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Rationale: Acute lung dysfunction of noninfectious etiology, known as idiopathic pneumonia syndrome (IPS), is a severe complication following hematopoietic stem cell transplantation (HSCT). Several mouse models have been recently developed to determine the underlying causes of IPS. A cohesive interpretation of experimental data and their relationship to the findings of clinical research studies in humans is needed to better understand the basis for current and future clinical trials for the prevention/treatment of IPS.

Objectives: Our goal was to perform a comprehensive review of the preclinical (i.e., murine models) and clinical research on IPS.

Methods: An ATS committee performed PubMed and OVID searches for published, peer-reviewed articles using the keywords “idiopathic pneumonia syndrome” or “lung injury” or “pulmonary complications” AND “bone marrow transplant” or “hematopoietic stem cell transplant.” No specific inclusion or exclusion criteria were determined a priori for this review.

Measurements and Main Results: Experimental models that reproduce the various patterns of lung injury observed after HSCT have identified that both soluble and cellular inflammatory mediators contribute to the inflammation engendered during the development of IPS. To date, 10 preclinical murine models of the IPS spectrum have been established using various donor and host strain combinations used to study graft-versus-host disease (GVHD). This, as well as the demonstrated T cell dependency of IPS development in these models, supports the concept that the lung is a target of immune-mediated attack after HSCT. The most developed therapeutic strategy for IPS involves blocking TNF signaling with etanercept, which is currently being evaluated in clinical trials.

Conclusions: IPS remains a frequently fatal complication that limits the broader use of allogeneic HSCT as a successful treatment modality. Faced with the clinical syndrome of IPS, one can categorize the disease entity with the appropriate tools, although cases of unclassifiable IPS will remain. Significant research efforts have resulted in a paradigm shift away from identifying noninfectious lung injury after HSCT solely as an idiopathic clinical syndrome and toward understanding IPS as a process involving aspects of both the adaptive and the innate immune response. Importantly, new laboratory insights are currently being translated to the clinic and will likely prove important to the development of future strategies to prevent or treat this serious disorder.

Keywords: hematopoietic stem cell transplant; pulmonary complications; lung injury; mouse models

INTRODUCTION

Acute pulmonary dysfunction is a frequently fatal complication following hematopoietic stem cell transplantation (HSCT). Previously, idiopathic pneumonia syndrome (IPS) was defined to describe a subset of these patients who have signs and symptoms of pneumonia, and evidence for widespread alveolar injury in the absence of lower respiratory tract infection (1).

Over the years, this definition has been adopted into clinical practice and has been useful in guiding medical management in afflicted patients. However, outcomes of patients diagnosed with IPS remain unacceptable; mortality rates are high, and time to death following diagnosis is short. An improved understanding of the clinical spectrum of disease and the mechanisms responsible for IPS is therefore essential for...
physician-scientists to make progress in the treatment and prevention of this disorder. Our goal was to perform a comprehensive review of the preclinical (i.e., murine models) and clinical research on IPS.

METHODS

An ATS committee, including representation from the adult and pediatric communities (national and international), was formed by invitation based on expertise in the care of patients with IPS after HSCT and/or expertise in preclinical models of IPS. Committee members performed PubMed and OVID searches for published, peer-reviewed articles using the keywords “idiopathic pneumonia syndrome” or “lung injury” or “pulmonary complications” AND “bone marrow transplant” or “hematopoietic stem cell transplant.” Each committee member was assigned to sections as outlined in this statement and asked to review the literature for the sections assigned. No specific inclusion or exclusion criteria were determined a priori for this review. All committee members reviewed and edited all drafts and the final version of this research statement, and reached consensus for its accuracy and relevance as a review of the current state of the science of IPS research.

RESULTS

The definition of IPS has been updated by the committee authoring this statement and is now shown in Table 1. The ATS defines IPS as an idiopathic syndrome of pneumopathy after HSCT, with evidence of widespread alveolar injury and in which infectious etiologies and cardiac dysfunction, acute renal failure, or iatrogenic fluid overload have been excluded. Moreover, IPS has been further classified into specific entities (Figure 1 and Table 2) based in large part on the primary anatomical sites of inflammation and dysfunction. The intent is to better characterize the clinical spectrum of disease and to more carefully match subtypes of IPS with specific preclinical models that have been developed to mimic and ultimately understand them. These animal models have proved valuable in defining specific contributors to the pathogenesis of inflammation and evaluating promising new therapeutic modalities. For example, results using novel approaches to use reduced intensity conditioning regimens or to neutralize tumor necrosis factor-α have been encouraging, and these approaches were developed primarily from insights gained from preclinical studies. The purpose of this statement is to provide a brief overview of the spectrum of noninfectious lung injury that occurs after HSCT, to relate these various forms of lung inflammation to the definition of IPS, and to provide a comprehensive review of data generated using experimental animal models that have defined our current understanding of mechanisms of this frequently fatal disorder.

IDIOPATHIC PNEUMONIA SYNDROME AND THE CLINICAL SPECTRUM OF LUNG INJURY AFTER HSCT

In 1993, a National Institutes of Health workshop proposed a broad definition of IPS that included widespread alveolar injury in the absence of active lower respiratory tract infection or cardiogenic causes after HSCT (1). As shown in Table 1, the diagnostic criteria of IPS have been updated and consider newly described pathogens (2), as determined on bronchoalveolar lavage (BAL) or lung biopsy (1, 3). In a practical sense, the diagnosis of IPS is based on exclusion of infectious organisms, cardiac dysfunction, acute renal failure, or iatrogenic fluid overload in the setting of pulmonary dysfunction after HSCT. IPS encompasses a spectrum of clinical presentations, in both children and adults, and is thought to result from a variety of lung insults, including toxic effects of HSCT conditioning regimens, immunologic cell-mediated injury, inflammatory cytokines, and occult pulmonary infections (1, 4–11).

Historically, the incidence of IPS in the first 120 days after allogeneic HSCT with myeloablative conditioning is 3 to 15% (12–19). Recently, the cumulative incidence of IPS after allogeneic HSCT was significantly lower following nonmyeloablative conditioning than conventional, full-intensity preparative regimens (2.2 versus 8.4%; P = 0.003) (15). Once established, however, pulmonary toxicity was severe in each group and resulted in respiratory failure in the majority of patients (15). These findings suggest that the intensity of HSCT conditioning plays an important role in the development of IPS, and are consistent with data generated from mouse models showing that the lung is sensitive to the combined effects of radiation and alloreactive T cells (7, 20).

The median time of onset for IPS was initially reported to be 6 to 7 weeks (range, 14–90 d) following allogeneic HSCT, but more recent studies have indicated an earlier median time of onset at 19 days (range, 4–106 d) (1, 14, 15). Mortality rates in allogeneic HSCT recipients range from 60 to 80% overall, and

### TABLE 1. DEFINITION OF IDIOPATHIC PNEUMONIA SYNDROME

<table>
<thead>
<tr>
<th>I: Evidence of widespread alveolar injury:</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Multilobar infiltrates on routine chest radiographs or computed tomography</td>
</tr>
<tr>
<td>b. Symptoms and signs of pneumonia (cough, dyspnea, tachypnea, rales)</td>
</tr>
<tr>
<td>c. Evidence of abnormal pulmonary physiology</td>
</tr>
<tr>
<td>1. Increased alveolar to arterial oxygen difference</td>
</tr>
<tr>
<td>2. New or increased restrictive pulmonary function test abnormality</td>
</tr>
<tr>
<td>II: Absence of active lower respiratory tract infection based upon:</td>
</tr>
<tr>
<td>a. Bronchoalveolar lavage negative for significant bacterial pathogens including acid-fast bacilli, Nocardia, and Legionella species</td>
</tr>
<tr>
<td>b. Bronchoalveolar lavage negative for pathogenic nonbacterial microorganisms:</td>
</tr>
<tr>
<td>1. Routine culture for viruses and fungi</td>
</tr>
<tr>
<td>2. Shell vial culture for CMV and respiratory RSV</td>
</tr>
<tr>
<td>3. Cytology for CMV inclusions, fungi, and Pneumocystis jirovecii (carinii)</td>
</tr>
<tr>
<td>4. Direct fluorescence staining with antibodies against CMV, RSV, HSV, VZV, influenza virus, parainfluenza virus, adenovirus, and other organisms</td>
</tr>
<tr>
<td>c. Other organisms/tests to also consider:</td>
</tr>
<tr>
<td>1. Polymerase chain reaction for human metapneumovirus, rhinovirus, coronavirus, and HHV6</td>
</tr>
<tr>
<td>2. Polymerase chain reaction for Chlamydia, Mycoplasma, and Aspergillus species</td>
</tr>
<tr>
<td>3. Serum galactomannan ELISA for Aspergillus species</td>
</tr>
<tr>
<td>d. Transbronchial biopsy if condition of the patient permits</td>
</tr>
<tr>
<td>III: Absence of cardiac dysfunction, acute renal failure, or iatrogenic fluid overload as etiology for pulmonary dysfunction</td>
</tr>
</tbody>
</table>

Definition of abbreviations: CMV = cytomegalovirus; HSV = herpes simplex virus; RSV = respiratory syncytial virus; VZV = varicella zoster virus. Updated from Reference 1.
are greater than 95% for patients requiring mechanical ventilation (3, 12–15, 21, 22). While IPS has been reported following autologous HSCT (22–29), the incidence is lower, the median time to onset is generally longer (63 d; range, 7–336 d), and the two entities differ sharply with respect to overall outcome. Inflammation in the autologous setting responds promptly to corticosteroids and is associated with a favorable prognosis, whereas IPS occurring in an allogeneic environment responds poorly to standard therapy and commonly results in rapid respiratory failure and death in the majority of patients.

Risk factors for IPS after allogeneic HSCT include full-intensity conditioning with total body irradiation (TBI), acute graft-versus-host disease (GVHD), older recipient age, and an underlying diagnosis of acute leukemia or myelodysplastic syndrome (MDS) (3, 12, 13, 15, 21, 22, 30). GVHD is a major cause of morbidity and mortality following HSCT and is caused by donor cell reactivity against host tissues, due to major and minor human leukocyte antigen (HLA) disparities, and primarily affects the gut, liver, and skin. While one report showed that a single, very low dose, nonmyeloablative conditioning regimen was associated with a reduced incidence of IPS (15), it remains to be fully determined what impact the frequent use of other more robust but reduced-intensity regimens will have on IPS. In a recent meta-analysis, investigators found that lung irradiation dose, cyclophosphamide dose, and the addition of busulfan were significantly associated with the development of interstitial pneumonitis and that lung shielding reduced the incidence of HSCT-related IPS (30). Although earlier studies identified methotrexate (MTX) as a risk factor for the development of interstitial pneumonia, these studies did not distinguish inflammation from infectious and noninfectious causes. More recent studies have reported that MTX is not a risk factor for IPS (3, 21, 22, 26, 31–34). Risk factors for IPS following autologous HSCT include older patient age, severe oral mucositis, TBI-based or bischloroethylnitrosourea (BCNU)-containing conditioning regimens, chest irradiation within 2 weeks of transplant, female sex, and an underlying diagnosis of solid tumor (24, 25, 27). To date, the impact that stem cell source (bone marrow versus peripheral blood versus umbilical cord blood) has on the development of IPS has not been fully evaluated. Thus, how the expanded use of peripheral blood and umbilical cord blood from unrelated donors will affect the incidence and severity of disease remains to be elucidated. Despite the identification and attempts to reduce risk factors, the overall mortality due to IPS remains unacceptably high (35, 36).

As initially defined by Clark and colleagues, the clinical spectrum of IPS encompasses several descriptive forms of pulmonary dysfunction (1, 37, 38). 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-HSCT period and is characterized by progressive shortness of breath, cough, and hypoxemia with or without fever. DAH has been reported in 5 to 12% of HSCT patients with a median time to onset of 19 days (range, 5–34 d) in allogeneic recipients and 12 days (range, 0–40 d) in autologous patients (24, 39–44). A disproportionately higher incidence of DAH post-HSCT has been reported in patients with mucopoly saccharidosis (45). The diagnosis of DAH is based on progressively bloodier return of BAL fluid, but frank hemoptyis is rare (24). The presence of greater than 20% hemosiderin-laden macrophages in the BAL fluid may give an indication of previous pulmonary hemorrhage. However, this finding has a very low specificity for DAH; BAL fluid hemosiderin-laden macrophages are also common in patients with low platelet counts or pulmonary hypertension (46). Mortality from DAH ranges from 60 to 100% despite treatment with high-dose corticosteroids (2 mg/kg/d to 1 g/m²/d), with death usually the result of multiorgan failure within 3 weeks of diagnosis (40, 41, 47). Majhail and colleagues recently compared patients with DAH and infection-associated alveolar hemorrhage who presented with similar clinical and radiographic findings in the setting of progressively bloodier BAL fluid following allogeneic HSCT. Alveolar hemorrhage from infectious and noninfectious causes were found to be related but distinct entities with extremely poor outcomes following therapy with conventional agents, including steroids (43).

Peri-engraftment respiratory distress syndrome (PERDS) represents another clinical subset of IPS. By definition, PERDS occurs within 5 days of engraftment and can account for 33% of IPS cases after allogeneic HSCT (48). Although the clinical presentation of PERDS after conventional allogeneic HSCT is similar to that observed in autologous patients or allogeneic patients who receive reduced-intensity conditioning regimens or T cell–depleted stem cell grafts, lung dysfunction is more responsive to corticosteroids and is associated with a better prognosis in the these patients (49–51). Delayed pulmonary toxicity syndrome (DPTS) has also traditionally fallen within the definition of IPS (52). The incidence of DPTS is 29 to 64% in autologous patients receiving chemotherapy with regimens that contain BCNU, cyclophosphamide, and cisplatin. The median time to onset of DPTS is 45 days (range, 21–149 d), and treatment with corticosteroids (1 mg/kg/d) leads to resolution in up to 92% of cases (52–54). High-dose inhaled corticosteroid prophylaxis may also be beneficial in these patients (55). The implication that DPTS is directly related to a specific HSCT conditioning regimen suggests that this entity should technically not be categorized as IPS per se.
Potential etiologies for IPS include direct toxic effects of HSCT conditioning regimens, occult pulmonary infections, and the release of inflammatory cytokines that have been implicated in other forms of pulmonary injury. As noted above, the dose intensity of HSCT conditioning may affect the frequency of IPS, and specific combinations of chemotherapeutic agents can predispose to pulmonary dysfunction in subsets of HSCT recipients. Historically, approximately one half of all pneumonias seen after HSCT have been secondary to infection, but the judicious use of broad-spectrum antimicrobial agents has tipped the balance to noninfectious causes. Together, broad-spectrum prophylaxis and the development and application of advanced diagnostic techniques, including immunohistochemical staining, indirect fluorescent antibody testing, and polymerase chain reaction (PCR), have significantly reduced the incidence of infectious pulmonary disorders. While there is a debate as to the diagnostic approach of a clinical disorder, the diagnosis of IPS is rarely made or confirmed by tissue biopsy given the significant mortality risks of open lung biopsy in the early transplant period. The lack of a pathognomonic histologic pattern for IPS and the heterogeneity of pulmonary pathology after allogeneic HSCT are complicated further by the nonspecific lung injury changes that may occur after mechanical ventilation and by the limited quality and quantity of transbronchial lung biopsy specimens obtained from HSCT recipients.

Unfortunately, categorizing patients with IPS based upon descriptive clinical terminology is equally unrewarding. With the help of new and improved diagnostic techniques in immunohistochemistry, radiology, and microbiology, we proposed to categorize the disease entities falling under the umbrella of IPS by the primary anatomic site of cellular damage, including the interstitial tissue, vascular endothelium, or airway epithelium (Table 1). While some cases may remain unclassifiable, this approach may focus future lines of investigation on specific pathways of tissue injury and thereby foster the development of therapeutic interventions that can be tailored to distinct subtypes of disease. To this end and to better define the pathogenesis of IPS, several murine models have been developed and used extensively to investigate immunologic mechanisms of injury that contribute to damage to the vascular endothelium as well as alveolar and bronchiolar epithelium. In this context, a wealth of experimental data has markedly improved our understanding of the pathophysiologic processes that regulate IPS, and has revealed that both cellular effectors and a variety of soluble effectors contribute to the inflammation engendered during disease progression.

**THE PATHOGENESIS OF IPS AFTER ALLOGENEIC HSCT**

**Mouse Models of Noninfectious Lung Injury after HSCT**

Several murine models of lung injury after HSCT have been established using a variety of strain combinations and conditioning regimens (Table 4). They include a lethally irradiated major histocompatibility complex (MHC)-matched, multiple minor antigen mismatch (6, 20, 68, 172); a complete MHC mismatch model with and without cyclophosphamide (7, 69), lethally irradiated, haploidentical, parent into F1 model (20, 146, 147, 165); a MHC class I mismatch (68, 70); a MHC class II mismatch (68); and a syngeneic HSCT model using high-dose chemotheraphy (71).

The model using a MHC class I and class II mismatched donor/recipient strain combination best reproduces acute, early-onset IPS. In this model, pulmonary toxicity is caused by the influx of host monocytes and donor T cells into the lungs of lethally irradiated mice within the first 2 weeks of HSCT (7). Intensifying the pre-HSCT conditioning with cyclophosphamide accelerates the development of IPS consistent with human data. From a classification standpoint (see Figure 1), lung injury is characterized by injured type II alveolar (ATII) epithelial cells, injured endothelial cells, and increased frequencies of cytotoxic T lymphocytes and cells expressing B7 family costimulatory ligands in the alveolar and interstitial space (7, 72). Lung dysfunction in this acute model reflects this interstitial and alveolar damage and presents as reduced specific compliance, decreased total lung capacity and increased wet and dry lung weights. BAL fluid of mice with IPS contained elevated levels of soluble mediators including cytokines as well as nitrites, lactate dehydrogenase, and total protein indicative of lung inflammation and pulmonary endothelial damage and leak (73). Similar findings of decreased compliance and oxidative stress were seen in the early-onset, syngeneic IPS model of DPTS in which mice are conditioned with high-dose chemotherapy alone (71).

Murine systems wherein donor and host differ at multiple minor HC antigens or at isolated MHC class I or class II loci more closely reflect conditions that are typically seen during...
TABLE 3. CLINICAL CATEGORIZATION, HISTOLOGY PATTERN, RADIOGRAPHIC FEATURES, AND MURINE HSCT MODEL SYSTEMS OF LUNG INJURY FOLLOWING HEMATOPOIETIC STEM CELL TRANSPLANTATION

<table>
<thead>
<tr>
<th>Clinical Categorization (Ref. no.)</th>
<th>Symptoms</th>
<th>Onset</th>
<th>Histologic Pattern</th>
<th>Radiographic Features</th>
<th>Murine HSCT Model? (Ref. no.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung parenchyma (interstitium and alveolar)</td>
<td>ARDS (59)</td>
<td>Fever, cough, dyspnea, hypoxemia</td>
<td>Acute onset, 2 to 6 mo after HSCT; typically &lt; 120 d</td>
<td>Diffuse alveolar damage</td>
<td>Bilateral interstitial infiltrates</td>
</tr>
<tr>
<td></td>
<td>Radiation (210), BCNU pneumonitis (211–212)</td>
<td>Dyspnea, nonproductive cough, rales, pleural friction rub, low-grade fever, restrictive PFTs, impaired DLCO</td>
<td>Subacute, 2 to 4 mo after therapy</td>
<td>Acute fibroin exudate with chronic fibrosis and sclerosis; often initially subtle pneumonitis with fibrosis in biopsy</td>
<td>Bilateral interstitial infiltrates, initial diffuse infiltrates around hilus</td>
</tr>
<tr>
<td></td>
<td>DPTS (52–54)</td>
<td>Fever, dry cough, dyspnea</td>
<td>Late, months to years after auto HSCT for breast cancer</td>
<td>Hyperplastic alveolar type II cells, septal thickening, interstitial fibrosis, small vessel endothelial injury</td>
<td>Bilateral interstitial infiltrates, ground glass opacities, abnormalities can initially be absent</td>
</tr>
<tr>
<td></td>
<td>PTLD (213–214)</td>
<td>Dyspnea, hypoxemia; diffuse lymphadenopathy, may also have more systemic symptoms (viral-like illness to fulminant sepsis)</td>
<td>1–5 mo, rarely years following allogeneic HSCT with T cell depletion</td>
<td>Lymphoid proliferation in association with EBV</td>
<td>Diffuse infiltrates preferentially located in the basal and subpleural areas</td>
</tr>
<tr>
<td></td>
<td>EP (215–217)</td>
<td>Cough, progressive dyspnea, BAL eosinophilia (&gt; 5%)</td>
<td>Late, 3 mo to years after HSCT</td>
<td>Alveolar and bronchiolar tissue eosinophilia, peripheral blood eosinophilia is also possible</td>
<td>Diffuse infiltrates, often of transient localization, subpleural localization</td>
</tr>
<tr>
<td></td>
<td>PAP (218–219)</td>
<td>Dyspnea, hypoxemia</td>
<td>Early (days) to late (1–2 yr)</td>
<td>BAL milky, extracellular, eosinophilic PAS+ material; alveolar filling with PAS+ material, normal BAL fluid possible</td>
<td>Bilateral patchy alveolar infiltrates; crazy paving pattern</td>
</tr>
<tr>
<td>Vascular endothelium</td>
<td>PERDS (50)</td>
<td>Fever, dyspnea, cough, hypoxemia, skin rash, weight gain, edema</td>
<td>Acute, within 5–7 d of neutrophil engraftment</td>
<td>Diffuse alveolar damage (but biopsy contraindicated due to thrombocytopenia), neutrophilic inflammation in BAL</td>
<td>Bilateral interstitial infiltrates, pleural effusions; intensity from mild to noncardiogenic pulmonary edema to ARDS</td>
</tr>
<tr>
<td></td>
<td>DAH (40)</td>
<td>Cough, progressive dyspnea, rarely hemoptysis, progressively bloomer BAL fractions</td>
<td>Acute, 1–3 mo after HSCT</td>
<td>Diffuse alveolar damage with intra-alveolar red blood cells and hemosiderin-laden macrophages</td>
<td>Diffuse infiltrates, initial appearance initially noted</td>
</tr>
<tr>
<td></td>
<td>PVOD (221–223)</td>
<td>Dyspnea</td>
<td>2–6 mo after HSCT</td>
<td>Fibrous intima proliferation of pulmonary venules, arteriolar involvement also possible</td>
<td>Infiltrates, cardiomegaly, pulmonary hypertension</td>
</tr>
<tr>
<td></td>
<td>TRALI (224)</td>
<td>Acute dyspnea, PaO2/FiO2 ratio &lt; 300 mm Hg, hypotension, fever, chills, leukopenia</td>
<td>Within 6 h of blood product transfusion</td>
<td>Granulocyte aggregation in pulmonary vasculature</td>
<td>Bilateral infiltrates</td>
</tr>
<tr>
<td></td>
<td>PCT (225)</td>
<td>Fever, cough, chest pain, progressive respiratory distress. Observed in pediatric HSCT recipients.</td>
<td>2–3 mo after HSCT</td>
<td>Open lung biopsy necessary for diagnosis showing thrombi consisting of basophilic, amorphous material which may extend through the vascular wall into adjacent tissue: hemorrhagic infarcts</td>
<td>CXR: normal in 25%, nodules, interstitial markings, atelectasis also seen. CT scan: multiple peripheral pulmonary nodules.</td>
</tr>
<tr>
<td></td>
<td>PAH (226–229)</td>
<td>Asymptomatic/insidious onset of dyspnea</td>
<td>Typically within the first 6 mo after HSCT</td>
<td>Intimal hyperplasia in small pulmonary arteries and arterioles, veins and venules remain unaffected</td>
<td>Pronounced pulmonary artery on CXR or CT</td>
</tr>
<tr>
<td>Airway epithelium</td>
<td>BOOP (36, 66)</td>
<td>Fever, dry cough, dyspnea; spirometry: restrictive findings, reduced FVC</td>
<td>2–12 mo after transplant</td>
<td>Organizing pneumonia (OP), intraluminal organizing fibrosis in distal airspaces with mild interstitial inflammation</td>
<td>Patchy airspace disease, ground glass appearance, nodular opacities</td>
</tr>
<tr>
<td></td>
<td>BOS (66)</td>
<td>Cough, dyspnea, wheezing, lack of fever; spirometry: diminished FEV1</td>
<td>3–24 mo after transplant</td>
<td>Bronchiolitis obliterans (BO), cellular and constrictive bronchiolitis</td>
<td>CXR: hyperinflation. Otherwise routinely normal.CT: (early) mosaic attenuation, air trapping; (late) septal lines, centrilobular nodules, bronchial dilatation.</td>
</tr>
</tbody>
</table>

Definition of abbreviations: AIP = acute interstitial pneumonitis; ARDS = acute respiratory distress syndrome; BCNU = bischloroethylnitrosourea; BOOP = bronchiolitis obliterans organizing pneumonia; BOS = bronchiolitis obliterans syndrome; DAH = diffuse alveolar hemorrhage; DPTS = delayed pulmonary toxicity syndrome; EP = eosinophilic pneumonia; PAH = pulmonary arterial hypertension; PAP = pulmonary alveolar proteinosis; PCT = pulmonary cytotoxic thrombi; PERDS = peri-engraftment respiratory distress syndrome; PTLD = post-transplant lymphoproliferative disease; PVOD = pulmonary veno-occlusive disease; TRALI = transfusion-related acute lung injury.
TABLE 4. MURINE HEMATOPOIETIC STEM CELL TRANSPLANTATION MODELS OF IDIOPATHIC PNEUMONIA SYNDROME

<table>
<thead>
<tr>
<th>Setting</th>
<th>Donor Cells</th>
<th>Recipient Conditioning</th>
<th>Histologic Pattern</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete MHC mismatch (Class I, II and minors)</td>
<td>CS7BL/6 into B10.BR</td>
<td>20 x 10^6 BM (T cell depleted) and 15 x 10^5 spleen cells from B6 donors</td>
<td>7.5 Gy TBI Day −1 ± Cy (120 mg/kg) Days −3 and −2</td>
<td>(7)</td>
</tr>
<tr>
<td></td>
<td>CS7BL/6 into B10.BR</td>
<td>20 x 10^6 BM (T cell depleted) and 11 x 10^5 spleen cells from BMT donors</td>
<td>7.5 Gy TBI Day −1 + Cy (120 mg/kg) Days −3 and −2</td>
<td>(69)</td>
</tr>
<tr>
<td></td>
<td>Class I mismatch CS7BL/6 into B6.C-H2m1/By</td>
<td>5 x 10^6 splenocytes from B6 donors</td>
<td>6.75 Gy TBI</td>
<td>(68)</td>
</tr>
<tr>
<td></td>
<td>Class II mismatch CS7BL/6 into B6.C-H2m12/KhEg</td>
<td>5 x 10^6 splenocytes from B6 donors</td>
<td>6.75 Gy TBI</td>
<td>(68)</td>
</tr>
<tr>
<td></td>
<td>Semi-allogeneic, Haploidentical: Parent into F1CS7BL/6 into B6D2F1 hybrid (CS7BL/6 × DBA)</td>
<td>5 x 10^8 BM and 2–10 x 10^6 splenic T cells from B6 donors</td>
<td>11–13 Gy TBI (split dose), 9 Gy of TBI</td>
<td>(20, 146, 165)</td>
</tr>
<tr>
<td></td>
<td>Semi-allogeneic, Haploidentical: Parent into F1 BALB/c into CB6F1 hybrid (CS7BL/6 × BALB/c)</td>
<td>10 x 10^8 Rag2−/− BM and 5 x 10^6 sorted splenic T cells from Tbet−/− or IFNγ−/− BALB/c donors (or IFNγR−/− recipients)</td>
<td>13 Gy TBI (split dose)</td>
<td>(147)</td>
</tr>
<tr>
<td>Minor MHC mismatch B10.BR into CBA</td>
<td>5 x 10^8 BM and 1 x 10^5 splenic T cells from B10.BR donors</td>
<td>11 Gy TBI (split dose)</td>
<td>Mononuclear infiltration, diffuse alveolar damage. DAH with LPS challenge</td>
<td>(6)</td>
</tr>
<tr>
<td>Minor MHC mismatch LJP into CS7BL/6</td>
<td>5 x 10^8 BM and 2 x 10^5 splenic T cells from LJP donors</td>
<td>13 Gy TBI (split dose)</td>
<td>Periluminal infiltration, interstitial inflammation</td>
<td>(172)</td>
</tr>
<tr>
<td>Syngeneic B6C3F1 hybrid (CS7BL/6 × C3H) into B6C3F1 hybrid (CS7BL/6 × C3H)</td>
<td>10 x 10^8 BM cells from B6C3F1 donors</td>
<td>Cy (105 mg/kg) + cisplatin (3.59 mg/kg) Days −5, −4, and −3 + BCNU (42.9 mg/kg) Day −2</td>
<td>Large, foamy alveolar macrophages, septal capillary congestion, reactive proliferation of bronchiolar epithelial cells, minimal edema</td>
<td>(71)</td>
</tr>
</tbody>
</table>

Definition of abbreviations: BCNU = bischloroethylnitrosourea; BM = bone marrow; Cy = cyclophosphamide; DAH = diffuse alveolar hemorrhage; LPS = lipopolysaccharide; MHC = major histocompatibility complex.

human HSCT and specifically model IPS that develops during the first 3 to 6 weeks after transplant and is characterized by leukocyte infiltration into the lung as observed on lung biopsy (59). Regardless of strain combination, two primary, reproducible abnormalities are apparent in these experimental models: a dense mononuclear cell infiltrate around both pulmonary vessels and bronchioles and an acute pneumonitis involving the interstitium and alveolar spaces representing each of the three anatomic categories outlined in Figure 1. When measured, alterations in pulmonary function are associated with lung histopathology demonstrating that the observed lung inflammation is physiologically relevant (6, 7, 75). The alveolar infiltrate is composed of macrophages, lymphocytes, epithelial cells, and scattered polymorphonuclear cells within a fibrin matrix, whereas the periluminal (around vascular and bronchial structures) infiltrates are composed primarily of activated donor T cells. Moreover, significant pulmonary endothelial apoptosis and dysfunction has been identified in this context; endothelial injury coincides with the onset of pulmonary pathology, is associated with elevations in BAL fluid TNF-α levels, and the extent of injury directly correlates with the severity of lung inflammation as IPS progresses (76). Despite the presence of endothelial damage, diffuse alveolar hemorrhage is not regularly seen in preclinical IPS models. The only exception to this is when mice are challenged with lipopolysaccharide (LPS). In this scenario, hemorrhage is only observed in mice with advanced GVHD and is associated with large increases in BAL fluid levels of neutrophils, TNF-α, and LPS (6, 11).

Collectively, a significant body of experimental data suggests that mouse models of IPS reproduce many of the histologic and functional changes observed during human disease. To this end, several investigators have used these models to uncover pathways of inflammation that may be operative during human disease in an attempt to identify opportunities to more effectively treat or prevent this complication.

THE ROLE OF SOLUBLE MEDIATORS IN THE DEVELOPMENT OF IPS

TNF-α and TNF Receptors

The mixed inflammatory alveolar infiltrates found in mice with IPS are associated with increased TNF-α in both lung tissue and BAL fluid (6, 11, 20, 77). A causal role for TNF-α in the development of IPS has been established by neutralizing this cytokine in experimental HSCT models (11, 77). Administration of a soluble, dimeric, TNF-binding protein (rhTNFR:Fc; Amgen Corp., Thousand Oaks, CA) from Week 4 to Week 6 after HSCT reduced the progression of lung injury during this time period (11). The use of mutant mice deficient in TNF-α and that cytokine production increases rather than host-derived TNF-α and that cytokine production from both donor accessory cells (macrophage monocytes) and T cells significantly contributes to this toxicity (78). TNF-α is likely to contribute to the development of IPS through both direct and indirect mechanisms. TNF-α increases MHC complex expression, modulates leukocyte migration, facilitates cell-mediated cytotoxicity, and is itself cytotoxic. Recently it has been shown that TNF-α contributes to pulmonary vascular endothelial cell apoptosis that accompanies lung
inflammation during the development of IPS (76). Moreover, donor-derived TNF-α serves as both a facilitator and an effector of lung injury. TNF-α secreted by donor T cells regulates the chemokine milieu in the lung within the first 2 weeks after HSCT, which directly contributes to the subsequent recruitment of monocytes/macrophages as lung injury progresses (78).

The actions of TNF-α are mediated by two receptors: a 55- to 60-kD type I receptor (TNFRI; p55/60; CD120a) and a 75- to 80-kD type II receptor (TNFRII; p75/80; CD120b) (79–81). These two receptors are co-expressed in almost every cell in the body. In contrast to the constitutive expression of TNFRI, the expression of TNFRII is strongly modulated by various cytokines and other inflammatory stimuli like LPS (82–84). TNFRI is generally regarded as the dominant receptor in TNF-α biology; it is the high-affinity receptor for soluble TNF-α (sTNF-α), and mediates many of the proinflammatory effects of this cytokine (85–88). While both sTNF-α and memTNF-α (transmembrane form of TNF-α) are able to activate TNFRI, TNFRII can only be fully activated by memTNF-α (89). TNFRII contributes to the cytoidal effects of TNF-α by its own signaling, and also by regulating the access of this cytokine to TNFRI thereby enhancing TNFRII signaling (90–93).

A role for TNFRI in the induction of GVHD, early after allo-HSCT, has been previously described (94). sTNF-α production is directly related to toxicity from pre-transplant conditioning and to the proinflammatory response that accompanies donor T cell activation and expansion early after allo-HSCT. It was demonstrated that absence of TNFRI in allo-HSCT recipients resulted in improved early post-HSCT survival that was associated with decreased pulmonary edema and improved lung compliance on Day 7 after HSCT (95). However, cellular infiltration into the lung, and BAL fluid levels of proinflammatory cytokines and chemokines were actually higher in BAL fluid of TNFRI-/- HSCT recipients compared with TNFRI+/+ controls. These findings were consistent with those reported when IPS was induced across minor H antigens or isolated MHC class I differences wherein interactions between TNFRII and TNF-α contributed to the leukocyte infiltration and pulmonary inflammation (96). In these studies, the reduction in lung injury observed in TNFRII-/- allo-HSCT recipients directly mirrored the more favorable outcome observed when TNF-α-/- mice were used as HSCT donors.

LPS

Increases in neutrophils and TNF-α in the lungs of allogeneic HSCT recipient without evidence of infection, suggest that host flora-derived LPS might play an important role in IPS pathophysiology. LPS is involved in initiating the innate immune response and is a potent enhancer of inflammatory cytokine release. In non-HSCT experimental models, intratracheal administration of LPS elicits a severe, acute inflammatory response in the lungs of animals (97–99). 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 (74, 100–105). 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 HSCT significantly amplifies lung injury (6). This amplification is only seen in mice with advanced GVHD and is associated with large increases in BAL fluid levels of TNF-α and LPS and the development of alveolar hemorrhage (6, 11).

Gut-Lung-Liver Axis of Inflammation

Collectively, these data demonstrate that the inflammatory mediators TNF-α and LPS both contribute to experimental IPS. Moreover, they support the hypothesis of a ”gut-liver-lung” axis of inflammation in IPS pathophysiology. Any process that ultimately results in large amounts of endotoxin and/or TNF-α in the pulmonary circulation may contribute to the development of lung injury in this setting. This hypothesis is consistent with the observation that serum and BAL fluid TNF-α levels are increased in patients with IPS (106, 107). A clinical linkage of hepatic dysfunction to lung injury after HSCT is also suggested by associations between veno-occlusive disease (VOD) and IPS and between hepatic failure and death from IPS (3, 14). Furthermore, evidence for cytokine activation and LPS amplification observed in the BAL fluid of patients with acute respiratory distress syndrome (ARDS) (108) has been demonstrated in patients with IPS after allogeneic HSCT; increased pulmonary vascular permeability and increases in BAL fluid levels of IL-1, IL-12, IL-6, TNF-α, LPS-binding protein (LBP), and soluble CD14 were also observed in these patients (8). The incomplete protection provided by abrogating the effects of TNF-α after HSCT by various strategies is consistent with reports from many groups (58, 74, 77, 100, 109–111), 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 (109, 112–114). For example, IL-1β, TGF-β, 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 (7, 73, 115).

Oxidative Stress and Pulmonary Dysfunction during IPS

Proinflammatory events after HSCT are frequently accompanied by enhanced production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) with a concomitant increase in oxidative and nitrosative stress (116–118). Exposure to radiochemotherapy further increases oxidant stress by depletion of antioxidant defenses, including reduced glutathione, the major antioxidant in the epithelial lining fluid of the lung (119, 120). These observations have been confirmed in murine IPS models after allogeneic and autologous HSCT (71, 121). HSCT is also associated with a state of iron excess in which free iron becomes available to catalyze the conversion of ROS intermediates to highly toxic free radicals such as hydroxyl radical (122). The lung is particularly sensitive because of its oxygen-rich environment. Exhaled nitric oxide is also increased in the lower respiratory tract after HSCT (123). Potential sources of nitric oxide are chemotherapy-induced inflammation of lung epithelium and lung-infiltrating inflammatory cells (118). When simultaneously generated in large amounts, nitric oxide rapidly reacts with superoxide to form peroxynitrite, a potent oxidant and nitrating species (73).

Oxidant/antioxidant imbalance may directly cause cellular injury, and may trigger multiple inflammatory signal transduction pathways (124). Direct evidence that oxidative stress plays a distinct role in the development of IPS injury in humans is lacking. However, experimental models indicate that recipient mice given high-dose chemotherapy and treated with N-acetylcysteine exhibit substantially less lung injury compared with mice receiving high-dose chemotherapy without N-acetylcysteine (71). The most likely mechanism for the protective effects of N-acetylcysteine is the replenishment and/or preservation of glutathione redox potential, although direct scavenging of...
reactive species by N-acetylcysteine may also contribute. Furthermore, overexpression of extracellular superoxide dismutase (EC-SOD), the major extracellular antioxidant enzyme in the lung, confers protection against radiation-induced acute lung injury (125, 126). Together, these data suggest that amelioration of oxidative stress may reduce the development of severe IPS injury; however, human studies using lung-targeted antioxidant approaches are lacking, and questions concerning the potential of these antioxidants to affect the efficacy of the chemotherapy need to be addressed.

Depletion of Pulmonary Surfactant during IPS

Pulmonary surfactant, produced by alveolar type II cells, is composed of a complex mixture of lipids and at least four surfactant proteins (SPs), named in the order of discovery. The presence of surfactant at the blood–gas interface is essential for the survival of air-breathing mammals. By reducing surface tension, surfactant decreases the work of breathing, allows alveoli to remain open at end expiration, and keeps alveoli dry. In addition, surfactant and particularly SP-A and SP-D have a major role in host defense and in regulating immune responses in the lung (127–131). Surfactant dysfunction caused by decreased synthesis in alveolar type II cells, inhibition of function by extravasated serum proteins, or degradation by lipases and oxidants (132, 133) likely contributes to the IPS clinical picture of hypoxemia, progressive dyspnea, and pulmonary edema (134, 135).

There are a limited number of experimental and human studies evaluating the role of surfactant during lung dysfunction following HSCT. In a model of severe IPS following lethal irradiation, mice lacking SP-A or SP-D exhibited exaggerated allogeneic T cell–dependent inflammation that was associated with enhanced markers of lung injury (136). Furthermore, transtracheal instillation of human SP-A attenuated the manifestations of IPS in mice (137). In humans, pre-transplant serum levels of SP-D were identified as a risk factor for the development of IPS and bronchiolitis obliterans after allogeneic HSCT (138). The investigators speculate that certain polymorphisms in the human SP-D gene cause subnormal levels of SP-D in the alveoli and serum (139). Low levels of SP-D may predispose these individuals to enhanced inflammatory responses after exposure to conditioning regimens and allogeneic immune responses that contribute to the development of IPS. Other investigators have shown that serum level of SP-D is a valuable biomarker in acute lung injury that can predict outcome (140). Similarly, serum SP-D may also be a useful test in following the clinical course of IPS, but the extent of this relationship first needs to be established in clinical trials. Further studies are required to determine the specific role of surfactant proteins during IPS injury.

Despite the overwhelming success in premature infants, results of surfactant replacement trials during acute lung injury have been variable (141, 142). Phase III studies are underway to determine the efficacy of novel surfactant preparations that closely resemble natural surfactant. If these investigations reveal beneficial effects on mortality, further trials of optimal surfactant preparation, dosing, and timing of administration need to be performed.

THE ROLE OF CELLULAR EFFECTORS IN THE DEVELOPMENT OF IPS

Donor-derived T Cell Effectors

The role of alloreactive, donor T cells in the pathogenesis of IPS has been a topic of considerable debate, particularly since the lung has not been considered a classic target organ of acute GVHD. The importance of lymphocytes to lung injury after experimental HSCT has, however, been shown by several groups (5, 7, 143, 144). Donor T cells are critical to the early proinflammatory events associated with lung injury that develops within the first week of HSCT 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 (7, 144). Furthermore, the infusion of donor T cell clones that recognize allelic differences in cell surface expression of the CD45 molecule into nonirradiated recipients results in a rapidly progressive pulmonary vasculitis within 3 days of their injection (5, 145).

The origin and functional capacity of T cells infiltrating the lung have been examined by using differences in the T cell Vβ repertoire between donor and recipient (144). Flow cytometric analysis demonstrated that the TCRαβ+ T cells found in the lung 6 weeks after allogeneic HSCT were of donor origin. When these donor T cells were recultured with irradiated host antigen-presenting cells (APCs), they proliferated vigorously and produced significant amounts of IFN-γ. Using a CD8-driven model of IPS wherein donor and host differ by class I MHC antigens only, it was shown that donor-derived T cells expressing IFN-γ and CXCR3 were present in the lungs of allo-HSCT recipients as early as Day 7. The influx of these cells correlated with elevations in protein levels of both CXCL9 and CXCL10, two of the principal ligands for CXCR3 (see below), and heralded the onset of significant pulmonary inflammation (70). While robust infiltration occurs reproducibly in these models when IFN-γ receptor–ligand interactions are completely intact, a recent study paradoxically showed that pulmonary inflammation is accelerated when IFN-γ signaling is absent and that this regulatory effect was entirely mediated by pulmonary parenchyma (146). These results were later independently confirmed and extended to show that IFN-γ negatively regulates the expansion of Th17+CD4+ T cells in the lungs during IPS (147). Furthermore, it has recently been demonstrated that in vitro differentiated Th17 cells mediate severe pulmonary pathology in a mouse model of GVHD (148). The role of Th17 cells in tissue inflammation is currently an area of intense study (149).

Role of Host APCs

While experimental data suggest that donor-derived T cell effectors can home to the lungs early after HSCT and contribute to physiologically significant tissue injury, the precise cytolytic targets of these cells and the process through which they are activated by host antigens remain unresolved. Each process is likely to be complex and to involve interactions with both pulmonary parenchymal cells and APCs (150, 151).

Likewise, the cells responsible for activating T cell effectors that are ultimately recruited to the lung and the specific location of these cellular activating events also remain enigmatic. Donor T cells contributing to IPS may initially encounter host APCs residing in more distant secondary lymphoid tissues and are subsequently specifically recruited to the lung following up-regulation of chemotactic receptors, including CXCR3. It is also possible that donor T cells are activated in the pulmonary microenvironment. The pulmonary dendritic cell (DC) is significantly more efficient than the alveolar macrophage (AM) in antigen presentation and functions as the dominant APC in the lung (152–154). Pulmonary DCs located in the pulmonary interstitium and the bronchial epithelium and submucosa are the sole source of MHC class II expression within the epithelial lining of the airway during
steady-state conditions (155, 156). The antigen-presenting capability of pulmonary DCs is regulated by AMs, which exist in close proximity to DCs in both the airway and lung parenchyma; the addition of AMs to in vitro DC cultures enhances this regulatory effect, whereas depletion of AMs in vivo enhances the APC function of freshly isolated DCs (156). Pulmonary DCs 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 (155, 157, 158). It is possible that radiosensitive host DCs persist longer in the lung than in other organs and allow for sustained presentation of host antigens in that organ (159). Activated donor T cells that can cause progressive lung injury might therefore remain within the pulmonary microvascular circulation because persistent host DCs serve as a continuing site of alloantigen presentation.

**Donor Accessory Cells**

The role of donor accessory cell populations (monocytes, macrophages, neutrophils) in the pathophysiology of IPS has been examined using HSCT donors that differ in their response to LPS by virtue of a genetic mutation in the Toll-like receptor 4 (TLR) gene (160) or the absence of CD14, a key cell surface receptor for the LPS-LBP complex (161). In each case, a significant decrease in lung injury was observed in recipients of LPS resistant donor cells compared with wild-type, LPS-sensitive donor cells. The results obtained using CD14-deficient donors are consistent with the report that monocytes recruited to an inflamed lung up-regulate CD14 expression and show enhanced sensitivity to LPS stimulation (162), and with the clinical evidence for cytokine activation and components of the LPS-activating system including LBP and soluble CD14 present in the BAL fluid of HSCT patients with IPS (8).

Polymorphonuclear cells are a major component of the BAL fluid of animals with IPS (6). In some mouse IPS models, the BAL fluid neutrophilia is prominent between Weeks 4 and 6 after HSCT and is associated with increases in BAL fluid levels of TNF-α and LPS (6, 11). Neutralization of TNF-α with rhTNFR:Fc during this time interval prevents the influx of neutrophils and reduces the progression of lung injury and dysfunction (11). Administration of rhTNFR:Fc following LPS challenge completely abrogates the recruitment of neutrophils into the lungs and prevents further damage (including hemorrhage), underscoring the relationship between neutrophils, TNF-α, and LPS in this setting (11). Neutrophil 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. Neutrophils are likely to play a role in patients with IPS as well; their appearance in the bloodstream is often temporally associated with the onset of lung inflammation (14).

**MECHANISMS OF LEUKOCYTE RECRUITMENT TO THE LUNG DURING THE DEVELOPMENT OF IPS**

**Chemokine Receptor–Ligand Interactions during IPS**

Cellular effectors play a significant role in the development of IPS, but the molecular mechanisms by which leukocytes traffic to the lung and cause damage have yet to be fully elucidated. The persistent elicitation of leukocytes into the lung after allogeneic HSCT requires an intercellular communication between infiltrating leukocytes, endothelium, parenchymal cells, and components of the extracellular matrix. The chemokines, by virtue of their specific cell surface receptor expression, can selectively mediate the local recruitment/activation of distinct leukocytes allowing for migration across the endothelium and beyond the vascular compartment along established chemotactic gradients.

The chemokine superfamily is divided into four subfamilies (C, CC, CXC, and CXC,C) based on the presence of conserved cysteine residues at the NH2-terminus (163, 164). CXC chemokines have been further subdivided on the basis of the presence or absence of the sequence glutamic acid–leucine–arginine (ELR) near the NH2-terminal. ELR+ CXC chemokines are neutrophil chemoattractants with angiogenic properties. Interferon-inducible ELR– CXC chemokines are chemoattractants of lymphocytes with angiostatic properties. CC chemokines predominantly recruit mononuclear cells. The C and CX3C subfamily predominantly attract lymphocytes. All chemokine action is mediated through seven-transmembrane–spanning G protein–coupled receptors (163, 164).

**CC Chemokine Family Members**

CCL2 (MCP-1) is a CC chemokine and its role in IPS was evaluated using the lethally irradiated haploidentical (B6 → B6D2F1) murine model. Investigators found increased expression of CCL2 in lungs undergoing IPS that paralleled the recruitment of leukocytes and cellular expression of CCR2 (165). Importantly, when CCR2-/- donor cells were used there was a reduction in lung infiltrating mononuclear cells resulting in the attenuation of IPS (165). Conversely, in a more aggressive lethally irradiated, MHC disparate IPS model (B10.BR → C57BL/6), the use of CCL2-/- or CCR2-/- as recipients resulted in a similar degree of host monocyte and donor T cell recruitment and IPS compared with wild type recipients (166). Collectively, these studies demonstrate that CCR2/CCL2 biology is more important in models where IPS evolves over the time course of two to four weeks and reflects the sequential recruitment of CCR2 expressing donor cells as compared with more aggressive forms of IPS where host APCs and other chemokines acting in parallel/series may play more important roles.

CCL3 (MIP-1α) is a potent chemoattractant of mononuclear cells expressing CCR1 and CCR5. Using the lethally irradiated, MHC disparate (C57BL/6 → B10.BR) murine model of IPS described above, investigators found elevated lung levels of CCL3 in the recipients of the CCL3+ donor cells compared with MHC disparate recipients (166). Collectively, these studies demonstrate the importance of specific CC chemokines in mediating the inflammatory response engendered in the lung during the pathogenesis of IPS.

**CX3C Chemokine Family Members**

Using the lethally irradiated haploidentical murine model of IPS, investigators also found augmented lung expression of CXCL10 (IP-10) and CXCL11 (I-TAC) that paralleled mononuclear cell recruitment during IPS (170). When other investigators used an irradiated murine MHC class I mismatched model of IPS, they too found elevated levels of CXCL9 (MIG) and CXCL10 that correlated with the recruitment of CXCR3-expressing CD8+ T cells to the lung by Day 7 after HSCT (70).
In vivo neutralization of CXCL9 or CXCL10, or HSCT using CXCR3−/− donor leukocytes led to a reduction of lung infiltrating CD8+ T cells and attenuated IPS demonstrating the importance of CXCR3/ligand biology in this model of IPS.

**Adhesion molecules and IPS**

In lethally irradiated allogeneic mouse models using ICAM−/− recipients, an aspect of the pathogenesis of IPS was found to be distinct from the development of GVHD in other traditional target organs (171, 172). The influx of T cells, macrophages, and neutrophils into the lungs and the production of inflammatory cytokines were dramatically decreased in ICAM−/− allogeneic HSCT recipients compared with wild-type controls. In contrast, systemic levels of these cytokines were unaffected, and GVHD-induced lesions in the liver and colon were at least as severe as those of the wild-type recipients. Furthermore, GVHD-mediated mortality was accelerated in ICAM−/− recipients at doses of allogeneic spleen cells that were otherwise not uniformly lethal, implying that ICAM-1 plays a critical role in the generation of IPS and may be a discerning element that segregates IPS from GVHD injury.

Attempts to block adhesion molecule interactions in humans undergoing HSCT have shown mixed results. In one series, patients with leukocyte adhesion deficiency (LAD) given allogeneic HSCT combined with anti-leukocyte function-associated antigen (LFA) and anti-CD2 treatment, achieved reasonable engraftment, but a majority of the patients developed GVHD (173). In a phase II study for high-risk ALL, graft failure and GVHD was prevented in patients given anti-LFA-1 and anti-CD2 (174). However, these patients tended to have long-lasting immunodeficiency and infectious complications.

**TARGETS OF INFLAMMATION AND INJURY DURING THE DEVELOPMENT OF IPS**

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 TNF-α and IFN-γ (175). It is conceivable, therefore, that pulmonary parenchymal cells can serve as targets for direct cell-mediated damage, but definitive proof for this has yet to be shown.

Allogeneic-mediated endothelial damage has been demonstrated by transfer of allogeneic lymphocytes to immune-deficient mice (176, 177). Endothelial cell (EC) injury has been observed after allogeneic HSCT and has been implicated as a direct contributor to the development of several complications, including GVHD, VOD, and thrombocytic microangiopathy (178). Clinical and experimental IPS is associated with evidence for EC injury and leak as demonstrated by pulmonary edema, enhanced total protein levels in BAL fluid, and increased wet-to-dry lung weight ratios. Moreover, leukocyte infiltration during IPS is accompanied by significant apoptosis of the pulmonary vascular endothelium (76). EC apoptosis coincides with the onset of pulmonary pathology, is associated with elevations in BAL fluid TNF-α levels, and is accompanied by evidence for EC activation as measured by enhanced mRNA expression of adhesion molecules (76). The administration of a soluble TNF-α–binding protein (rhTNFR:Fc) from Week 4 to Week 6 after allogeneic HSCT significantly reduces EC apoptosis and lung histopathology observed in mice (76).

By contrast, epithelial apoptosis, generally ascribed to T cell–mediated injury and considered pathognomonic for acute GVHD in other target tissues, has not been consistently observed in allogeneic HSCT recipients with lung injury. The unique aspects of epithelial anatomy in the lung may help explain this discrepancy. Since there is no stratification or layering of pulmonary epithelial cells as in the skin or intestine, identification of epithelial cell apoptosis by histologic criteria can be more challenging. Experimental studies have, however, provided evidence for epithelial injury during IPS. Panoskaltis-Mortari and coworkers demonstrated that IPS was associated with injured alveolar type II cells and increased frequencies of cytotoxic T lymphocytes in a model of early-onset IPS (7). The same group later showed that keratinocyte growth factor, a mediator of epithelial cell proliferation and a growth factor for type II pneumocytes, diminished IPS injury by dampening the immune response to chemoradiotherapy and by accelerating repair of the damaged tissue, specifically alveolar type II epithelial cells (72). It was further shown in a murine model of obliterative bronchiolitis after HSCT that bronchial epithelial cells expressed MHC class II and were surrounded by cells expressing granzyme B prior to the occlusion of the airways (69).

**PRECLINICAL INTERVENTIONS FOR IPS**

Keratinocyte growth factor (KGF), or FGF7, is a mediator of epithelial cell proliferation and mesenchymal–epithelial interactions, and a growth factor for type II pneumocytes (179–181). KGF is protective against chemotherapy- and radiation-induced injury in various rodent models (182). In mouse HSCT models, in vivo administration of exogenous KGF, completed prior to conditioning, ameliorates GVHD (183, 184). In investigations targeting pulmonary injury, KGF was protective in lethal models of radiation-, hyperoxia-, acid-, and bleomycin-induced lung injury in rats (185–187), possibly by facilitating repair of DNA damage in alveolar epithelial cells (188, 189). Furthermore, KGF induces increased lung surfactant levels (190, 191), potentiates alveolar fluid clearance by increasing Na,K-ATPase activity (192), decreases hyperoxia-induced apoptosis of alveolar type II cells, and may detoxify reactive oxygen species generated by injured cells (reviewed in Reference 193). In the acute, early-onset IPS mouse model, KGF diminished IPS injury by dampening the immune response to chemoradiotherapy and by accelerating repair of the damaged tissue, specifically alveolar type II (ATII) cells (72). Furthermore, an association between the ability of rHuKGF to up-regulate the expression of SP-A and to limit IPS injury in recipients of allogeneic HSCT was observed (194–196). To establish a causal relationship, rHuKGF was administered subcutaneously, for 3 consecutive days before irradiation, to SP-A–sufficient (SP-A+/+) and SP-A–deficient (SP-A−/−) mice given inflammation-inducing allogeneic T cells at the time of HSCT. rHuKGF failed to suppress the high levels of TNF-α, IFN-γ, and nitric oxide contained in BAL fluid collected on Day 7 after HSCT from SP-A−/− mice compared with wild-type, SP-A+/+ mice.

KGF-treated mice also had significantly lower levels of serum TNF-α than non–KGF-treated counterparts. This finding was consistent in both the complete MHC mismatched and the parent into F1 mouse HSCT models (184, 197). The association of lower TNF-α and LPS levels with less severe manifestations of GVHD and lung injury post-HSCT is consistent with the hypothesis that TNF-α and LPS contribute to IPS injury.

**THE DIAGNOSTIC AND THERAPEUTIC APPROACH TO PATIENTS WITH IPS**

The approach to HSCT patients with acute pulmonary dysfunction is complex and requires a variety of diagnostic tests and possible consultation with experts in the fields of pulmonology,
cardiology, nephrology, radiology, and critical care medicine. Because symptoms of respiratory distress can progress rapidly once established, the timely coordination of care is essential to optimizing outcomes. In addition, there are several diagnostic challenges to address in this setting as both pulmonary and nonpulmonary causes of respiratory compromise are possible. Determination of the severity of respiratory dysfunction, including an assessment of the need for supplemental oxygen support, overall fluid balance, renal function, and cardiac output, should be followed by radiographic imaging. In general, an initial chest X-ray or CT scan will identify the presence of lobar, multilobar, or diffuse pulmonary infiltrates. While such findings may impact the decision-making process, they are nondiagnostic in and of themselves.

In the absence of obvious left-sided heart failure or iatrogenic fluid overload, bronchoscopy with BAL should be strongly considered when multilobar or diffuse infiltrates are present. BAL fluid samples should be sent for a variety of diagnostic tests to determine the presence of community-acquired, hospital-acquired, and otherwise opportunistic infections. While accepted at many large transplant centers, the need to complete a BAL in HSCT recipients with significant respiratory compromise remains a matter of debate, particularly in those patients who are critically ill. Recently, Yanik and colleagues reported on the results of 444 bronchoscopy procedures completed on 300 (20% of all patients) who received HSCT at the University of Michigan from 2001 to 2007 (198). Procedural complications were noted in only 3.6% of cases, including hypoxemia (1.8%), bleeding (1.7%), and hypotension (0.2%). Importantly, only 2% of patients that were breathing spontaneously before bronchoscopy required endotracheal intubation and mechanical ventilation within the initial 48 hours after the procedure. While only 13% of BAL specimens collected in the first 30 days of HSCT were positive for infection, this number increased to 33% between Days 31 and 100. Hence, although the majority of HSCT patients requiring BAL within the first 100 days may have IPS, a significant number of individuals will have evidence for infection. To this end, BAL data resulted in changes in medical management in approximately 60% of cases, and modifications in antimicrobial therapy in just under half. Of note, only one recipient of allogeneic HSCT had evidence for Pneumocystis, underscoring the effect antimicrobial prophylaxis has on the scope of subsequent infectious complications. Since medical management of IPS (immunosuppression) and infection (antimicrobial therapy) are rather divergent, making the appropriate diagnosis has significant merit.

Current standard treatment strategies for IPS include supportive care measures in conjunction with broad-spectrum antimicrobial agents and intravenous corticosteroids. Unfortunately, responses to standard therapy are limited, and the mortality of patients diagnosed with IPS remains unacceptably high. Response to corticosteroid therapy has shown mixed but limited efficacy. On the one hand, high-dose corticosteroid therapy (> 4 mg/kg/d of methylprednisolone equivalent) has not been shown to improve outcome when compared with lower doses of corticosteroids (≤ 2 mg/kg/d) in patients diagnosed with IPS (15). On the other hand, in the presence of the DAH form of IPS, high-dose steroids may have increased efficacy with improved mortality (91% versus 67%) (199) and the addition of aminocaproic acid may further improve outcomes (41). However, the overall response to steroids for patients presenting with DAH remains disappointing (41, 47). Advances in supportive care, including the early institution of continuous venovenous hemofiltration (200), may help to improve survival in some patients, but prospective studies addressing the treatment of IPS, including the specific use of steroids, are lacking in the literature.

Insights generated from preclinical models of IPS suggest that neutralization of TNF-α may be a useful therapeutic strategy for IPS. In a recent translational research effort, etanercept was given subcutaneously at a dose of 0.4 mg/kg twice weekly for a maximum of eight doses in combination with systemic steroids and empiric antimicrobial therapy to a total of 15 patients with IPS (198). All patients had evidence for systemic inflammation and pulmonary vascular dysfunction prior to enrollment on study. Therapy was well tolerated overall. Ten of the 15 patients were able to completely withdraw from supplemental oxygen support within 28 days of therapy, and survival at Day 28 and Day 56 (from the first etanercept dose) was significantly improved. Based on these encouraging results, larger phase II (pediatric) and phase III (adult) trials are ongoing within the Bone Marrow Transplant Clinical Trials Network (phase III) and the Children’s Oncology Group (phase II).

Consistent with animal studies, not all patients with IPS responded to etanercept therapy. Categorizing patients with IPS based upon the presumed anatomic site of primary injury and exploiting mechanistic insights gained in the laboratory, as described above, may avail the use of other promising, non–cross-reactive agents for the treatment of IPS. It is conceivable that approaches to 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 (201, 202). Specifically, KGFs have been shown to be efficacious in reducing epithelial damage and the severity of acute GVHD in mice and humans (183, 184, 203) and experimental IPS after allogeneic HSCT (72), as well as protecting pulmonary endothelium from oxygen-induced injury. Similarly, future studies examining the role of surfactant replacement therapy, perhaps in combination with etanercept, might prove useful in overcoming the effects of epithelial injury and dysfunction on ventilation and oxygenation.

Finally, the role for specific chemokine receptor:ligand interactions in the development of experimental IPS suggests that these proteins may also be operative in the clinical setting as well, particularly if disease onset is later and inflammation by cellular, rather than soluble, effector predominates. Since IPS develops and progresses to respiratory failure despite significant systemic immunosuppression, it is possible that novel strategies directed toward inhibiting pathways of leukocyte recruitment to the lung may serve as future adjuncts to standard therapy intended to prevent or treat this serious complication.

**CONCLUSIONS AND FUTURE DIRECTIONS**

IPS remains a frequent and severe complication of allogeneic HSCT, and despite significant advances in critical care medicine, mortality rates remain unacceptably high with standard therapy. Although such lung injury occasionally occurs after autologous transplants, the allogeneic setting significantly exacerbates toxicity and increases the likelihood of treatment failure. Recent efforts in pre-HSCT prognostic risk assessments for developing IPS, including evaluation of pulmonary function (FEV₁ and DLCO), BAL fluid inflammatory mediator levels, and genetic analyses (single-nucleotide polymorphisms), show promise for identifying those patients at highest risk of developing IPS (45, 204–207).

In addition, the use of established preclinical models of IPS has resulted in a more robust understanding of the mechanisms that contribute to inflammation during the development of IPS. To this end, the findings of this statement support a shift away
from the current paradigm, in which noninfectious lung injury after HSCT is viewed simply as an idiopathic clinical syndrome and toward a process in which the lung is the target of a complex cytotoxic and immune-mediated attack. Soluble inflammatory mediators like TNF-α, LPS, and reactive oxygen species 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. Donor-derived cellular effectors may also be recruited to the lung by using specific receptor-ligand interactions that allow them to traverse through an inflamed pulmonary vascular endothelium and into the parenchyma, where they contribute to epithelial damage and dysfunction. Donor bone marrow–derived cells, epithelial progenitors, and other stem cells with multipotential differentiative capacity may also be recruited to the lungs after HSCT. The roles of such cells in repairing the injured lung after transplant is currently the subject of much debate and anticipation (reviewed in References 208–209). It is hoped that mechanistic insights from experimental models will form the basis for translational clinical research protocols with the goal of treating or preventing IPS after HSCT. As our understanding of pathways involved with the development of IPS improves, determining which patient will respond best to the new agents available in the therapeutic armamentarium will be critical to improving outcomes for HSCT patients.

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