Mini review

Acute lung injury after allogeneic stem cell transplantation: is the lung a target of acute graft-versus-host disease?

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Summary:

Allogeneic hematopoietic stem cell transplantation (SCT) is an important therapeutic option for a number of malignant and nonmalignant conditions but the broader application of this treatment strategy is limited by several side effects. In particular, diffuse lung injury is a major complication of SCT that responds poorly to standard therapeutic approaches and significantly contributes to transplant-related morbidity and mortality. Historically, approximately 50% of all pneumonias seen after SCT have been secondary to infection, but the judicious use of broad-spectrum antimicrobial prophylaxis in recent years has tipped the balance of pulmonary complications from infectious to noninfectious causes. This mini review will discuss the definition, risk factors and pathogeneses of noninfectious lung injury that occurs early after allogeneic SCT.

Over the last several decades, allogeneic hematopoietic stem cell transplantation (SCT) has emerged as an important therapeutic option for a number of malignant and nonmalignant conditions. Unfortunately, this treatment strategy is limited by several side effects including pulmonary toxicity. Diffuse lung injury is a major complication of SCT that occurs in 25–55% of SCT recipients and can account for approximately 50% of transplant-related mortality.[1–6] Noninfectious lung injury can be either acute or chronic depending on the time of onset after SCT and the tempo of disease progression. Historically, approximately 50% of all pneumonias seen after SCT have been secondary to infection, but the judicious use of broad-spectrum antimicrobial prophylaxis in recent years has tipped the balance of pulmonary complications from infectious to noninfectious causes.[7] Importantly, noninfectious lung injury is associated with significant morbidity and mortality and responds poorly to standard therapeutic approaches. This mini review will discuss the definition, risk factors and pathogeneses of noninfectious lung injury that occurs early after allogeneic SCT.

Acute lung injury: idiopathic pneumonia syndrome (IPS)

Definition and clinical course

In 1993, a panel convened by the NIH defined widespread alveolar injury following SCT that occurs in the absence of an active lower respiratory tract infection and cardiogenic causes as idiopathic pneumonia syndrome (IPS).[8] The panel was careful to stress that they considered this definition to be a clinical syndrome with variable histopathologic correlates and several potential etiologies.[8] As shown in Table 1, diagnostic criteria of IPS include signs and symptoms of pneumonia, nonlobar radiographic infiltrates, abnormal pulmonary function and the absence of infectious organisms as determined by broncho-alveolar lavage (BAL) or lung biopsy.[2,8] Histopathologic findings associated with IPS include diffuse alveolar damage with hyaline membranes and lymphocytic bronchitis and bronchiolitis obliterans organizing pneumonia (BOOP). However, the most frequently reported pattern is interstitial pneumonitis, a term historically used interchangeably with IPS.[3,9,10] Interstitial pneumonitis is seen in association with diffuse alveolar damage and hemorrhage early after SCT and is accompanied by bronchiolar inflammation and epithelial damage at later time points.[10]

Several studies have reported the incidence of IPS in the first 120 days after allogeneic SCT with myeloablative conditioning to be 3–15% (Table 2).[3,5] The median time of onset for IPS was initially reported to be 6–7 weeks (range 14–90 days) after the infusion of donor stem cells.[8] Mortality rates ranged from 60 to 80% overall, and were greater than 95% for patients requiring mechanical

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ventilation.[1,3,5–8,11] A retrospective study from Seattle showed a lower incidence and earlier onset of IPS than previously reported, but the typical clinical course involving the rapid onset of respiratory failure leading to death remained unchanged.[6] A recent review from the University of Michigan Medical Center demonstrated that the frequency of IPS after allogeneic SCT ranged from 5 to 25% depending upon the donor source and the degree of antigenic mismatch between donor and recipient.[12] The median time for development of IPS at Michigan was 14 days after transplant, and the overall day 100 mortality was 80%. Of note, the median time to death from diagnosis of IPS was 13 days despite aggressive treatment with high-dose steroids and broad-spectrum antimicrobial therapy.[12]

The definition of IPS encompasses various forms of pulmonary toxicity (Table 3). In one small subset of patients with IPS, acute pulmonary hemorrhage or hemorrhagic alveolitis occurs. Diffuse alveolar hemorrhage (DAH) generally develops in the immediate post-SCT period and is characterized by progressive shortness of breath, cough and hypoxemia with or without fever.[7,13–15] Progressively bloodier aliquots of BAL fluid have traditionally diagnosed DAH, but frank hemoptysis is rare.[13]

Table 1 Definition of idiopathic pneumonia syndrome

Evidence of widespread lung injury
(a) Multilobar infiltrates on chest radiograph or computed tomography
(b) Signs and symptoms of pneumonia (cough, dyspnea, rales)
(c) Abnormal pulmonary physiology: increased alveolar to arterial oxygen gradient; or new or increased restrictive lung findings

Absence of lower respiratory tract infection based upon
(a) Broncho-alveolar lavage negative for bacterial pathogens and/or lack of improvement with broad-spectrum antibiotics
(b) Broncho-alveolar lavage negative for pathogenic non-bacterial microorganisms
(c) Transbronchial lung biopsy if condition permits
(d) Ideally, second confirmatory negative test for infection 2–14 days following the initial procedure[8]

Sloan et al[16] identified acute hemorrhagic pulmonary edema (the histologic correlate of DAH) in a subset of SCT recipients at autopsy, 80% of which had received a non-HLA identical SCT and had previous acute graft-versus-host disease (GVHD). Similarly, a Hopkin's report showed that death from pulmonary hemorrhage was associated with grade II or greater GVHD.[17] Mortality from DAH is as high as 75% despite aggressive treatment with high-dose (2 mg/kg to 1 gm/m^2) steroids, and death usually occurs within 3 weeks of diagnosis.[14]

Periengraftment respiratory distress syndrome (PERDS) and delayed pulmonary toxicity syndrome (DPTS) are also included within the definition of IPS.[7] PERDS and DPTS typically occur after autologous SCT,[7] and both are characterized by fever, dyspnea and hypoxemia.[18–20] By definition, PERDS occurs within 5 days of engraftment, whereas the onset of DPTS may be delayed for months and commonly occurs following conditioning regimens that contain cyclophosphamide, cisplatin and bischloroethylinitrosurea (BCNU) as used in SCT for breast cancer.[20] Although PERDS after autologous SCT appears similar to IPS after allogeneic SCT with respect to clinical presentation and time of onset, the two entities differ sharply with respect to overall outcome, and this difference is directly related to the clinical context within which lung injury is observed. Injury from PERDS after autologous SCT or non-myeloablative SCT with significant *in vivo* T cell depletion, even when requiring mechanical support, typically responds promptly to corticosteroids and is associated with a favorable prognosis.[18,21,22] By contrast, PERDS occurring after unmanipulated allogeneic SCT responds poorly to standard therapy and is associated with significant morbidity and mortality.[21,23] This difference in mortality is clearly exemplified in a recent report by Cahill and co-workers; although a noncardiogenic capillary leak syndrome (CLS) was identified with similar frequency following autologous and allogeneic SCT, mortality was significantly higher in allo-SCT recipients (61 *vs* 27%). In

Table 2 Incidence and mortality of idiopathic pneumonia syndrome

Author	Autologous[a] (n)	Allogeneic[a] (n)	Incidence (%)	Median age (range)	Median onset[b] (days)	Time to death[c] (days)	Mortality[d] (%)	TBI[e]
Pecago et al[121]	70	—	4.3	27 (4–61)	27	NS[h]	NS	Yes
Granena et al[122]	73	230	7.4	25 (4–51)	63	NS	83	Yes
Kantrow et al[6]	209	956	7.7	NS	21	NS	74	Yes
Meyers et al[25]	—	525	12.0	NS	42	NS	60	Yes
Weiner et al[3]	—	923	78	21 (1–59)	52	NS	78	Yes
Wingard et al[9]	—	386	14.7	21 (0–50)	39	NS	82	Yes
Crawford and Heckman[2]	—	41	NS	25 (1–58)	35	148	71	Yes
Yanik et al[12]	—	651	9.0	36 (1–61)	15	26	74	No
Fukuda et al[28]	—	183[f]	2.2	53 (1–73)	16	15	75	Yes
Fukuda et al[28]	—	917[g]	8.4	41 (1–66)	22	14	74	Yes

[a]Number of patients analyzed.
[b]Number of days post transplant.
[c]Number of days post onset of IPS.
[d]Mortality listed as IPS-related mortality, or mortality within 56 days of onset of IPS.
[e]TBI administered to >50% of patients.
[f]Nonmyeloablative conditioning regimen.
[g]Myeloablative conditioning regimen.
[h]NS = not stated.

Table 3 The spectrum of noncardiogenic, pulmonary toxicity defined by IPS

Interstitial pneumonitis (IP)
- Clinical symptoms: fever, cough, dyspnea, hypoxemia
- Onset: within first 100 days post transplant
- Etiology: infectious (ie CMV, PCP) or noninfectious factors (chemotoxicity: BCNU, bleomycin, busulfan, methotrexate)
- Radiographic findings: bilateral interstitial infiltrates

Bronchiolitis obliterans syndrome (BOS)
- Clinical symptoms: cough, dyspnea, wheezing, lack of fever
- Pulmonary function testing: obstructive findings (diminished $FEV_{1.0}$, or $FEV_{1.0}/FVC$)
- Onset: 3–24 months post transplant.
- Radiographic findings: hyperinflation. Otherwise routinely normal
- Computed tomography (CT): bronchiectasis, centrilobular nodules, septal lines, ground glass appearance
- Histology: lymphocytic bronchitis. Bronchiolar inflammation with luminal obliteration

Bronchiolitis obliterans organizing pneumonia (BOOP)
- Clinical symptoms: fever, dry cough, dyspnea
- Onset: 2–12 months post transplant
- Radiographic findings: patchy airspace disease, ground glass appearance, nodular opacities
- Histology: peribronchiolar infiltration and fibrosis and the presence of intraluminal granulation tissue

Diffuse alveolar hemorrhage (DAH)
- Clinical symptoms: progressive dyspnea, cough, rare hemoptysis
- Key finding: progressively bloodier aliquots of lavage fluid
- Onset: early, within first 100 days post transplant
- Radiographic findings: diffuse infiltrates, central appearance initially noted
- Histology: diffuse alveolar damage with alveolar hemorrhage

Periengraftment respiratory distress syndrome (PERDS)
- Clinical symptoms: fever, dyspnea, hypoxemia
- Onset: very early, within 5–7 days of engraftment, classically after autologous SCT
- Radiographic findings: bilateral interstitial infiltrates

Delayed pulmonary toxicity syndrome (DPTS)
- Clinical symptoms: fever, dry cough, dyspnea
- Onset: late, months to years following autologous SCT for breast cancer
- Responds to corticosteroid therapy

Noncardiogenic capillary leak syndrome (CLS)
- Clinical symptoms: dyspnea, cough, weight gain, edema
- Onset: early, within first 30 days post transplant
- Radiographic findings: bilateral perihilar infiltrates, pulmonary edema, pleural effusions

Table 4 Risk factors for idiopathic pneumonia syndrome

GVHD prophylaxis (methotrexate)[3]
Acute GVHD (grades II–IV)[2,25]
Acute GVHD (grade IV)[3,6]
Increasing recipient age (≥21 years)[3,25] (>40 years)[28]
Total body irradiation (TBI) ≥1200 cGy[2,3,28]; dose rate of
TBI (≥6 Cgy/min)[3]
Myeloablative conditioning
High-dose 1-3 bis chloroethyl-1 nitrosurea[25]
Decreased pre-transplant performance status[3]
Longer duration from diagnosis to transplant[3]
Transplantation for malignancy other than leukemia[6]
Transplantation for hematologic malignancy[9]
HLA disparity (donor:recipient)[12]

addition, CLS was diagnosed in eight of 10 patients receiving mismatched or URD SCT, and was associated with acute GVHD in 11 of 18 (62%) of patients, overall suggesting a contribution of a graft-versus-host immunologic response.

Risk factors of IPS

Risk factors for IPS have included conditioning with total body irradiation (TBI), acute GVHD, older recipient age, initial diagnosis of malignancy other than leukemia and the use of methotrexate (MTX) for GVHD prophylaxis.[5,9,24,25] (Table 4). Moreover, the likelihood of developing IPS increases with the number of identified risk factors.[3]

Although recipient age and the use of MTX are not always risk factors, the use of TBI and the development of acute GVHD have been identified as factors in multiple studies.[2,5,6,25–27] Fukuda et al[28] have recently compared the incidence and outcome of IPS among patients who underwent allogeneic SCT after nonmyeloablative (n = 183) and conventional (n = 917) conditioning. The cumulative incidence of IPS was significantly lower at 120 days following nonmyeloablative conditioning than after conventional conditioning (2.2 vs 8.4%; P = 0.003). However, once diagnosed, IPS progressed rapidly and was associated with a high mortality rate (75%) despite aggressive support in both subsets of patients. Grades III–IV acute GVHD remained prognostic for IPS after adjusting for other risk factors, confirming previous reports and supporting the hypothesis that the lung may be a target of graft-versus-host reaction. Importantly, other risk factors are likely to be involved because even though acute GVHD occurred with similar frequency in the two study populations, the incidence of IPS was lower after nonmyeloablative conditioning. In addition, greater patient age (>40 years) and diagnosis of acute leukemia or MDS were associated with significantly increased risks for IPS. Among the older patients who received conventional conditioning, high-dose (≥12 Gy) TBI was associated with an increased risk for IPS than were non-TBI-based regimens (16 vs 5.8%; P = 0.001). Although there are limitations to the conclusions that can be drawn from this study, the results suggest nonmyeloablative or targeted dose chemotherapy-based myeloablative regimens may reduce the risk of IPS in patients aged 40 years or older. Moreover, the findings suggest that the intensity of SCT conditioning plays an important role in the development of IPS, and they are consistent with data generated from two mouse SCT models showing that the lung is sensitive to the combined effects of radiation and alloreactive T cells.[29,30]

Etiology of IPS

As shown in Table 5, potential etiologies for IPS are several and include direct toxic effects of SCT conditioning regimens, occult pulmonary infections and the release of inflammatory cytokines that have been implicated in other forms of pulmonary injury.[31–35] Other immunologic factors may also be important as suggested by the association of

Table 5 Possible etiologic factors contributing to IPS

Toxicity effects of chemoradiotherapy
Occult pulmonary infections

Immunologic dysregulation
- Enhanced pro-inflammatory cytokine/chemokine expression (TNFα)
- Release of endogenous endotoxin (LPS)
- Donor-derived cellular effectors
  Alloreactive T cells
  Mononuclear cells/macrophages
  Neutrophils
Endothelial cell apoptosis/activation

IPS and severe GVHD in several large series.[2,3,5–7,11] Moreover acute GVHD often precedes IPS, suggesting a possible causal relationship between the two disorders.[5,9,36,37] Although the lung is not recognized as a classic target organ of GVHD, the clinical association between lung injury and GVHD, along with the demonstration of pathologic lung changes in rodents with acute GVHD, make this possibility intriguing.[2,3,5,6,38–42]

The role of GVHD and specifically of alloreactive donor T lymphocytes in the pathogenesis of IPS remains a topic of considerable debate. Epithelial apoptosis is usually attributed to T cell-mediated injury and is considered pathognomonic for acute GVHD. Although observed in the lungs of some patients with IPS,[10] epithelial apoptosis has not been consistently identified in allogeneic SCT recipients with pulmonary dysfunction.[16,37,43,44] Lymphocytic bronchitis was, however, reported as a potential histopathologic correlate of GVHD of the lung.[37] This pattern was initially observed in allogeneic SCT recipients with GVHD but not in patients receiving autologous SCT or in untransplanted controls, but this association was not confirmed in subsequent reports.[16,43,44] More recently, Yousem[10] described the histologic spectrum of pulmonary GVHD in 17 allogeneic SCT recipients. Findings ranged from diffuse alveolar injury early after SCT to cicatrical bronchiolitis obliterans, which represented a late and irreversible form of lung injury. In this report, bronchitis/bronchiolitis with interstitial pneumonitis (BIP) was the most common finding and included a lymphocytic infiltration around bronchial structures along with a mononuclear inflammation in the perivascular zones and alveolar septa.

In contrast to epithelial apoptosis, our group has recently demonstrated that pulmonary histopathology after experimental allogeneic SCT is accompanied by significant apoptosis of the pulmonary vascular endothelium[45] Endothelial cell (EC) injury has been observed after allogeneic BMT and has been implicated as a direct contributor to the development of several complications including GVHD, veno-occlusive disease (VOD) and endothelial leak syndrome (ELS).[46,47] In our studies, EC apoptosis coincided with the onset of pulmonary pathology, was associated with elevations in broncho-alveolar lavage (BAL) fluid tumor necrosis factor alpha (TNFα) levels, and was accompanied by evidence for EC activation. Moreover, administration of a soluble TNFα binding protein (rhTNFR:Fc) from week 4 to week 6 after allogeneic

BMT significantly reduces EC apoptosis and lung histopathology observed in this setting.[45]

The heterogeneity of pulmonary histopathology after allogeneic SCT is complicated further by the nonspecific changes that occur after mechanical ventilation and by the limited quality and quantity of lung biopsy tissue. Despite the lack of classic acute GVHD histopathology, several lines of evidence suggest that the lung is a target of immunologically mediated damage after allogeneic SCT.

The pathogenesis of IPS

Murine models of lung injury after allogeneic SCT

Several laboratories have explored the relationship between alloreactivity and IPS in rodent SCT models and have consistently shown that animals with systemic GVHD develop lung injury, whereas those receiving syngeneic SCT do not (Figure 1).[38,41,48,49] Even under tightly controlled experimental conditions, several patterns of lung injury have emerged, including acute hemorrhagic alveolitis, late onset interstitial pneumonitis and lymphocytic bronchiolitis 38. In several models where the GVH reaction is induced to (1) minor H antigens, (2) class I or class II MHC antigens only or (3) both major and minor H antigens, two major abnormalities are apparent after allogeneic SCT: a dense mononuclear cell infiltrate around both pulmonary vessels (Figure 1c) and bronchioles (Figure 1d) and an acute pneumonitis involving the interstitium and alveolar spaces
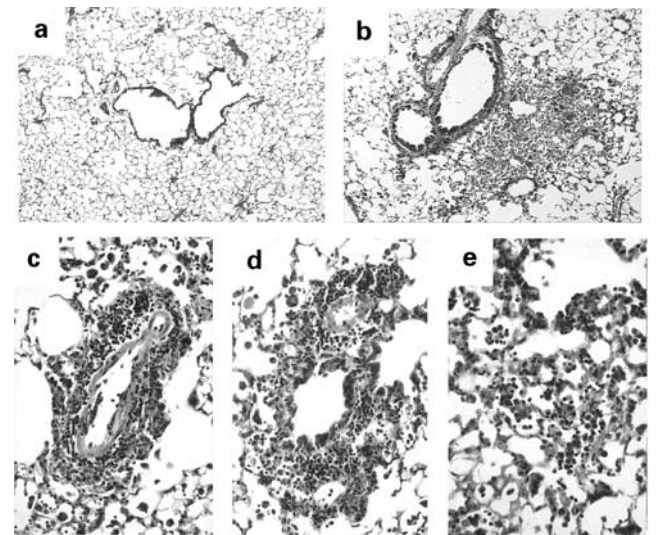

Figure 1 IPS pathology. Following lethal irradiation, mice received hematopoietic SCT from either allogeneic (MHC matched) or syngeneic donors. Lungs were harvested and prepared for microscopic analysis. At 6 weeks, lungs of mice receiving syngeneic SCT maintain virtually normal histology (**a**), whereas allogeneic SCT recipients develop significant lung histopathology (**b–e**). Two major abnormalities are apparent after allogeneic SCT. First, dense mononuclear cell infiltrates are observed around both pulmonary vessels (**c**) and bronchioles (**d**). Second, an acute pneumonitis is present involving both the interstitial and alveolar spaces (**e**). The alveolar infiltrate is composed of macrophages, lymphocytes, epithelial cells and scattered polymorphonuclear cells within a fibrin matrix. Original magnification: × 200 (**a, b**); × 400 (**c–e**).

(Figure 1e).[41,50,51] The alveolar infiltrate is composed of macrophages, lymphocytes, epithelial cells and scattered polymorphonuclear cells within a fibrin matrix. Both of these histopathologic patterns closely resemble those of the nonspecific, diffuse interstitial pneumonias seen in allogeneic SCT recipients.[8,10,16,37] Evidence for diffuse alveolar injury including alveolar hemorrhage, edema and hyaline membranes has not however been demonstrated in these models.

In studies where it has been measured, pulmonary function is significantly decreased in animals with IPS, demonstrating that the observed lung pathology is physiologically relevant.[42,49] In one report, mice with IPS showed significant reductions in both dynamic compliance and airway conductance compared with syngeneic controls, consistent with changes expected from both the interstitial and peribronchial infiltrates respectively.[42] Lung injury correlated with the presence but not the severity of GVHD in that study, consistent with clinical reports of IPS in allogeneic SCT recipients whose signs and symptoms of GVHD were mild or absent.[12,52–56]

The inflammatory effectors TNFα and lipopolysaccharide (LPS)

The mixed inflammatory alveolar infiltrates found in mice with IPS are accompanied by significant increases in the number of total number of cells, lymphocytes, macrophages and neutrophils in the broncho-alveolar compartment.[41] These infiltrates are also associated with increased TNFα in both lung tissue and BAL fluid.[39–41,57,58] A causal role for TNFα in the development of IPS has been established by neutralizing this cytokine in experimental SCT models.[57,58] Administration of a soluble, dimeric, TNF binding protein (rhTNFR:Fc; Immunex Corp. Seattle, WA) from week 4 to week 6 after SCT reduced the progression of lung injury during this time period.[58] Moreover, the use of mutant mice, deficient in TNFα, has shown that lung injury after allogeneic SCT is dependent upon donor, rather than host-derived TNFα and that cytokine production from both donor accessory cells (macrophage/monocytes) and T cells significantly contributes to this toxicity.[59] The incomplete protection provided by abrogating the effects of TNFα after SCT by either strategy is consistent with reports from many groups[38,57,60–64] and suggests that other inflammatory and cellular mechanisms such as the Fas-FasL pathway that mediate acute GVHD may also contribute to the development of IPS.[50,62,65,66] For example, IL-1β, TGFβ and nitrating species including nitric oxide and peroxynitrite have also been implicated in the development of IPS, particularly when cyclophosphamide is included in the conditioning regimen.[49,67,68]

Increases in neutrophils and TNFα in the absence of infection suggested that endogenous endotoxin (LPS) might play an important role in IPS pathophysiology. LPS is a component of the innate immune response and is a potent enhancer of inflammatory cytokine release. In non-SCT experimental models, intratracheal administration of LPS elicits a severe, acute inflammatory response in the lungs of animals.[69–71] Recent work has also demonstrated

that LPS is an important effector molecule in the development of acute GVHD; translocation of LPS across a gut mucosa damaged early in the post transplant period provides access to the systemic circulation where it stimulates leukocytes to release inflammatory mediators that subsequently contribute to GVHD target organ damage and dysfunction.[60,61,72–76] LPS levels are elevated in the BAL fluid of mice with IPS, and LPS stimulates the release of inflammatory cytokines that directly contribute to lung damage: intravenous LPS injection 6 weeks after allogeneic SCT significantly amplifies lung injury.[41] This amplification is only seen in mice with advanced GVHD and is associated with large increases in BAL fluid levels of TNFα and LPS, and the development of alveolar hemorrhage.[41,58]

Collectively, these data demonstrate that the inflammatory mediators TNFα and LPS both contribute to experimental IPS. Moreover, they support the hypothesis of a 'gut–liver–lung' axis of inflammation in IPS pathophysiology (Figure 2). Any process that ultimately results in large amounts of endotoxin and/or TNFα in the pulmonary circulation may contribute to the development of lung injury in this setting. This hypothesis is consistent with the observation that serum TNFα levels are increased in patients with IPS.[77] A clinical linkage of hepatic dysfunction to lung injury after SCT is also suggested by associations between VOD and IPS and between hepatic failure and death from IPS.[2,12] Furthermore, evidence for cytokine activation and LPS amplification observed in the BAL fluid of ARDS patients[78] has been demonstrated in patients with IPS after allogeneic SCT; increased pulmonary vascular permeability and increases in BAL fluid levels of IL-1, IL-12, IL-6, TNFα, LPS binding protein (LBP) and soluble CD14 were also observed in these patients.[1]

The role of donor-derived T cell effectors

Although the induction of GVHD fundamentally depends upon interactions between donor T cells and host antigen-presenting cells (APCs),[79] the role of alloreactive donor T cells in the pathogenesis of IPS has been a topic of considerable debate. Pulmonary endothelial and epithelial cells can express MHC Class I, MHC Class II and minor histocompatibility (H) antigens, and the expression of these molecules on vascular endothelium is enhanced by TNFα and IFNγ.[80] It is conceivable, therefore, that pulmonary parenchymal cells can serve as targets for direct cell-mediated damage. The importance of lymphocytes to lung injury after experimental SCT has been shown by several groups.[39,49,81,82] Donor T cells are critical to the early proinflammatory events associated with lung injury that develops within the first week of SCT across MHC antigens, whereas in minor H antigen mismatch systems, donor lymphocytes continue to respond to host antigens and contribute to physiologically significant lung injury at later time points.[42,49] Donor T cell clones that recognize CD45 polymorphisms result in a rapidly progressive pulmonary vasculitis within 3 days of their injection into nonirradiated recipients.[39,83] The origin and functional capacity of T cells infiltrating the lung have been examined by using differences in the T cell Vβ repertoire between
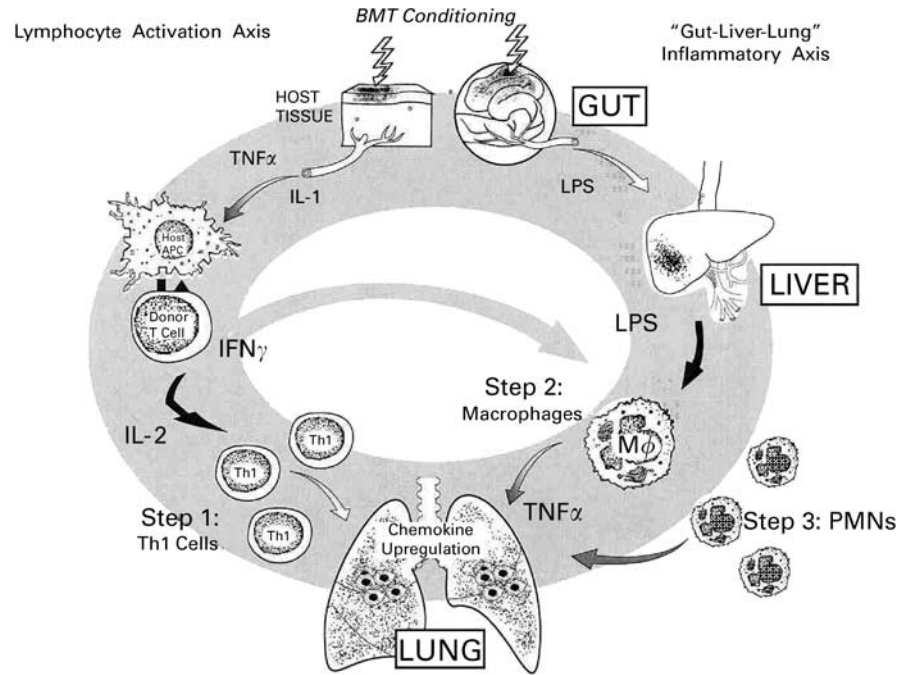
Figure 2 Pathophysiology of IPS after allogeneic SCT. Data generated using murine SCT models have been incorporated into a working hypothesis of IPS physiology. This schema postulates that the lung is susceptible to two pathways of immune-mediated injury that occur along a T-lymphocyte activation axis and an inflammatory cytokine axis. The lymphocyte activation pathway fundamentally depends upon interactions between donor T cells and host APCs, whereas the inflammatory cytokine pathway focuses on the relationship between the cellular activating effects of LPS and the downstream production of TNFα as it occurs along a gut–liver–lung axis of inflammation. Although distinct, these two pathways are inter-related; donor macrophages, primed by IFNγ that is produced by activated T cells, are recruited to the lung where they are triggered by LPS to secrete inflammatory cytokines like TNFα, resulting in enhanced chemokine expression, the recruitment of neutrophils to the lung and increased tissue damage.

donor and recipient.[42] Flow cytometry demonstrated that the TCRαβ+ T cells found in the lung 6 weeks after allogeneic SCT were of donor origin. When these donor T cells were recultured with irradiated host APCs, they proliferated vigorously and produced significant amounts of IFNγ.[42] These experimental data support the hypothesis that the alveolar lymphocytosis associated with IPS after clinical SCT represents a pulmonary manifestation of GVHD.[84]

Despite these compelling experimental data supporting a role for alloreactive donor lymphocytes in the development of IPS, noninfectious lung injury has been reported in patients in whom systemic GVHD is mild or absent, making a causal relationship between the two entities difficult to establish.[10,52–56] The relationship between lung injury and GVHD severity has been examined in an SCT model across minor H antigens. T cell depletion (TCD) at the time of allogeneic SCT reduced the number of T cells by greater than 99% and eliminated evidence of clinical or histologic GVHD. Nevertheless, significant lung injury was noted after allogeneic TCD SCT, and donor lymphocytes reactive to host antigens were present in the BAL fluid, but not the spleens, of these animals.[42] This intriguing result suggested that the lung may be particularly sensitive to the effects of small numbers of host-reactive donor T cells even when systemic tolerance has been established. Consistent with these findings, 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.[85] Furthermore, pulmonary toxicity has been reported after nonconditioned allogeneic SCT for severe combined immunodeficiency (SCID) where donor lympho-cytes were noted in the lung during a period of rapid engraftment without evidence of systemic GVHD. Suppres-sion of cellular immunity with high-dose methylpredniso-lone resulted in complete resolution of lung disease.[86]

The role of APCs

Experimental and clinical data suggest that donor-derived, IFNγ-secreting T cells home to the lungs early after SCT, persistently respond to host antigens and can cause clinically and histologically significant tissue injury even when systemic GVHD is limited or absent. However, 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 involve interactions with pulmonary APCs.[87,88] Pulmonary dendritic cells 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 of lung allografts.[89–92] The necessity of host APCs for the genera-tion of acute GVHD has been demonstrated in a CD8+ T cell driven GVHD model.[79] These results were recently extended by Teshima *et al*,[93] who showed that alloantigen expression on host APCs alone is both necessary and sufficient to induce a graft-versus-host reaction and that

GVHD target organ damage can be mediated by inflammatory cytokines. Radioresistant host DCs may persist longer in the lung than in other organs and thus may allow for sustained presentation of host antigens in that organ. In experiments using congenic rats, host DC populations in tracheal epithelium were depleted by 80% 3 days after 1000 cGy of TBI and were completely eliminated 14 days after syngeneic SCT. By contrast, lung parenchymal DCs were only reduced by 50% at day 3 and 60% by day 6 and declined at a rate that was intermediate between airway and epidermal DC populations.[94] Activated donor T cells that can cause progressive lung injury might therefore remain within the pulmonary microvascular circulation because persistent host DCs function as a continuing site of alloantigen presentation. This scenario could account for the apparent 'sanctuary' status of the lung with respect to alloreactive donor T cells and may have important implications with regard to the evaluation and treatment of IPS after allogeneic SCT even when clinical GVHD is absent.

The role of donor accessory cells

A significant body of experimental data suggests that synergistic interactions between cells from the lymphoid and myeloid lineage are critical to the development of GVHD.[61,73,74] The contribution of donor accessory cells (monocytes/macrophages) to IPS has been investigated using several models. Kinetic studies of macrophage recruitment to the lung after allogeneic SCT show that the percentage of donor macrophages in the BAL fluid increases from approximately 40% at week 1 to >90% by week 4. Additional experiments showed that these donor-derived macrophages are major sources of TNFα after SCT.[59] The role of accessory cell populations in the pathophysiology of IPS was further examined using SCT donors that differ in their response to LPS.[95] A genetic mutation in the Toll-like receptor 4 (Tlr 4) gene makes C3H/Hej mice resistant to LPS (LPS-r).[95–98] SCT from LPS-r donors results in a significant decrease in lung injury when compared to SCT using wild-type, LPS-sensitive (LPS-s) donors even though T cell responses to host antigens are identical between the two donor strains.[61] BAL cells collected from LPS-r recipients produce 30-fold less TNFα when restimulated with LPS compared to cells collected after LPS-s SCT, reproducing the phenotype of naïve LPS-r and LPS-s donor cells.[58] Lung injury is also reduced when animals deficient in CD14, a cell surface receptor that is critical to the innate immune response and is an important receptor for LPS, are used as donors.[99] These results are consistent with the report that monocytes recruited to an inflamed lung upregulate CD14 expression and show enhanced sensitivity to LPS stimulation[100] and with the clinical observation that components of the LPS activating system are elevated in the BAL fluid of SCT patients with IPS.[1] Collectively, these data demonstrate that donor macrophages/monocytes cells are recruited to the lungs of allogeneic SCT recipients, and their ability to secrete TNFα in response to LPS stimulation directly correlates with IPS severity. Strategies that disrupt the innate immune response by targeting interactions between

CD14/Tlr4 and LPS may, therefore, reduce the severity of IPS or prevent its development.

The role of neutrophils/polymorphonuclear (PMN) cells

Although not traditionally considered essential to the induction of GVHD, neutrophils are consistently identified in target tissues collected from both mice and humans.[61,93,101] Neutrophilia is also prominent finding in several forms of immune-mediated lung injury including acute respiratory distress syndrome (ARDS) and in bronchiolitis obliterans syndrome (BOS), characteristic of lung allograft rejection.[102–107] PMN products such as elastase, myeloperoxidase, metalloproteinases and oxidants are abundant in the BAL fluid of patients with ARDS and are believed to contribute to the endothelial and epithelial damage that occurs in this setting.[102,107] Furthermore, increases in PMN activation markers may also be early indicators of BOS after lung transplant.[104] Neutrophils are likely to play a role in lung injury after SCT as well. PMNs are a major component of the inflammatory infiltrates seen in animals with IPS,[41] and their appearance in the bloodstream is often temporally associated with lung injury in the clinical setting; more than 60% of patients diagnosed with IPS at the University of Michigan developed signs and symptoms of pulmonary dysfunction within 7 days of neutrophil engraftment.[12] In mouse IPS models, the influx of neutrophils into BAL fluid is prominent between weeks 4 and 6 after SCT and is associated with increases in BAL fluid levels of TNFα and LPS.[41,58] Neutralization of TNFα with rhTNFR:Fc during this time interval prevents the influx of neutrophils and reduces the progression of lung injury and dysfunction.[58] Administration of rhTNFR:Fc following the injection of LPS in mice with GVHD completely abrogates the influx of PMNs into the lungs and prevents further damage (including hemorrhage) underscoring the relationship between neutrophils, TNFα and LPS after allogeneic SCT. In sum, neutrophils may play a role in pulmonary toxicity incurred during IPS, thus further supporting the concept that components of the innate immune response significantly contribute to this process.

Mechanisms of leukocyte recruitment to the lung after allogeneic SCT

Cellular effectors play a significant role in development of IPS, but the molecular mechanisms by which white blood cells (WBCs) traffic to the lung and cause inflammation have yet to be determined. WBC migration to sites of inflammation is a complex process involving interactions between leukocytes and endothelial cells that are mediated by adhesion molecules, chemokines and their receptors.[108,109] The recruitment of leukocytes from the vascular space and into target tissue can be divided into four steps: (1) weak adhesion of WBCs to the vascular endothelium, (2) firm adhesion of WBCs to endothelial cells, (3) transmigration of leukocytes through the vascular wall and (4) migration of cells through the extracellular matrix along a chemotactic gradient. A subset of chemoattractant molecules called chemokines likely contribute to steps 2, 3 and 4 of this process as it relates to the recruitment of

leukocytes into GVHD target tissues. Chemokines secreted at the site of tissue inflammation are retained within the extracellular matrix and on the surface of the overlying endothelial cells.[110] Leukocyte rolling is facilitated by selectin molecules and brings WBCs into contact with chemokines present on the endothelial surface. Chemokine signaling activates leukocyte integrin molecules resulting in arrest and extravasation. Once through the vascular wall, the WBC enters the tissue space where it is exposed to an existing chemokine concentration gradient surrounding the inflammatory stimulus.

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,[111,112] 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 allogeneic 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 et al,[49] 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 weeks after SCT. Despite these findings, a mechanistic relationship between chemokines and recruitment of cells to the lung during IPS has not been fully elucidated. Although a role for macrophage inflammatory protein-1 alpha (MIP-1α) in the recruitment of leukocytes to the lung after SCT was noted in one report,[113] subsequent work by the same group suggested that the use of MIP-1$^{-/-}$ mice as allo-SCT donors exacerbated rather than reduced early lung injury.[114]

We have recently studied the contribution of CCR2 and its primary ligand monocyte chemoattractant protein-1 (MCP-1) to the development of IPS using a lethally irradiated parent→F1 mouse SCT model.[45] Compared to syngeneic controls, the pulmonary expression of MCP-1 and CCR2 mRNA was significantly increased after allo-SCT. Transplantation of CCR2-deficient (CCR2$^{-/-}$) donor cells resulted in a significant reduction in IPS severity compared to SCT with wild-type (CCR2$^{+/+}$) cells. The reduction in histopathology after CCR2$^{-/-}$ SCT was associated with decreased macrophages, CD8 + lymphocytes and levels of TNFα and TNFRI in the BAL fluid. Similar findings were observed when recipients of wild-type SCT were treated with polyclonal antibodies to MCP-1 from day 10 to 28 after transplant. Importantly, experimental data correlated with preliminary clinical findings; patients with IPS have elevated levels of MCP-1 in the BAL fluid at the time of diagnosis.

Treatment strategies for IPS after allogeneic SCT

Current standard treatment regimens for IPS include supportive care measures in conjunction with broad-spectrum antimicrobial agents with or without intravenous

Table 6 Therapeutic considerations for IPS

Supportive therapy
- Supplemental oxygen, mechanical ventilation
- Emperic broad-spectrum antimicrobial agents pending culture results
- Diuresis: furosemide ± thiazide diuretic
- Continuous veno-venous hemofiltration (CVVH)

Immunosuppressive therapy
- Corticosteroids (2 mg/kg/day)
- Investigational
     cytokine inhibitors, including anti-TNF agents.
     ? use of KGF as an agent to prevent epithelial/endothelial injury
     ? use of chemokine receptor antagonists

corticosteroids (Table 6).[6,12] Although anecdotal reports of responses to standard therapy are available, such responses are limited and the mortality of patients diagnosed with IPS remains unacceptably high.[7] Advances in supportive care including the early institution of continuous veno-venous hemofiltration may help to improve survival in some patients.[115] However, prospective studies addressing the treatment of IPS, including the specific use of steroids, are lacking in the literature and no agent or combination of agents has been proven superior. In light of the poor response rate to standard treatment, the lack of prospective treatment trials and preclinical and clinical data that suggest a potential role for TNFα in the development of noninfectious lung injury after SCT, etanercept (Enbrel, Amgen Corp., Thousand Oaks, CA, USA) was administered to three consecutive pediatric patients at the University of Michigan SCT program who met criteria for IPS.[12] In all three patients, BAL fluid was negative for infection, and pulmonary edema from fluid overload or cardiogenic factors was also ruled out prior to the administration of etanercept. Each patient received empiric broad-spectrum antimicrobial therapy and methylprednisolone (2 mg/kg/day) prior to and during etanercept therapy. The administration of etanercept was well tolerated, and in combination with standard immunosuppressive therapy it was associated with clearing of radiographic infiltrates (Figure 3) and significant improvements in pulmonary function within the first week of therapy.[12] Clinical trials using etanercept for the treatment of IPS are now ongoing.

Use of other noncrossreactive strategies may also hold promise in the future. As noted above, our group has demonstrated that EC apoptosis mediated by TNFα is associated with the pathogenesis of IPS and suggest that injury to the pulmonary vascular endothelium may be critical to both the initiation and propagation of this process. Thus, it is conceivable that strategies that maintain EC integrity may be effective at preventing or treating IPS. The administration of molecules that function as survival factors for ECs has been successful in preventing endothelial damage and mortality from septic shock and radiation injury.[116,117] Specifically, keratinocyte growth factors (KGF) have been shown to be efficacious in reducing epithelial damage and the severity of acute GVHD[118,119] and pulmonary injury after allogeneic SCT,[120] as well as protecting pulmonary endothelium from oxygen-induced injury. Phase I/II clinical trials using KGF along with
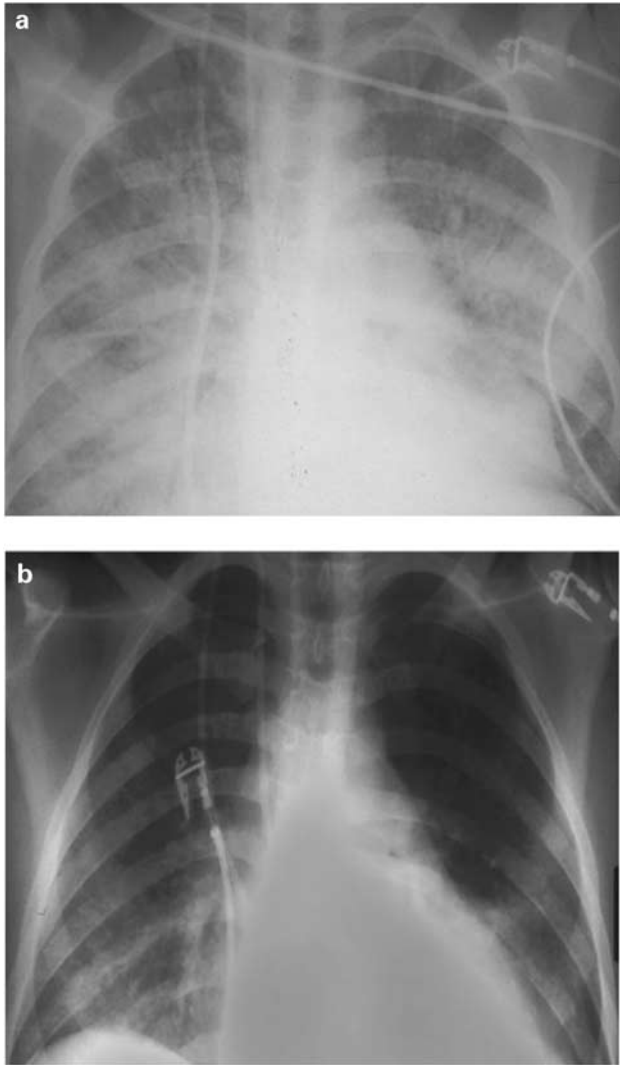
Figure 3 Chest radiographs obtained on day 0 (Figure 3a) and day 4 (Figure 3b) of therapy with etanercept for a 14-year-old patient who developed IPS 8 days following a 4/6 matched PBSCT for recurrent renal cell carcinoma.

standard GVHD prophylaxis are in progress and the effects of this strategy on the development of IPS is of great interest. Finally, as noted above, CCR2/MCP-1 interactions contribute to the development of lung injury in mice and BAL fluid MCP-1 levels are elevated in patients with IPS, suggesting that specific chemokine receptor–ligand interactions may be operative in the clinical setting as well. Since IPS develops and progresses to respiratory failure despite significant systemic immunosuppression, these findings suggest that novel strategies directed toward inhibiting pathways of effector cell recruitment to the lung may serve as future adjuncts to standard therapy intended to prevent or treat this serious complication.

Conclusions

Despite significant advances in critical care medicine, noninfectious lung injury remains a frequently fatal complication following allogeneic SCT. Although our understanding of the pathogenesis of IPS is limited by the absence of controlled clinical studies and the resultant paucity of human data, these limitations have been overcome in part by observations made using animal models that have clearly shown immunologic mechanisms to be operative. TNFα and LPS are significant, albeit not exclusive, contributors to IPS, and cells of both lymphoid and myeloid origin play a direct role in lung injury that occurs in this setting. In particular, the contribution of donor, nonlymphoid, accessory cells may be linked to the cellular activating effects of LPS and the ultimate secretion of TNFα within a 'gut–liver–lung' axis of inflammation, whereas donor T cell effectors can home to and damage the lung even when systemic GVHD is mild or absent. These findings have led to the hypothesis that the lung is susceptible to two distinct but interrelated pathways of injury after SCT involving aspects of both the adaptive and the innate immune response (Figure 2). These findings support a shift away from the current paradigm of acute lung injury after SCT as an idiopathic clinical syndrome toward a process in which the lung is the target of an alloantigen-specific, immune-mediated attack.

Is the lung a target of GVHD? The weight of conceptual and experimental evidence seems to us to favor rather than disfavor this possibility. The lung, like the gut and skin, serves as an interface between the sterile body sanctuary and the outside environment, and the pulmonary defense system is well designed to maintain this barrier; the lung is a rich source of histocompatibility antigens and professional APCs and is the site of complex immunologic networks involving cytokine production and lymphocyte activation. As noted above, inflammatory cytokines along with donor-derived T cell effectors, which are known to play a role in acute GVHD, also directly contribute to acute lung injury in animal SCT models and have been identified in the BAL fluid of patients with IPS. Clinically, evidence supporting the concept that the lung is a target organ of acute GVHD is limited, and the major obstacle has been the lack of apoptotic epithelial injury. However, other GVHD target organs such as the thymus do not express this particular form of injury, and recent experimental data demonstrate that direct recognition of alloantigen on host epithelium by cytotoxic effectors is not required for GVHD induction or target organ injury. Moreover, the unique aspects of epithelial anatomy in the lung may significantly contribute to this discrepancy. Since there is no stratification or layering of pulmonary epithelial cells as in the skin or intestine, the histopathologic repertoire of pulmonary damage is very limited making a potential diagnosis of acute GVHD in the lung by histologic criteria difficult. As animal models of lung injury after SCT yield further insights, our understanding of this clinical complication should improve. Indeed mechanistic insights from these experimental models should form the basis for translational clinical research protocols that will ultimately lead to successful therapeutic strategies to diagnose, treat and prevent pulmonary toxicity in recipients of allogeneic SCT.

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