg* 1990;49:424.
138. Ferrara JLM, Deeg HJ. Graft versus host disease. *N Engl J Med* 1991;324:667–674.
139. Kradin RL, Xia W, Pike M, et al. Interleukin-2 promotes the motility of dendritic cells and their accumulation in lung and skin. *Pathobiology* 1996;64:180.
140. Suda T, Callahan RJ, Wilkerson RA, et al. Interferon-gamma reduces Ia+ dendritic cells traffic to the lung. *J Leukocyte Biol* 1996;60:519.
141. Mackay CR. Chemokines: immunology's high impact factors. *Nature Immunol* 2001;2:95–101.
142. Luster AD. Chemokines—chemotactic cytokines that mediate inflammation. *N Engl J Med* 1998;338:436–445.
143. Rollins BJ. Chemokines. *Blood* 1997;90:909–928.
144. Nelson PJ, Krensky AM. Chemokines, chemokine receptors, and allograft rejection. *Immunity* 2001;14:377–386.
145. Gerard C, Rollins BJ. Chemokines and disease. *Nature Immunol* 2001;2:108–115.
146. Luster AD. The role of chemokines in linking innate and adaptive immunity. *Curr Opin Immunol* 2002;14:129–135.
147. Panoskaltsis-Mortari A, Strieter RM, Hermanson JR, et al. Induction of monocyte- and T-cell-attracting chemokines in the lung during the generation of idiopathic pneumonia syndrome following allogeneic murine bone marrow transplantation. *Blood* 2000;96:834–839.
148. Serody JS, Burkett SE, Panoskaltsis-Mortari A, et al. T-Lymphocyte production of macrophage inflammatory protein-1alpha is critical to the recruitment of CD8(+) T cells to the liver, lung, and spleen during graft-versus-host disease. *Blood* 2000;96:2973–2980.
149. Murai M, Yoneyama H, Harada A, et al. Active participation of CCR5(+)CD8(+) T lymphocytes in the pathogenesis of liver injury in graft-versus-host disease. *J Clin Invest* 1999;104:49–57.
150. Cerveri I, Fulgoni P, Giorgiani G, et al. Lung function abnormalities after bone marrow transplantation in children: has the trend recently changed? *Chest* 2001;120:1900–1906.

151. Ringden O, Remberger M, Ruutu T, et al. Increased risk of chronic graft-versus-host disease, obstructive bronchiolitis, and alopecia with busulfan versus total body irradiation: long-term results of a randomized trial in allogeneic marrow recipients with leukemia. *Blood* 1999;93:2196–2201.
152. Clark JG, Crawford SW, Madtes DK, et al. Obstructive lung disease after allogeneic marrow transplantation. Clinical presentation and course. *Ann Intern Med* 1989;111:368–376.
153. Ralph DD, Springmeyer SC, Sullivan KM, et al. Rapidly progressive air-flow obstruction in marrow transplant recipients. Possible association between obliterative bronchiolitis and chronic graft-versus-host disease. *Am Rev Respir Dis* 1984;129:641–644.
154. Sullivan KM, Agura E, Anasetti C, et al. Chronic graft-versus-host disease and other late complications of bone marrow transplantation. *Semin Hematol* 1991;28:250–259.
155. King TE Jr. Overview of bronchiolitis. *Clin Chest Med* 1993;14:607–610.
156. Crawford SW, Clark JG. Bronchiolitis associated with bone marrow transplantation. *Clin Chest Med* 1993;14:741–749.
157. Cooper JD, Billingham M, Egan T, et al. A working formulation for the standardization of nomenclature and for clinical staging of chronic dysfunction in lung allografts. International Society for Heart and Lung Transplantation. *J Heart Lung Transplant* 1993;12:713–716.
158. Ooi GC, Peh WC, Ip M. High-resolution computed tomography of bronchiolitis obliterans syndrome after bone marrow transplantation. *Respiration* 1998;65:187–191.
159. Bankier AA, Van Muylem A, Knoop C, et al. Bronchiolitis obliterans syndrome in heart-lung transplant recipients: diagnosis with expiratory CT. *Radiology* 2001;218:533–539.
160. Beinert T, Dull T, Wolf K, et al. Late pulmonary impairment following allogeneic bone marrow transplantation. *Eur J Med Res* 1996;1:343–348.
161. Depledge MH, Barrett A, Powles RL. Lung function after bone marrow grafting. *Int J Radiat Oncol Biol Phys* 1983;9:145–151.
162. Gore EM, Lawton CA, Ash RC, et al. Pulmonary function changes in long-term survivors of bone marrow transplantation. *Int J Radiat Oncol Biol Phys* 1996;36:67–75.
163. Tait RC, Burnett AK, Robertson AG, et al. Subclinical pulmonary function defects following autologous and allogeneic bone marrow transplantation: relationship to total body irradiation and graft-versus-host disease. *Int J Radiat Oncol Biol Phys* 1991;20:1219–1227.
164. Belperio JA, Keane MP, Burdick MD, et al. Critical role for the chemokine MCP-1/CCR2 in the pathogenesis of bronchiolitis obliterans syndrome. *J Clin Invest* 2001;108:547–556.
165. Belperio JA, DiGiovine B, Keane MP, et al. Interleukin-1 receptor antagonist as a biomarker for bronchiolitis obliterans syndrome in lung transplant recipients. *Transplantation* 2002;73:591–599.
166. Elssner A, Jaumann F, Dobmann S, et al. Elevated levels of interleukin-8 and transforming growth factor-beta in bronchoalveolar lavage fluid from patients with bronchiolitis obliterans syndrome: proinflammatory role of bronchial epithelial cells. Munich Lung Transplant Group. *Transplantation* 2000;70:362–367.
167. El-Gamel A, Sim E, Hasleton P, et al. Transforming growth factor beta (TGF-beta) and obliterative bronchiolitis following pulmonary transplantation. *J Heart Lung Transplant* 1999;18:828–837.
168. Piguet P, Collart M, Grau G, et al. Tumor necrosis factor/cachectin plays a key role in bleomycin-induced pneumopathy and fibrosis. *J Exp Med* 1989;170:655–663.
169. Piguet P, Vesin C. Treatment by human recombinant soluble TNF receptor of pulmonary fibrosis induced by bleomycin or silica in mice. *Eur Respir J* 1994;7:515–518.
170. Reynaud-Gaubert M, Thomas P, Gregoire R, et al. Clinical utility of bronchoalveolar lavage cell phenotype analyses in the postoperative monitoring of lung transplant recipients. *Eur J Cardiothorac Surg* 2002;21:60–66.
171. Leonard CT, Soccal PM, Singer L, et al. Dendritic cells and macrophages in lung allografts: a role in chronic rejection? *Am J Respir Crit Care Med* 2000;161:1349–1354.
172. Ward C, Whitford H, Snell G, et al. Bronchoalveolar lavage macrophage and lymphocyte phenotypes in lung transplant recipients. *J Heart Lung Transplant* 2001;20:1064–1074.
173. Szeto WY, Krasinskas AM, Kreisel D, et al. Donor antigen-presenting cells are important in the development of obliterative airway disease. *J Thorac Cardiovasc Surg* 2000;120:1070–1077.
174. Yamada A, Konishi K, Cruz GL, et al. Blocking the CD28-B7 T-cell costimulatory pathway abrogates the development of obliterative bronchiolitis in a murine heterotopic airway model. *Transplantation* 2000;69:743–749.

175. Shankar G, Cohen DA. Idiopathic pneumonia syndrome after bone marrow transplantation: the role of pre-transplant radiation conditioning and local cytokine dysregulation in promoting lung inflammation and fibrosis. *Int J Exp Pathol* 2001;82:101–113.
176. Coker R, Laurent G. Pulmonary fibrosis: cytokines in the balance. *Eur Respir J* 1998;11:1218–1221.
177. Ortiz L, Lasky J, Lungarella G, et al. Upregulation of the p75 but not the p55 TNFα receptor mRNA after silica and bleomycin exposure and protectin from lung injury in double receptor knockout mice. *Am J Respir Cell Mol Biol* 1999;20:825–833.
178. Miyazaki Y, Araki K, Vesin C, et al. Expression of a tumor necrosis factor-α transgene in murine lung causes lymphocytic and fibrosing alveolitis. *J Clin Invest* 1995;96:250–259.
179. Payne L, Chan CK, Fyles G, et al. Cyclosporine as possible prophylaxis for obstructive airways disease after allogeneic bone marrow transplantation. *Chest* 1993;104:114–118.
180. Whitford H, Walters EH, Levvey B, et al. Addition of inhaled corticosteroids to systemic immunosuppression after lung transplantation: a double-blind, placebo-controlled trial. *Transplantation* 2002;73:1793–1799.
181. Rabitsch W, Deviatko E, Keil F, et al. Successful lung transplantation for bronchiolitis obliterans after allogeneic marrow transplantation. *Transplantation* 2001;71:1341–1343.
182. Xun CQ, Thompson JS, Jennings CD, et al. Effect of total body irradiation, busulfan–cyclophosphamide, or cyclophosphamide conditioning on inflammatory cytokine release and development of acute and chronic graft-versus-host disease in H-2-incompatible transplanted SCID mice. *Blood* 1994;83:2360–2367.
183. O'Garra A, Murphy K. Role of cytokines in determining T-lymphocyte function. *Curr Opin Immunol* 1994;6:458–466.
184. Swain SL, Weinberg AD, English M, et al. IL-4 directs the development of Th2-like helper effectors. *J Immunol* 1990;145:3796–3806.
185. Swain SL, Bradley LM, Croft M, et al. Helper T-cell subsets: phenotype, function and the role of lymphokines in regulating their development. *Immunol Rev* 1991;123:115–144.