# Vigorous inflammatory responses in noninfectious pulmonary complication induced by donor lymphocyte infusion

*Miyuki Nishie,[1] Nobuharu Fujii,[1] Yusuke Mimura,[2] Takeru Asano,[1] Yuka Mimura-Kimura,[2] Keisuke Aoe,[2,3] Michinori Aoe,[4] Hiromi Nakashima,[1] Hideaki Fujiwara,[1] Hisakazu Nishimori,[1] Ken-Ichi Matsuoka,[1] Eisei Kondo,[1] Yoshinobu Maeda,[1] and Mitsune Tanimoto[1]*

**BACKGROUND:** Donor lymphocyte infusion (DLI) is used for treatment of hematologic malignancy relapse or mixed chimerism after allogeneic hematopoietic stem cell transplantation. Although graft-versus-host disease is well recognized as one of the adverse effects of DLI, there are limited reports on noninfectious pulmonary complications (NIPCs) after DLI.

**CASE REPORT:** A 55-year-old woman with acute myeloid leukemia received DLI for conversion from recipient predominant to complete donor chimerism on Day +193 after allogeneic HSCT. Eight weeks later, she complained of dyspnea with fever; chest computed tomography revealed diffuse, bilateral, ground glass opacity and reticular appearance. She was diagnosed as having NIPC based on serum and bronchoalveolar lavage fluid (BALF) findings. She was successfully treated with prednisolone (PSL) and completely recovered.

**DISCUSSION:** We analyzed the cell profile from the BALF and 27 cytokines and chemokines in the serum using the Bio-Plex platform. The cells consisted of recipient predominant macrophages and T cells. The serum cytokine and chemokine profile showed significant elevation of interleukin (IL)−1$\beta$, IL-6, IL-8, tumor necrosis factor-$\alpha$, macrophage inflammatory protein (MIP)−1$\alpha$, and MIP-1$\beta$, which declined with the improvement of symptoms after initiation of PSL treatment.

**CONCLUSION:** Inflammatory effectors by recipient cells, rather than allogeneic responses by donor cells, played an important role in the pathogenesis of NIPCs after DLI in the present case.

Allogeneic hematopoietic stem cell transplantation (HSCT) is used to treat hematologic malignant diseases, often providing prolonged survival; however, relapse is the major cause of treatment failure. Withdrawal of immunosuppressive drugs is the usual initial step, and donor lymphocyte infusion (DLI) is performed for intervention of disease reinduction.[1] DLI is also used to convert from mixed to complete donor chimerism for prevention of graft failure or disease relapse.[2] The most significant adverse effect of DLI is graft-versus-host disease (GVHD), which typically develops 32 to 42 days after infusion in approximately 50% to 60% of patients.[3-5]

Among numerous DLI clinical trials,[3-8] only two reports on pulmonary complications induced by DLI have

**ABBREVIATIONS:** BALF = bronchoalveolar lavage fluid; DLI = donor lymphocyte infusion; IPS = idiopathic pneumonia syndrome; MIP = macrophage inflammatory protein; NIPC = noninfectious pulmonary complication; PSL = prednisolone; VEGF = vascular endothelial growth factor.

From the [1]Department of Hematology and Oncology and the [4]Department of Laboratory Medicine, Okayama University Hospital, Okayama; and the [2]Department of Clinical Research, NHO Yamaguchi-Ube Medical Center, Ube, Japan; and the [3]Department of Medical Oncology, NHO Yamaguchi-Ube Medical Center, Ube, Japan.

This work was supported by the Japan Society for Promotion of Science KAKENHI (Grant 23591417 to NF).

*Address reprint requests to*: Nobuharu Fujii, MD, PhD, Department of Hematology and Oncology, Okayama University Hospital, Okayama, Japan 2-5-1 Shikata-cho, Kita-ku, Okayama 700-8558, Japan; e-mail: nfujii@md.okayama-u.ac.jp.

Received for publication April 18, 2015; revision received July 18, 2015; and accepted July 21, 2015.

doi:10.1111/trf.13283



**TRANSFUSION** 2016;56;231–236

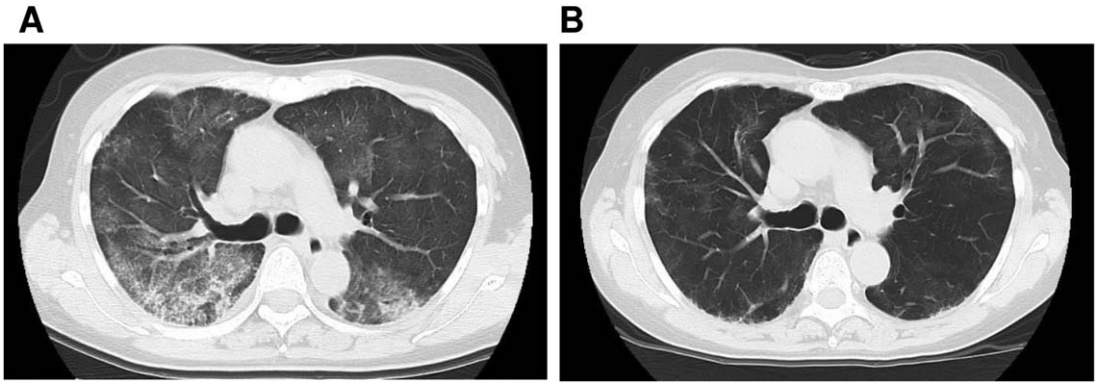


**Fig. 1. Chest CT scan. (A) Scan on Day 63 after DLI. (B) Scan on Day 100 after DLI (Day 12 after start of PSL treatment).**

been published. Lee and colleagues[9] reported that 9 of 38 patients receiving prophylactic DLI died of idiopathic pneumonia syndrome (IPS). They speculated that the high rate of fatal IPS may be associated with prior radiation to the chest. In another report, Badros and colleagues[10] found that interstitial pneumonitis developed in two patients after DLI. Neither of these reports included any detailed clinical information, and the underlying pathophysiologic mechanisms of this pulmonary complication remain unknown. In this report, we present detailed clinical information of a noninfectious pulmonary complication (NIPC) after DLI and further investigated the pathophysiologic mechanisms using serum and bronchoalveolar lavage fluid (BALF) findings.

## CASE REPORT

A 55-year-old Japanese woman with acute myeloid leukemia received allogeneic bone marrow transplantation (BMT) from an HLA-DRB1 allele 1 locus-mismatched unrelated donor in the first remission phase. The conditioning regimen consisted of 3.2 mg/kg busulfan for 4 days and 30 mg/m$^2$ fludarabine for 5 days. GVHD prophylaxis consisted of 0.03mg/kg tacrolimus and short-term methotrexate. Rapid engraftment was observed, and chimerism analysis demonstrated that 89% of whole blood cells were donor derived by Day +29 after BMT. Marrow aspiration on Day +53 revealed no blasts, confirming complete remission. No acute GVHD signs or symptoms were observed.

On Day +74, chimerism analysis of whole blood cells showed decreased donor-derived cells (66.3%). Concurrent lineage-specific chimerism analysis demonstrated that only 10.5% of CD3+ cells were donor-derived cells, although 73.5% of CD3– mononuclear cells (MNCs) and more than 95% of granulocytes were donor derived. Based on these findings, tacrolimus was tapered off and then discontinued; however, neither recovery of donor-derived CD3+ cells nor GVHD symptoms were observed. Approximately 1 month after discontinuation of the immunosuppressant, DLI (CD3+ dose of $4.5 \times 10^6$/kg recipient body weight) was performed from the same donor on Day +193 after allo-BMT. On Day 14 after DLI, the patient complained of spontaneous itchy and dry skin, with eruption on the face suspected to be GVHD. The skin eruption continued until Day 42 without progression and improved without treatment by Day 50. On Day 56, the patient complained of very mild dyspnea with a low-grade fever, which gradually worsened until Day 60. Chest radiography on Day 62 showed diffuse shadows in both lower lung fields. Chest computed tomography (CT) on Day 63 revealed diffuse, bilateral, ground glass opacity and reticular appearance predominantly in the lower lung fields (Fig. 1A). Pulmonary function tests showed a decline in forced vital capacity, from 3.21 L to 1.98 L, as well as in percentage of vital capacity from 128% to 80% and almost no change (78% to 80%) in forced expiratory volume in 1 second (FEV1), indicating restrictive lung dysfunction. On Day 78, fiber optic bronchoscopy and a bronchoalveolar lavage were performed. Due to the small sample, histologic findings of the transbronchial lung biopsy only showed trivial alveolar edema without evidence of leukemia infiltration; the specimen did not include bronchiole for differential diagnosis of COP/BOOP or for identification of the BO as chronic GVHD. Analysis of the BALF showed a total cell count of $3.4 \times 10^5$ cells/mL, with differential cell counts of 36% macrophages, 63% lymphocytes, and 1% granulocytes. Of the lymphocytes, 98% were CD3+ T cells, and the CD4/8 ratio was 1.72 (CD4, 62.4%; CD8, 36.3%). Bacterial, mycobacterial, and fungal cultures of the BALF were negative. A cytomegalovirus antigenemia test was also negative. The BALF was also shown to be negative for influenza A (including subtypes H1 and H3), influenza B, RSV A and B, HMPV, PIV 1-4, coronavirus OC43, 229E, NL63, HKU1, rhinovirus/enterovirus, adenovirus, and bocavirus by a respiratory viral panel fast assay (Luminex xTAG, Luminex Diagnostics, Toronto, Canada).

**TABLE 1. Chimerism analysis of PBMNCs and BALF cells before PSL treatment**

| Cells | Peripheral blood | BALF |
|---|---|---|
| CD3+ MNCs | 9.4% | 10.7% |
| CD3– MNCs | 86.8% | 80.5% |
| Neutrophils | 100% | NA |

Based on these findings, we diagnosed NIPC induced by DLI. On Day 88, prednisolone (PSL) was administered at a dose of 1 mg/kg/day, which led to rapid clinical improvement. The follow-up CT 12 days after initiation of PSL treatment showed remarkable improvement of the abnormal shadows (Fig. 1B). The PSL dose was tapered off and discontinued 9 months after onset, without recurrence of NIPC.

## MATERIALS AND METHODS

### Collection of sera and BALF

All blood samples for cytokine determination were collected from the patient before PSL treatment, as well as at four weekly time points after the initiation of PSL treatment, with written informed consent. Cells from the BALF were also collected for phenotypic and chimerism analysis; BALF serum and cells were separated immediately and stored at −20°C until the assays were performed.

### Chimerism analysis of peripheral blood cells and invaded cells in BALF

To clarify whether the NIPC after DLI was induced only by the infused donor T cells, chimerism of peripheral blood mononuclear cells (PBMNCs) and BALF was assessed by polymerase chain reaction analysis of short tandem repeat loci using a cell line identification system (Cell ID, Promega Corp., Madison, WI), according to the manufacturer's instructions.

### Multiplex immunoassay analysis of cytokines

Cytokines in sera were quantitated using a suspension array system with a pro human cytokine group 27-plex panel (Bio-Plex, Bio-Rad Laboratories Inc., Hercules, CA). This panel includes the cytokines and chemokines interleukin (IL)-$\beta$, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p70, IL-13, IL-15, IL-17A, eotaxin, fibroblast growth factor (FGF)-basic, granulocyte–colony-stimulating factor (G-CSF), granulocyte-macrophage–colony-stimulating factor (GM-CSF), interferon (IFN)-$\gamma$, IFN-$\gamma$ inducible protein-10, MCP-1, macrophage inflammatory protein (MIP)−1$\alpha$, MIP-1$\beta$, platelet-derived growth factor (PDGF)-bb, RANTES, tumor necrosis factor (TNF)-$\alpha$, and vascular endothelial growth factor (VEGF), according to the manufacturer's specifications.[11]

## RESULTS

### T-cell chimerism in BALF was predominantly patient T cells

As shown in Table 1, approximately 10.7% of CD3+ MNCs and 80.5% of CD3– MNCs were of donor origin in the BALF, suggesting that T-cell chimerism was predominantly patient T cells. The BALF chimerism was approximately comparable to that of the peripheral blood of the patient.

### Cytokine profile

Serum analysis revealed a significant elevation of nine cytokines and chemokines (IL-1$\beta$, IL-2, IL-6, IL-7, IL-8, TNF-$\alpha$, MIP-1$\alpha$, MIP-1$\beta$, and VEGF) at the onset of NIPC, which declined soon after initiation of PSL treatment (Fig. 2A). The kinetics after PSL treatment are shown in Fig. 2B; gray shadow indicates the range of normal concentration calculated by median ± 2SD, using five healthy donors. The IL-2, IL-7, and VEGF levels were not significantly different from the normal range of the healthy controls. IL-5, IL-15, and GM-CSF were below the detection limit. The concentrations of the other 15 cytokines and chemokines were also measured, but did not change during the clinical course after PSL treatment (figure not shown).

## DISCUSSION

Since the first report of DLI for treatment of CML was published by Kolb and colleagues in 1990,[3] numerous clinical trials of DLI against various hematological malignancies have been reported.[4-8] Although the efficacy of DLI depends on the disease type and status, DLI has been shown to have a direct graft-versus-leukemia effect by infusion of alloreactive donor T cells. On the other hand, DLI may induce severe adverse effects, including severe GVHD. When our patient complained of dyspnea with fever, we suspected that the complication was pulmonary GVHD by alloreactive donor T cells recognizing alloantigens. Previous studies have suggested that infused lymphocytes lead to lung injury in murine GVHD models, although the lungs have not been generally considered a classic target organ of acute GVHD.[12-14] To clarify whether NIPC of the patient was pulmonary GVHD induced by infused donor lymphocytes, we performed chimerism analysis of T cells in the BALF and demonstrated that the cells recruited to the patient's lung were not exclusively derived from donor T cells. These results suggested that the pathogenesis of DLI-induced NIPC was not due to attack by donor-derived cytotoxic T cells directly recognizing the patient's pulmonary tissue, but by migrated patient T cells that were activated after DLI.

Analysis of the cytokine profile revealed the kinetics of inflammatory mediators, such as proinflammatory cytokines and chemokines in the serum. IL-1$\beta$, IL-6, IL-8, TNF-$\alpha$, MIP-1$\alpha$, and MIP-1$\beta$ were elevated at the onset of the

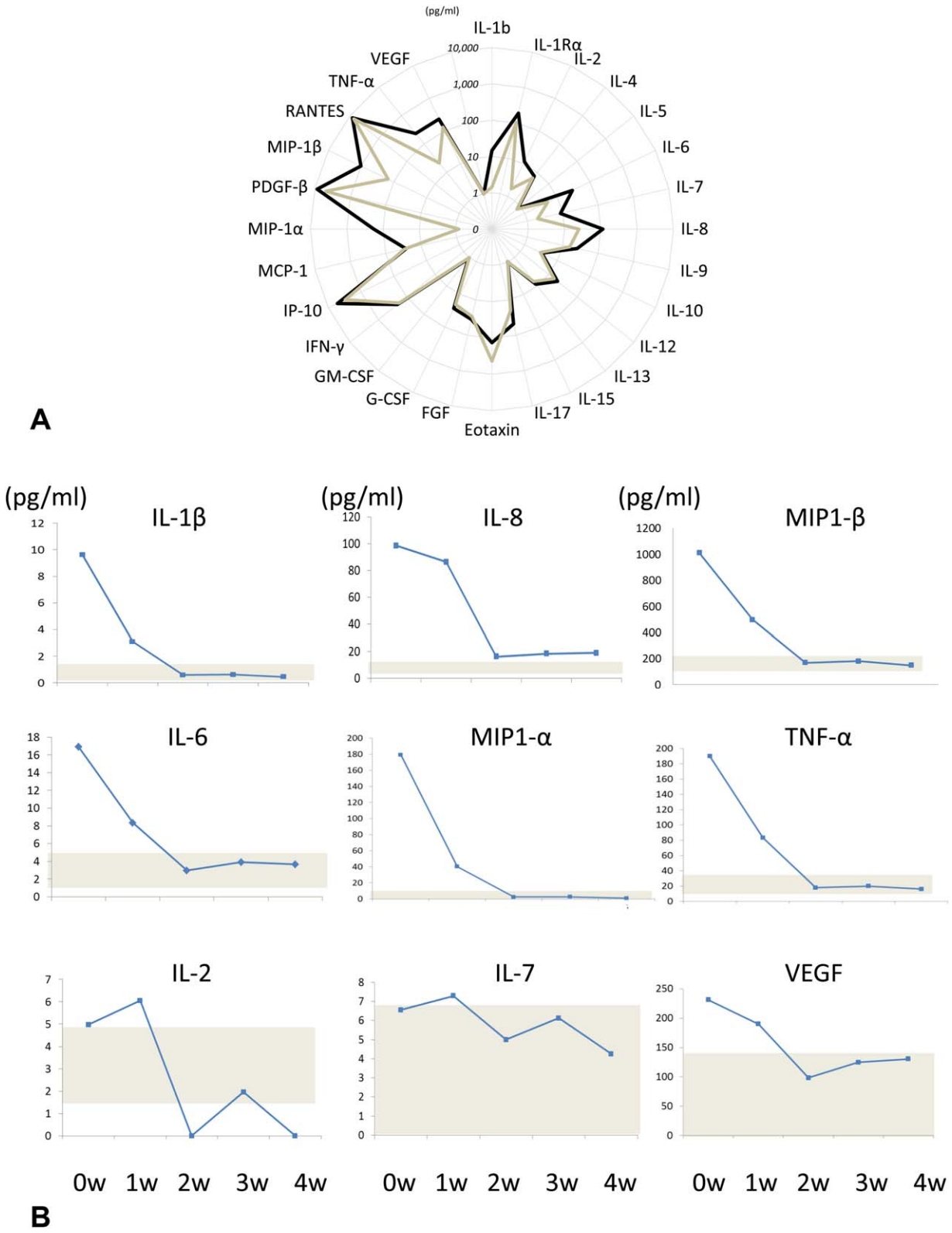


**Fig. 2. Multiplex immunoassay analysis of 27 cytokines and chemokines. (A) Black line indicates the levels before PSL treatment. Gray line indicates the levels 4 weeks after PSL treatment. (B) Weekly kinetics of the elevated serum cytokines and chemokines after PSL treatment. 0w indicates the levels before PSL treatment. Gray shadow indicates the range of normal concentration calculated by median ± 2SD using five healthy donors.**

patient's pulmonary symptoms and decreased after treatment with PSL. This phenomenon has previously been reported in IPS occurring in the early period after transplantation in humans.[15,16] The importance of chemokines in IPS has also been reported in murine models.[17,18]

IPS has frequently been a fatal complication after allo-HSCT, with a 3% to 15% incidence and median onset time between the second and third week after HSCT.[19,20] The clinical outcome for patients with IPS is poor, with mortality ranging from 50% to 80% and median time from diagnosis to death as short as 13 days. Risk factors for classical IPS include full-intensity conditioning with total body irradiation and acute GVHD.[21] Based on previous findings, it is thought that potential etiologies for IPS include the direct toxic effects of conditioning regimens and the release of inflammatory cytokines. The cytokine profile of the present case suggested that NIPC was a result of systemic immune response stimulated by DLI.

In conclusion, the elevated cytokines and chemokines measured in the present case may have played an important role in the pathogenesis of NIPCs after DLI. Further study of cytokine and chemokine profiles after DLI in a number of patients may clarify the pathophysiology of NIPC.

#### ACKNOWLEDGMENTS

The authors express their appreciation for the contributions from all the physicians and nurses at Okayama University Hospital.

#### CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

## REFERENCES

1. Elmaagacli AH, Beelen DW, Trenn G, et al. Induction of a graft-versus-leukemia reaction by cyclosporin a withdrawal as immunotherapy for leukemia relapsing after allogeneic bone marrow transplantation. Bone Marrow Transplant 1999;23:771-7.
2. Dey BR, McAfee S, Colby C, et al. Impact of prophylactic donor leukocyte infusions on mixed chimerism, graft-versus-host disease, and antitumor response in patients with advanced hematologic malignancies treated with nonmyeloablative conditioning and allogeneic bone marrow transplantation. Biol Blood Marrow Transplant 2003;9:320-9.
3. Kolb HJ, Schattenberg A, Goldman JM, et al. Graft-versus-leukemia effect of donor lymphocyte transfusions in marrow grafted patients. Blood 1995;86:2041-50.
4. Collins RH Jr, Shpilberg O, Drobyski WR, et al. Donor leukocyte infusions in 140 patients with relapsed malignancy after allogeneic bone marrow transplantation. J Clin Oncol 1997; 15:433-44.
5. Porter DL, Collins RH Jr, Hardy C, et al. Treatment of relapsed leukemia after unrelated donor marrow transplantation with unrelated donor leukocyte infusions. Blood 2000; 95:1214-21.
6. Dazzi F, Szydlo RM, Cross NC, et al. Durability of responses following donor lymphocyte infusions for patients who relapse after allogeneic stem cell transplantation for chronic myeloid leukemia. Blood 2000;96:2712-6.
7. Guglielmi C, Arcese W, Dazzi F, et al. Donor lymphocyte infusion for relapsed chronic myelogenous leukemia: prognostic relevance of the initial cell dose. Blood 2002;100: 397-405.
8. Schmid C, Labopin M, Nagler A, et al. Donor lymphocyte infusion in the treatment of first hematological relapse after allogeneic stem-cell transplantation in adults with acute myeloid leukemia: a retrospective risk factors analysis and comparison with other strategies by the EBMT Acute Leukemia Working Party. J Clin Oncol 2007;25:4938-45.
9. Lee CK, de Magalhaes-Silverman M, Hohl RJ, et al. Prophylactic T cell infusion after T cell-depleted bone marrow transplantation in patients with refractory lymphoma. Bone Marrow Transplant 2002;29:615-20.
10. Badros A, Barlogie B, Morris C, et al. High response rate in refractory and poor-risk multiple myeloma after allotransplantation using a nonmyeloablative conditioning regimen and donor lymphocyte infusions. Blood 2001;97:2574-9.
11. Oishi K, Mimura-Kimura Y, Miyasho T, et al. Association between cytokine removal by polymyxin B hemoperfusion and improved pulmonary oxygenation in patients with acute exacerbation of idiopathic pulmonary fibrosis. Cytokine 2013;61:84-9.
12. Watanabe T, Kawamura T, Kawamura H, et al. Intermediate TCR cells in mouse lung: their effector function to induce pneumonitis in mice with autoimmune-like graft-versus-host disease. J Immunol 1997;158:5805-14.
13. Clark JG, Madtes DK, Hackman RC, et al. Lung injury induced by alloreactive Th1 cells is characterized by host-derived mononuclear cell inflammation and activation of alveolar macrophages. J Immunol 1998;161:1913-20.
14. Cooke KR, Krenger W, Hill G, et al. Host reactive donor T cells are associated with lung injury after experimental allogeneic bone marrow transplantation. Blood 1998;92:2571-80.
15. Clark JG, Madtes DK, Martin TR, et al. Idiopathic pneumonia after bone marrow transplantation: cytokine activation and lipopolysaccharide amplification in the bronchoalveolar compartment. Crit Care Med 1999;27:1800-6.
16. Yanik GA, Ho VT, Levine JE, et al. The impact of soluble tumor necrosis factor receptor etanercept on the treatment of idiopathic pneumonia syndrome after allogeneic hematopoietic stem cell transplantation. Blood 2008;112: 3073-81.
17. Panoskaltsis-Mortari A, Hermanson JR, Taras E, et al. Acceleration of idiopathic pneumonia syndrome (IPS) in the absence of donor MIP-1 alpha (CCL3) after allogeneic BMT in mice. Blood 2003;101:3714-21.

18. Hildebrandt GC, Duffner UA, Olkiewicz KM, et al. A critical role for CCR2/MCP-1 interactions in the development of idiopathic pneumonia syndrome after allogeneic bone marrow transplantation. Blood 2004;103:2417-26.
19. Yanik G, Hellerstedt B, Custer J, et al. Etanercept (Enbrel) administration for idiopathic pneumonia syndrome after allogeneic hematopoietic stem cell transplantation. Biol Blood Marrow Transplant 2002;8:395-400.
20. Fukuda T, Hackman RC, Guthrie KA, et al. Risks and outcomes of idiopathic pneumonia syndrome after nonmyeloablative and conventional conditioning regimens for allogeneic hematopoietic stem cell transplantation. Blood 2003;102:2777-85.
21. Weiner RS, Horowitz MM, Gale RP, et al. Risk factors for interstitial pneumonia following bone marrow transplantation for severe aplastic anaemia. Br J Haematol 1989;71:535-43.