Idiopathic pneumonia syndrome after bone marrow transplantation: the role of pre-transplant radiation conditioning and local cytokine dysregulation in promoting lung inflammation and fibrosis

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Summary. Pulmonary complications and graft-vs.-host disease (GVHD) remain severe threats to survival after bone marrow transplantation (BMT). Idiopathic pneumonia syndrome (IPS) accounts for nearly 50% of all the cases of interstitial pneumonitis after BMT. IPS is characterized by an early inflammatory phase followed by chronic inflammation and fibrosis of lung tissue; however, the immunopathogenesis of this disease is not yet clearly understood. This biphasic syndrome has been reported to be associated with pre-transplant radiation conditioning in some studies while others have suggested that GVHD or autoimmune phenomena may be responsible for its development. The early post-BMT phase is characterized by the presence of inflammatory cytokines whose net effect is to promote lymphocyte influx into lungs with minimal fibrosis, that leads to an acute form of graft-vs.-host reaction-mediated pulmonary tissue damage. Gradual changes over time in leucocyte influx and activation lead to dysregulated wound repair mechanisms resulting from the shift in the balance of cytokines that promote fibrosis. Using data from new animal models of IPS and information from studies of human IPS, we hypothesize that cytokine-modulated immunological mechanisms which occur during the acute and chronic phases after bone marrow transplantation lead to the development of the progressive, inflammatory, and fibrotic lung disease typical of idiopathic pneumonia syndrome.

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Idiopathic pneumonia syndrome following bone marrow transplantation

In the past few decades, bone marrow transplantation (BMT) has become an integral part of the therapy of several haematologic and nonhaematologic disorders. Despite the increasing clinical success rate of BMT, lung disease has remained a significant cause of morbidity and mortality among patients (Thomas *et al*. 1977;

Breuer *et al*. 1993; Quabeck 1994; Soubani *et al*. 1996). Pulmonary disease is also a major cause of death after BMT in humans, although generally the lung has not been considered a critical target organ in animal models of graft-vs.-host disease (GVHD). While the occurrence of infectious complications of the lung after BMT is high among humans transplanted with bone marrow, these do not generally occur in experimental animals maintained in pathogen-free environments. Yet chronic lung interstitial pneumonitis and fibrosis can still occur in these controlled experimental circumstances. Clinically, the development of pulmonary inflammation and fibrosis after bone marrow transplantation in the absence of identifiable infectious agents has been termed idiopathic pneumonia syndrome (IPS). This syndrome accounts for as many as 50% of the total cases of pneumonia after BMT (Meyers *et al*. 1982; Cordonnier *et al*. 1986; Weiner *et al*. 1989; Ettinger & Trulock 1991). IPS in humans is a severely debilitating disease with an average survival time from onset of clinical symptoms to death ranging between 4 and 6 years. Once established, the mortality rate of IPS can be as high as 70% (Quabeck 1994) and there are currently no promising therapeutic options available for treatment.

IPS is characterized pathologically by the presence of interstitial and alveolar pneumonitis, and interstitial fibrosis, in the absence of an identifiable infectious agent. Interstitial pneumonitis and fibrosis lead to alveolar congestion and decreased lung compliance, which is clinically manifested as dyspnea, tachypnea and hypoxemia. The cellular and molecular mechanisms that give rise to IPS remain an enigma.

Chronic lung inflammation and fibrosis

The lung is constantly exposed to the external environment with the attendant airborne allergens, pathogens, toxic chemicals, and natural and synthetic dusts. The natural defence against such offensive environmental agents is primarily clearance by resident alveolar macrophages. However, ineffective clearance and degradation by alveolar macrophages and/or tissue damage by the insulting agent is commonly followed by an inflammatory response by leucocytes into the lungs (referred to as interstitial pneumonitis), which aids in effective clearing of insulting environmental agents from the lungs. Pulmonary inflammation may also represent the initial component of a progressive disorder such as fibrotic lung disease. Lung 'fibrosis' is a general term referring to inflammatory disorders of the lower respiratory tract associated with peri-luminal, intra-alveolar, and interstitial collagen deposition (Crystal *et al*. 1984; Crystal *et al*. 1991). Collagen deposition in the pulmonary interstitium and intra-alveolar spaces is a process of normal wound healing; but while this normal process is localized in space and confined in time, fibrotic lung diseases may involve the entire organ and are often chronic, progressive processes (Crouch 1990; Wolff & Crystal 1997). The severity of fibrosis has been found to correlate with the degree of inflammatory cellularity in fibrotic lungs (Fulmer *et al*. 1979; Cherniack *et al*. 1991). Current concepts on the pathogenesis of pulmonary fibrosis propose an initial stage involving an influx of inflammatory cells into the interstitium, which together with activated resident cells, are thought to release a variety of cytokines and other polypeptide mediators that stimulate fibroblast proliferation and collagen secretion. Genetic factors have also been proposed to influence the development of fibrosis, although there is little evidence for the presence of a 'fibrosis gene' (Marshall *et al*. 1997). A more likely genetic influence on fibrosis is the genetic control of cytokine synthesis and/or the response of cells to these cytokines in lung tissue. A number of cytokines secreted by inflammatory cells and resident lung cells have been implicated in the pathogenesis of pulmonary fibrosis. Pro-inflammatory cytokines such as interleukin-1β (IL-1β), tumour necrosis factor-α (TNF-α), and IL-6 (Agelli & Wahl 1986; Martinet *et al*. 1996; White & Das 1997), chemokines such as IL-8, macrophage inflammatory protein-1α (MIP-1α), and macrophage chemotactic protein-1 (MCP-1) (Berman *et al*. 1990; Driscoll 1994; Martinet *et al*. 1996), and cell surface adhesion molecules (Dupuis & Mcdonald 1997; Southcott *et al*. 1998), are produced by cells in the lungs during inflammatory processes. Cytokines such as IL-2, interferon-γ (IFN-γ), and IL-12, that direct type-1 helper T cell (T_h1) responses, and IL-4, IL-10, and IL-13, that promote type-2 helper T cell (T_h2) responses, have also been identified in the lungs during acute and chronic inflammation (Agelli & Wahl 1986; Marshall *et al*. 1996; Martinet *et al*. 1996). Collagen gene expression by fibroblasts is known to be regulated by various cytokines, such as IL-1β, TNF-α, transforming growth factor-β (TGF-β), and IFN-γ (Elias *et al*. 1990a; Grande *et al*. 1997; Coker & Laurent 1998). Importantly, TNF-α (Piguet *et al*. 1993; Grande *et al*. 1997), TGF-β (Khalil *et al*. 1996; Grande *et al*. 1997), IL-6 (Shahar *et al*. 1996), and IL-1β (Kline *et al*. 1993; Zhang *et al*. 1993) have all been demonstrated in the fibrotic lungs of patients (Cantin *et al*. 1988). Additionally, chemokines such as

MIP-1 (Standiford *et al*. 1993b), MCP-1 (Standiford *et al*. 1993a; Smith *et al*. 1995), and IL-8 (Ogushi *et al*. 1997) and cell adhesion molecules such as endothelial leucocyte adhesion molecule-1 (ELAM-1), intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) (Nakao *et al*. 1995), have also been hypothesized to play key roles in the recruitment of leucocytes to fibrotic lungs and promotion of the pro-fibrogenic environment therein. Furthermore, reduced levels of antifibrogenic factors such as prostaglandin E2 (Wilborn *et al*. 1995) and IFN-γ (Narayanan *et al*. 1992) have also been found in fibrotic lungs, suggesting that the interplay between pro- and antifibrotic factors in the lung may be critical for maintaining homeostatic levels of collagen in lung tissue.

Association of IPS with graft-versus-host disease

The onset of IPS after BMT often coincides with the occurrence of graft-vs.-host disease (GVHD). Although a massive amount of information on GVHD mechanisms has accumulated over the years through animal experimentation, few studies have considered the lung as a critical target organ of GVHD. The major target organs are typically considered to be skin, spleen, gastrointestinal tract, and liver; however, the designation of IPS as a complication of BMT has increased awareness of the need to understand the mechanisms by which the lung becomes a target of injury in those patients who develop IPS and to elucidate whether IPS is a unique disease or yet another target site of GVHD. The graft-vs.-host reaction is complex, involving recognition of host histocompatibility antigens by donor T cells, followed by cellular activation and cytokine secretion at both the afferent and efferent phases of the reaction (Ferrara 1993; Ferrara *et al*. 1996). The incidence of GVHD is dependent not only upon the extent of donor-host histoincompatibility, but also on sex mismatch, recipient age, insufficient T cell-depletion of bone marrow, pre-transplant myeloablative conditioning, post-transplant immunosuppressive therapy, and status of the underlying disease (Chao 1992). It has been shown that mature T cells contaminating the marrow graft are responsible for the initiation of the two forms of GVHD: acute and chronic (Cantor 1972; Korngold & Sprent 1978; Vanbekkum 1980; Ferrara & Deeg 1991). Purging the marrow of T lymphocytes prior to transplant, an approach used by some BMT centres, has been found to correlate with decreased risk and severity of both forms of GVHD (Kernan *et al*. 1986; Mitsuyasu *et al*. 1986; Ash *et al*. 1990); however, the transfusion of T cell-depleted bone marrow causes other complications, such as poor engraftment and a higher risk of cancer relapse.

Acute GVHD by definition occurs within the first 100 days following BMT and is observed in 30–80% of allogeneic transplant patients (Ferrara & Deeg 1991; Chao 1992). The target organs for acute GVHD are usually considered to be the skin, liver, gastrointestinal tract, and the immune system, leading to profound immunosuppression. In the acute GVHD response, alloactivated donor T cells primarily produce T_h1-type cytokines, IL-2 and IFN-γ. These and other cytokines can, in turn, activate macrophages to secrete pro-inflammatory cytokines, including IL-1β and TNF-α (Ferrara 1993; Ferrara *et al*. 1996). This sequence of cytokine production which is associated with acute GVHD (Jadus & Wepsic 1992; Ferrara 1993; Ferrara *et al*. 1996) has been termed the 'cytokine storm' (Antin & Ferrara 1992). By inducing elevated expression of cell adhesion molecules in target organs, inflammatory cytokines produced during the acute GVHD process may facilitate binding of inflammatory cells in target tissues thereby increasing the possibility of recipient cell damage (Norton & Sloane 1991; Ferrara *et al*. 1996). The second form of GVHD, chronic GVHD, affects approximately 30–60% of patients and is defined as occurring beyond 100 days after grafting (Chao 1992). Clinical manifestations of chronic GVHD include scleroderma, liver dysfunction, rare gastrointestinal involvement, and the development of abnormalities in immune responsiveness such as hypergammaglobulinaemia and autoantibody production. Pulmonary involvement in chronic GVHD is quite common (Soubani *et al*. 1996). Infiltration and activation of $CD4^+$ T cells have been associated with chronic GVHD in animal studies (Parkman 1986). While the role of T_h1-type cytokines has been elucidated in acute GVHD, the network of pathogenic cytokines that cause and maintain chronic GVHD in BMT patients has not been established. However, studies in animal models have implicated a role for B cell-activating T_h2-type cytokines such as IL-4 (Doutrelepont *et al*. 1991; Ushiyama *et al*. 1995), IL-5 (Dobashi *et al*. 1987), IL-10 (Dewit *et al*. 1993), and a selective loss of T_h1-type cytokine production, such as that of IFN-γ, during chronic GVHD (Allen *et al*. 1993; Rus *et al*. 1995).

Idiopathic pneumonia syndrome temporally overlaps the two forms of graft-vs.-host disease (GVHD) (Soubani *et al*. 1996). Work from several groups (Stein-Streilein *et al*. 1981; Piguet *et al*. 1989b; Breuer *et al*. 1993) including our own (unpublished observations) has delineated the importance of graft-vs.-host reactivity.

Both lung disease and GVHD fail to develop in mice reconstituted with T cell-depleted allogeneic BM alone, demonstrating a common requirement for mature alloreactive T cells in the development of GVHD and pneumonitis (Shankar *et al*. 1998). Analyses of cytokine mRNA expression in lung tissue of GVHD mice have consistently shown that steady-state levels of IL-1β, TNF-α, IFN-γ, and IL-12 are significantly elevated at 3 weeks post-BMT (acute, nonfibrotic phase). Mice transplanted with allogeneic bone marrow in the absence of alloreactive T cells also displayed elevated expression of these cytokines with the exception of IFN-γ, indicating that expression of cytokines may have been induced by pre-transplant conditioning and/or the transplant procedure and is unrelated to the presence of alloreactive T cells in the graft. In contrast, at 12 weeks after transplantation (chronic, fibrotic phase) only TNF-α and IL-12 levels remained elevated in GVHD mice, suggesting prolonged macrophage activation. Moreover, expression of TNF-α and IL-12 in the chronic phase was dependent on the presence of alloreactive T cells in the recipient. Lung cytokine mRNA expression during the disease could not be classified strictly as a T_h1-or T_h2-type (Shankar *et al*. 1998) and since *in vitro* stimulated lung lymphocytes could secrete both T_h1 and T_h2 cytokines (unpublished observations) we believe that IPS may not involve cytokine changes characteristic of either 'classical' acute or chronic GVHD in the lungs.

Pre-transplant radiation conditioning and IPS

Total body irradiation has played an important role in the development of bone marrow transplantation therapy. While current human BMT protocols offer life-saving treatment for lethal malignancies and immunodeficiency diseases, a significant proportion of BMT recipients die from treatment-related side-effects such as GVHD and IPS. The development of IPS has been found to be associated with pre-transplant conditioning. A number of studies have demonstrated that the lungs are quite susceptible to tissue damage by irradiation (Travis 1980; Down & Steel 1983; Hill 1985). Lung shielding during radiation conditioning (Wilms *et al*. 1982; Lawton *et al*. 1989; Labar *et al*. 1992; Giacchino *et al*. 1993) and fractionated total body irradiation (Miale *et al*. 1987; Giacchino *et al*. 1993; Cosset *et al*. 1994; Penny *et al*. 1994) have been shown to reduce (albeit not eliminate) the incidence of IPS in patients, suggesting that radiation-induced lung damage may participate in the initiation of this syndrome.

The severity of GVHD has been related to the extent of pre-transplant radiation conditioning (Deeg *et al*. 1991; Xun *et al*. 1994; Hill *et al*. 1997; Holler *et al*. 1997; Shankar *et al*. 1999). Acute GVHD has been found to be closely associated with radiation conditioning in both humans and animals (Deeg *et al*. 1991; Biggs *et al*. 1992) while its role in chronic GVHD is unresolved. Inflammatory reactions induced by radiation injury very likely play a role in the induction of immune responses (Milburn *et al*. 1990; Holler *et al*. 1997) and may play an important role in localized GVHD. For example, irradiated skin was found more permissive to GVHD lesion formation than unirradiated skin in the setting of systemic GVHD (Desbarats *et al*. 1994). Indeed, modulation of pre-transplant radiation conditioning paradigms has been shown to ameliorate post-BMT mortality and injury to target organs (Cosset *et al*. 1994; Penny *et al*. 1994; Vriesendorp *et al*. 1994). Studies utilizing animal models have shown that pre-transplant radiation conditioning when combined with allogeneic BMT significantly enhances GVHD-associated mortality (Lehnert *et al*. 1986; Truitt & Atasoylu 1991). Whether pre-transplant radiation exposure also sensitizes BMT recipients to develop IPS is not clear. Radiation dose fractionation is a suggested option at several BMT centres since it has been demonstrated to reduce the incidence of restricted ventilation and impaired gas exchange (Tait *et al*. 1991). The occurrence of IPS among patients treated by BMT was correlated in one study with the absolute dose of radiation to the lung (Keane *et al*. 1981).

Cytokines induced by pre-transplant radiation conditioning and GVHD

Is IPS a result of synergy between pre-transplant radiation-induced lung damage and the graft-vs.-host reaction? This question has not yet been conclusively answered. We have demonstrated in a murine model that BMT with alloreactive T cells and radiation conditioning act synergistically to induce GVHD and IPS under conditions when neither could do so individually; GVHD and IPS could also be induced in the absence of radiation, but only if a tenfold higher dose of alloreactive T cells were transplanted (Shankar *et al*. 1999). This observation suggests that radiation may lower the threshold for development of IPS and GVHD via increased expression of cytokines, alloantigens and possibly costimulatory molecules. The high incidence of IPS in BMT patients with GVHD compared to those without ongoing GVHD, suggests that the lung must also be considered as a critical target tissue for the graft-vs.-host reaction and that lung disease may be due to the additive or synergistic effects of both radiation injury

and the graft-vs.-host reaction. Cytokines have been implicated in mediating GVHD, and both $CD4^+$ and $CD8^+$ T cells are sources of these factors (Jadus & Wepsic 1992). However, it must be noted that many of the T cell cytokines implicated in GVHD can also be secreted by a variety of non-T cells.

Radiation exposure is known to cause local and systemic cytokine production in the early period after irradiation and can persist for several weeks or months after radiation exposure (Tartakovsky *et al*. 1993; Morgan & Breit 1995; Rubin *et al*. 1995). The importance of radiation-induced cytokines in the development of BMT-related GVHD was addressed in a study by Xun *et al*. (Xun *et al*. 1994). These investigators found that BMT after a critical 'window period' of 4–7 days following irradiation alleviated acute GVHD. Total body irradiation of severe combined immunodeficiency (SCID) mice rapidly induced synthesis of IL-1β, IL-6 and TNF-α. These pro-inflammatory cytokines were postulated to prime host tissues for the optimal expression of target antigens or, alternatively, prime donor T-cells for maximal allo-activation.

There are several direct effects of radiation on lung tissue which could promote IPS. Ionizing radiation exposure to the lungs has been demonstrated to induce synthesis of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α, fibroblast growth factors such as TGF-β, and vascular adhesion molecules such as ICAM-1, ELAM-1, and selectins (Hallahan *et al*. 1989; Krivenko *et al*. 1992; Johnston *et al*. 1996; Thornton *et al*. 1996; Hallahan & Virudachalam 1997b). Moreover, direct irradiation of several cell types *in vitro* can increase expression of histocompatibility molecules (Hauser *et al*. 1993) and adhesion molecules such as E-selectin, ICAM-1, and VCAM-1 (Gaugler *et al*. 1997; Hareyama *et al*. 1998; Heckmann *et al*. 1998), which could promote better extravasation into lung tissues and enhance allorecognition by T cells. Finally, gamma irradiation can enhance the ability of stimulated monocytes to produce hydrogen peroxide (Gallin & Green 1987) and nitric oxide (Ibuki & Goto 1997), which may suggest an increased capacity for causing tissue damage via peroxidation of proteins, lipids, or nucleic acids, by peroxides and peroxynitrites.

Thoracic irradiation of mice has been shown to induce cytokine expression (Rubin *et al*. 1995; Johnston *et al*. 1996). We have discovered that pro-inflammatory cytokines are expressed in the lungs of mice that received either total body irradiation or thoracic irradiation (unpublished observations). The expression of TGF-α in the lungs following radiation has been shown to correlate with development of radiation pneumonitis and pulmonary fibrosis in both humans and rodents (Anscher *et al*. 1994; Finkelstein *et al*. 1994). Recent studies in transgenic animals have shown that constitutive expression of TNF-α by lung epithelial cells leads to progressive mononuclear cell infiltration and lung fibrosis (Miyazaki *et al*. 1995; Thrall *et al*. 1997; Sime *et al*. 1998). Thus, local production of cytokines in the lungs clearly can affect the development of lung inflammation and fibrosis. However, the contribution of circulating cytokines produced by cells outside of the lung after total body irradiation cannot be excluded and could exacerbate direct radiation effects in the lung.

A recent study on pneumonitis following allogeneic BMT demonstrated that alveolar macrophages from BMT patients were abnormal with respect to an increase in the number of alveolar monocytes and a decrease in expression of HLA class II (Milburn *et al*. 1993). However, whether alveolar or interstitial macrophages also display altered production of cytokines has not been addressed in humans with IPS. In contrast, several animal studies have given hints about the immunopathogenesis of IPS after BMT. Piguet *et al*. demonstrated using a murine GVHD model that a progressive lung disorder displaying signs of diffuse alveolar haemorrhage, alveolitis, and mononuclear interstitial pneumonitis, developed in recipients that received allogeneic donor T cells and thereby had ongoing GVHD. The lungs of these mice exhibited elevated levels of mRNA for TNF-α (Piguet *et al*. 1989b), a finding consistent with studies in our laboratory (Shankar *et al*. 1998). In the murine model of bleomycin-induced lung fibrosis TNF-α levels have been found to be elevated (Piguet *et al*. 1989a). It has also been reported that blocking radiation-induced TNF-α reduced the incidence of GVHD (Holler *et al*. 1995) inhibited the development of lung inflammation (Ulich *et al*. 1994).

A hypothetical mechanism for the induction of IPS after BMT: roles of radiation conditioning and graft-versus-host reactivity

The response of alloreactive T cells against class I and II MHC molecules or minor histocompatibility antigens is an attractive hypothesis for initiating and sustaining IPS. Although it is unclear which cells mediate GVHD and IPS, or what the exact specificity of the reactive cell is, it is reasonable to hypothesize at this juncture that chronic activation of macrophages and T cells, and subsequent cytokine release in the interstitial space, is responsible for tissue remodelling after injury – a consequence that results in fibrosis in the lungs. Given the importance of both $CD4^+$ and $CD8^+$ T cells in GVHD (Jadus & Wepsic 1992; Kelemen *et al*. 1993) and the well-described

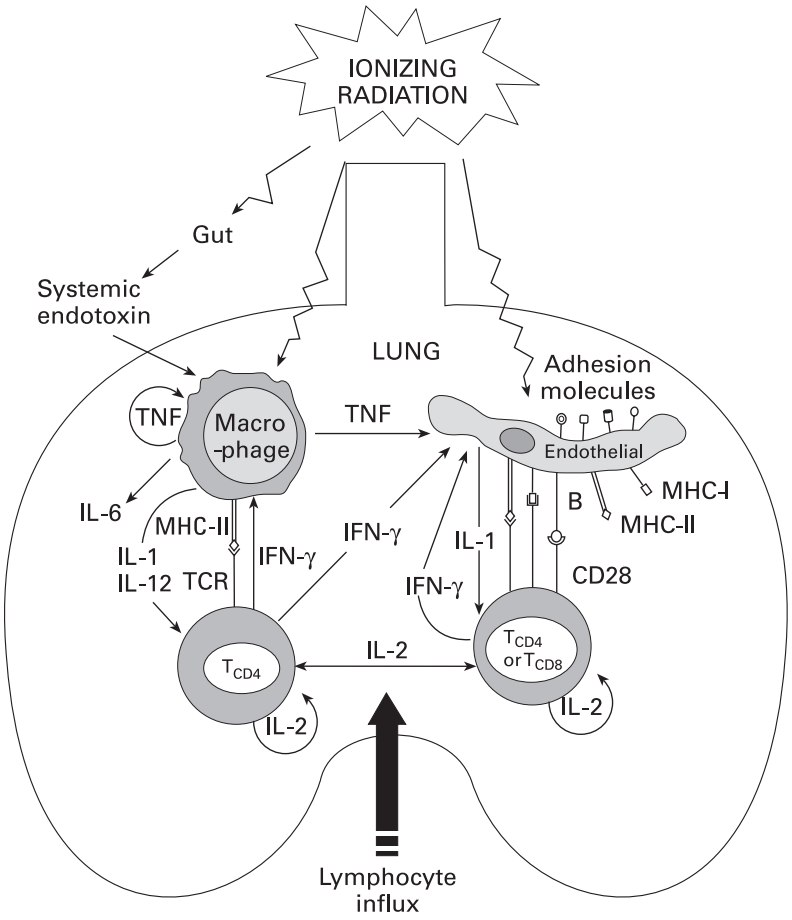

Figure 1. Pre-transplant irradiation can induce cytokines that lead to the expression of MHC, costimulatory and/or cell adhesion molecules on pulmonary tissue. Cytokine networks may induce an environment that promotes homing and activation of allogeneic T cells in the lungs.

importance of T cells in a variety of chronic lung injury models, it is highly conceivable that T cells contribute to the onset and progression of IPS, directly through effector cell functions and indirectly through cytokine release and cytokine-mediated effects on other cells. The importance of progressive development of $CD4^+$ T cell inflammation in the development of fibrosis may be two-fold. First, these T cells can amplify the disruption of the normal tissue architecture by directly contributing to local injury via release of cytokines. Second, inflammatory T cells may promote the accumulation of fibroblasts in the local milieu and thus contribute to the repair process and the development of fibrosis. It is equally likely that interstitial and alveolar macrophages participate in development of IPS through release of cytokines such as IL-1β, TNF-α and IL-12. Over the last decade, considerable evidence has emerged to suggest that cytokines constitute important stimuli for collagen deposition in pulmonary fibrosis (Agelli & Wahl 1986; Elias *et al*. 1990b; Rubin *et al*. 1995; Martinet *et al*. 1996; Coker & Laurent 1998). Resident lung cells of the host and donor-derived alloreactive T cells may release cytokines after BMT that can modulate fibroblast proliferation and collagen secretion.

Given the association of lung irradiation with IPS one can envision how lung irradiation could lead to tissue damage or could enhance the damage caused by another ongoing process, such as GVHD (Figure 1). Radiation injury can induce pro-inflammatory cytokine secretion such as IL-1β from macrophages or endothelial cells. Macrophage-derived IL-12, a potent inducer of T_h1 responses mediated by $CD4^+$ T cells, can also be induced by irradiation. Interferon-γ secreted by activated T cells in turn can activate macrophages. Cytokines such as IFN-γ and TNF-α can directly or indirectly upregulate endothelial adhesion molecules (Hanseleit *et al*. 1995; Shen *et al*. 1997; Mohamadzadeh *et al*. 1998) thereby promoting lymphocyte homing to the lungs (Morgan & Breit 1995; Panes *et al*. 1995; Hallahan & Virudachalam 1997a). Lung irradiation may also directly induce or upregulate the expression of histocompatibility (MHC class I and II) antigens on lung epithelial and endothelial cells. In addition, several cytokines are also known to promote the expression of

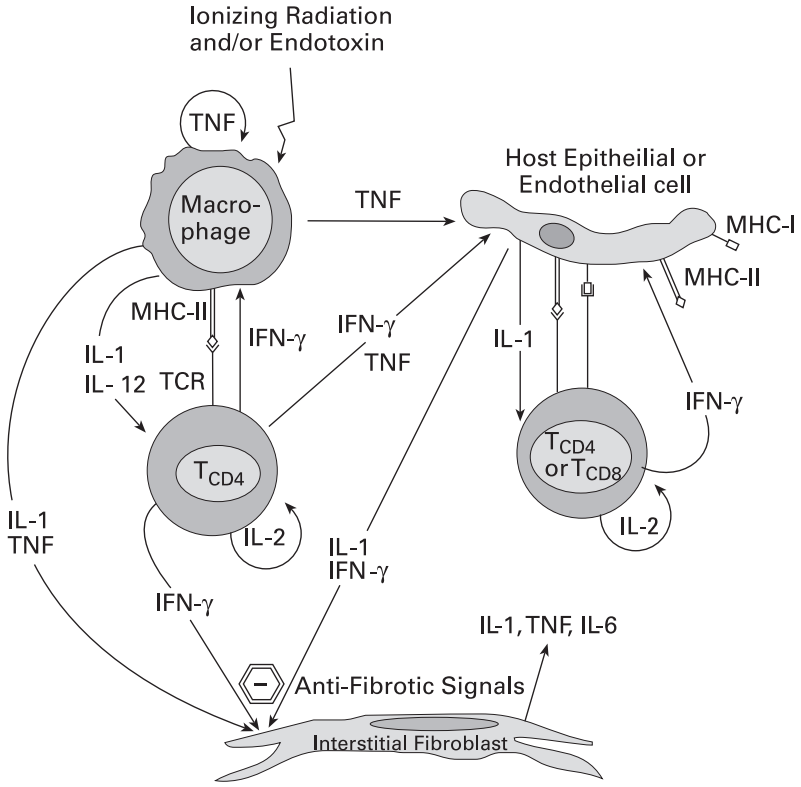

Figure 2. The initial inflammatory phase in the lungs after bone marrow transplantation is an antifibrotic phase. Homing and activation of alloreactive T cells to the lungs results in a cytokine milieu that promotes cellular influx into lungs while maintaining a nonfibrotic microenvironment.

costimulatory molecules such as B-7 (CD80/CD86) and CD40 on the surface of endothelial cells (Behrends *et al*. 1994; Briscoe *et al*. 1997) which, along with MHC class II expression, can provide these cells with a potent capacity to activate $CD4^+$ T cells. Finally, extraneous factors such as increased circulating endotoxin due to radiation-induced gastrointestinal tract damage (Hill *et al*. 1997), may activate resident macrophages (Nestel *et al*. 1992) and further induce pro-inflammatory cytokines in the lung. Endotoxin (bacterial lipopolysaccharide, LPS) has been shown to superinduce radiation-induced adhesion molecule expression *in vivo* (Eissner *et al*. 1996) and the presence of LPS in bronchoalveolar lavage fluid has been reported to correlate with the extent of lung injury (Cooke *et al*. 1996). In the presence of alloreactive T cells, these radiation-induced events could initiate a lung-specific immune reaction or non-specific tissue injury leading to the inflammatory and fibrotic lesions characteristic of idiopathic pneumonia syndrome. Indeed, modulation of pre-transplant radiation conditioning has been suggested as a means to control post-BMT mortality that is associated with noninfectious lung complications (Cosset *et al*. 1994; Penny *et al*. 1994; Vriesendorp *et al*. 1994). While high-dose thoracic irradiation alone (i.e. in the absence of BMT) can cause pneumonitis, low doses up to 10–11 Gy have been shown to be relatively noninflammatory in murine models (Travis 1980; Travis *et al*. 1980). However, studies utilizing animal models have also shown that radiation treatment in this range, when combined with allogeneic BMT, significantly enhanced GVHD-associated mortality (Lehnert *et al*. 1986; Truitt & Atasoylu 1991). We have demonstrated that a TBI dose as low as 6 Gy in conjunction with allogeneic BMT in mice was able to enhance the development of GVHD as well as IPS (Shankar *et al*. 1999). Thus, even low-level pre-transplant irradiation can enhance graft-vs.-host reactions.

In our experience with a murine model of IPS, the kinetics of lung disease differ markedly from the development of GVHD (Shankar *et al*. 1998; Shankar *et al*. 1999). While GVHD pathology is evident in liver, gut, and skin, early after BMT ('acute phase', up to 3 weeks post-BMT), lung pathology develops progressively beginning around 7–9 weeks post-BMT and remains unresolved beyond 25 weeks ('chronic phase'). This late onset IPS is similar to the onset of post-BMT lung disease in many patients (Soubani *et al*. 1996). Histological analysis of lung tissue from GVHD mice in the acute phase shows minor immunopathologic changes compared to control mice. In addition, there is a

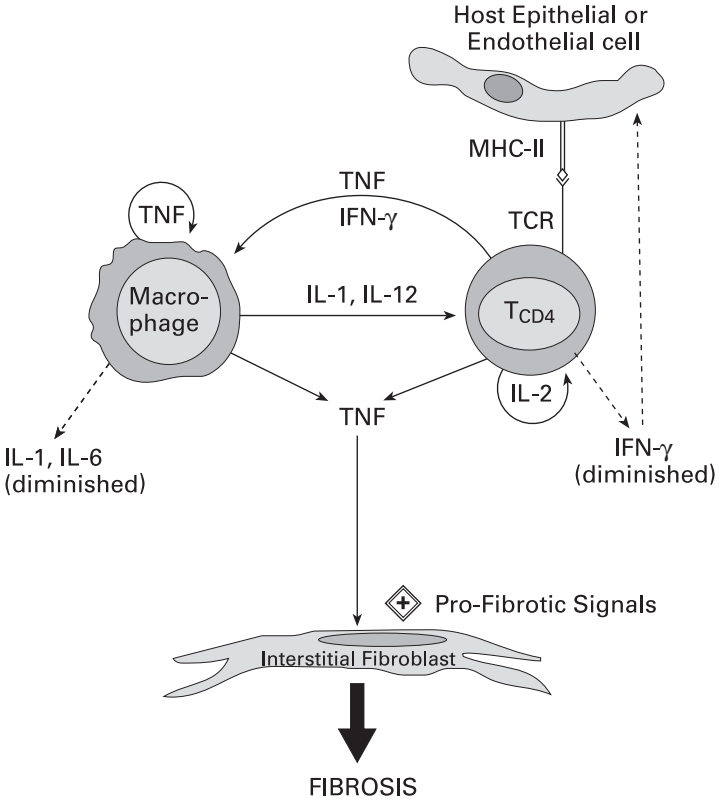

Figure 3. The late phase of IPS is characterized by a chronic fibrotic phase in the lungs. The balance of cytokines in the pulmonary microenvironment shifts to higher expression of tumour necrosis factor (TNF) and reduced expression of IFN-γ which promotes collagen secretion by interstitial fibroblasts, thereby leading to lung fibrosis.

complete lack of collagen deposition at the acute stage. In contrast, lungs of GVHD mice in the chronic phase display histopathological hallmarks of interstitial pneumonitis, such as prominent periluminal mononuclear cell infiltration, areas of alveolar congestion, and significant periluminal and diffuse interstitial fibrosis. Flow cytometric analyses of lung interstitial cells of GVHD mice revealed an increase of $CD8^+$ T cells during the acute phase, which decreased to normal levels at the later chronic phase. Conversely, the percentage of $CD4^+$ T cells, which is normal in the acute phase, increased progressively during the chronic phase. Analyses of cytokine mRNA expression in lung tissue in several experiments have shown that the steady-state levels of IL-1β, TNF-α, IFN-γ, and IL-12 are significantly elevated in the acute phase in lungs of mice with GVHD compared to untreated controls. In contrast, in the chronic phase after BMT only TNF-α and IL-12 levels remained elevated in GVHD mice, suggesting prolonged macrophage activation.

As mentioned earlier, pulmonary complications during acute GVHD are minimal in humans and are most commonly diagnosed during chronic GVHD (Soubani *et al*. 1996). A recently published review article on lung fibrosis by Coker and Laurent (Coker & Laurent 1998) concluded that fibrosis resulted from an imbalance in the normal cytokine regulatory pathways within the lung. Thus, based on our own observations and those of other laboratories, we propose that the acute phase may be characterized by the prevalence of an antifibrotic cytokine milieu that progressively changes into a chronic, pro-fibrotic environment. As shown in Figure 2, the first event in the initiation of an alloreactive response in the lungs after BMT may be mediated by donor-derived mature $CD8^+$ and/or $CD4^+$ T cells that recognize recipient-specific alloantigens in the context of MHC class I or class II, respectively. Secretion of IFN-γ as a result of T cell activation may act directly on macrophages that subsequently secrete IL-1β, TNF-α and IL-12. Radiation exposure can have direct effects such as induction of cytokine secretion and cell surface molecule expression on various host cell types including macrophages, lymphocytes, endothelial cells, epithelial cells, and fibroblasts (Goldstein & Lewis 1973; Eissner *et al*. 1996; Thornton *et al*. 1996; Heckmann *et al*. 1998). Activated endothelial cells, for instance, possess enhanced capability for augmentation of T cell-derived IFN-γ, via costimulatory molecules (Briscoe *et al*. 1997). IFN-γ can in turn induce adhesion molecule and costimulatory molecule expression by endothelial and

epithelial cells (Westphal *et al*. 1993). Endothelial cell expression of adhesion molecules, induced by pro-inflammatory cytokines (Ray *et al*. 1997; Shen *et al*. 1997; Mohamadzadeh *et al*. 1998) and ionizing radiation (Gaugler *et al*. 1997; Heckmann *et al*. 1998), may be key to the influx of alloreactive cells into the lungs (Hanseleit *et al*. 1995). Importantly, the key antifibrogenic stimuli in the acute phase may be provided by IFN-γ in combination with IL-1β and TNF-α. While IFN-γ is a potent inhibitor of fibroblast growth and function (collagen synthesis), IL-1β and TNF-α are each known to be pro-fibrogenic. Numerous *in vitro* studies have demonstrated that IL-1β, IL-6, TGF-β, or TNF-α individually are pro-fibrogenic, promoting fibroblast proliferation and collagen production (Matsushima *et al*. 1985; Elias *et al*. 1987a; Elias 1988; Elias & Lentz 1990; Elias & Reynolds 1990; Elias *et al*. 1990a; Elias *et al*. 1990b), while IL-1β and TNF-α in combination with IFN-γ deliver inhibitory, and thereby antifibrogenic, signals to fibroblasts (Elias *et al*. 1987b; Elias *et al*. 1988a; Elias *et al*. 1988b; Elias *et al*. 1990b; Narayanan *et al*. 1992). The pro-fibrogenic role of TNF-α is well documented since expression of a TNF-α transgene in lungs induces spontaneous lung inflammation and fibrosis (Miyazaki *et al*. 1995; Sime *et al*. 1998), and blocking of TNF-α in lungs inhibits lung inflammation and fibrosis (Ulich *et al*. 1994). The secretion of chemokines and pro-inflammatory cytokines may be sufficient to attract the influx of T cells into the lungs. However, the presence of high levels of IL-1β, TNF-α, and IFN-γ may preclude the development of fibrosis in the lungs in the acute phase after BMT.

As shown in Figure 3, the chronic phase after BMT is characterized mainly by the presence of $CD4^+$ T cells and elevated levels of TNF-α. Other pro-inflammatory cytokines return to normal constitutive levels at this time (Shankar *et al*. 1998). These basal levels of IL-1β and IFN-γ may be sufficient for their homeostatic roles in the immune response, such as T cell and macrophage stimulation. However, the reduced expression of IFN-γ and the continued presence of TNF-α, in the absence of elevated IL-1β, may shift the balance from an antifibrogenic environment to a profibrogenic milieu in lung tissue. We suggest that one mechanism by which fibrosis occurs after BMT may be due to elevated levels of TNF-α, which promotes fibrosis via stimulation of collagen expression by fibroblasts in an environment containing reduced levels of IL-1β and IFN-γ.

Hence, the outcome of pulmonary fibrosis following BMT is likely the result of an abnormal balance between cytokine signals that modulate the activity of fibroblasts in the lung. What regulates the progressive resolution of elevated levels of IFN-γ and IL-1β, yet maintains sustained elevated levels of TNF-α, needs to be categorically elucidated. It is hoped that the recently described animal models of IPS may be the necessary tools to discern the roles of these cytokines in the immunopathogenesis of this BMT-associated lung disease.

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