T-cell depletion prevents from *bronchiolitis obliterans* and bronchiolitis obliterans with organizing pneumonia after allogeneic hematopoietic stem cell transplantation with related donors

Markus Ditschkowski, Ahmet H. Elmaagacli, Rudolf Trenschel, Rudolf Peceny, Michael Koldehoff, Claudia Schulte, Dietrich W. Beelen

From the Department of bone marrow transplantation, University Hospital of Essen, Hufelandstr. 55, 45122 Essen, Germany.

Manuscript received August 28, 2006.
Manuscript accepted February 15, 2007.

Correspondence:
Markus Ditschkowski, MD, University Hospital of Essen, Department of bone marrow transplantation, Hufelandstrasse 55, 45122 Essen, Germany. E-mail: markus.ditschkowski@uni-essen.de

ABSTRACT

Bronchiolitis obliterans (BO) and bronchiolitis obliterans organizing pneumonia (BOOP) are late-onset non-infectious pulmonary complications (LONIPCs) following allogeneic hematopoietic stem cell transplantation (HSCT). In the present study 10 of 197 conventionally prepared stem cell recipients developed BOOP after 365 days and 6 patients developed BO 333 days post-transplant. No BOOP or BO was diagnosed following T-cell depletion ($p<0.05$). Chronic GVHD was ascertained in all BOOP patients and appeared significantly ($p<0,001$) more frequent in the conventional transplant group. The data confirm a strong association between T-cell activity, chronic GVHD, BO and BOOP and point out the impact of T lymphocytes in the pathomechanism of BOOP.

Key words: bronchiolitis obliterans, pneumonia, stem cell transplantation

Haematologica 2007; 92:558-561

Pulmonary complications develop in 30-60% of allogeneic haematopoietic stem cell transplantation (SCT) recipients and are considered as major cause of morbidity and mortality.[1] The use of prophylactic antimicrobial agents changed the spectrum of pulmonary complications from infectious to noninfectious, in particular late-onset noninfectious pulmonary complications (LONIPCs).[2] LONIPCs occur 3 months after transplantation in 10-15% of cases and are life-threatening complications which can significantly reduce quality of life.[3,4] Although the classification of LONIPCs is undefined, the most common categorizations include bronchiolitis obliterans (BO), bronchiolitis obliterans organizing pneumonia (BOOP) and interstitial pneumonia (IP) or idiopathic pneumonia syndrome (IPS).[5]

BOOP is less common than BO with an incidence of 1–1.7% in allogeneic SCT recipients.[5] Among SCT-recipients with histologic BOOP, 5-year survival was estimated at 33%.[6] Acute GVHD affecting the skin and chronic GVHD involving the gut and oral cavity were found to be the most important risk factors for the development of BOOP following SCT and there is some evidence that alloreactive T-lymphocytes in the context of acute and chronic GVHD may play an important role.[6] Recent reports suggest that mutations of the NOD2 and TLR-4 genes may influence the risk of severe acute GVHD after allogeneic SCT.[7] Since NOD2/ CARD15 is also expressed on bronchial epithelial cells the question was raised whether NOD2/ CARD15 and TLR-4 variants are also involved in the development of BO or BOOP.

This retrospective single-center study aimed to evaluate the impact of in vivo and in-vitro T-cell depletion or mutations of the NOD2 and TLR-4 (Thr399Ile) genes on the post-transplant development of BO and BOOP.

Design and Methods

Two-hundred and eighty-one patients undergoing BMT (n=33) or PBSCT (n=248) from genotypic HLA-identical (n=242) or HLA-mismatched (n=39) sibling donors between January 1998 and December 2003 were consecutively included. Patients were divided into two treatment groups characterized by either conventional transplantation of PBSC or BM (group 1, n=197) or *in vivo* T-cell depletion by administration of anti-CD52-antibody followed by transplantation of an unmanipulated graft (n=14), or receiving a CD34-purified transplant (n=70) (group 2,

Tabke 1. Clinical and demographic profile and transplant related data of the patient groups.

	Group 1 n=197	*Group 2 n=84*	
Patient gender			
male	110 (56%)	43 (51%)	
female	87 (44%)	41 (49%)	
Patient age [years] (median; range)	40 (16-63)	42 (20-62)	
Donor age [years] (median; range)	40 (12-72)	42 (18-66)	
Diagnosis			
Acute leukemia	93	11	
Chronic myeloid leukemia	69	53	
Myelodysplastic syndrome	23	4	
Non Hodgkin's lymphoma	4	8	
Idiopathic myelofibrosis	8	2	
Multiple myeloma	0	6	
Advanced disease stages	48 (24%)	11 (13%)	
Non advanced disease stages	149 (76%)	73 (87%)	$p<0.05$
Donor match			
HLA-identical	159 (81%)	83 (99%)	$p<0.05$
HLA-nonidentical	38 (19%)	1 (1%)	$p<0.05$
Gender constellation donor-recipient			
Female donor-male recipient	56 (28%)	29 (34%)	
Graft source			
Bone marrow transplantation (BMT)	31 (16%)	2 (2%)	p<0.05
Peripheral blood stem cell transplantation (PBSCT)	166 (84%)	82 (98%)	
Conditioning regimen			
Total body irradiation (TBI) + Cyclophosphamide	160 (81%)	79 (94%)	
Treosulfan + Cyclophosphamide	17 (9%)	2 (2%)	
Busulfan + Cyclophosphamide	20 (10%)	3 (4%)	
GVHD prophylaxis			
CD34 purification	0	70 (83%)	
Cyclosporin A (CSA) + Methotraxate (MTX)	197	0	
Alemtuzumab+Cyclosporin A (CSA)	0	14 (17%)	
Acute GVHD			
grade 0	50 (25%)	58 (69%)	$p<0.05$
grades I-II	109 (55%)	21 (25%)	$p<0.05$
grades III-IV	38 (19%)	5 (6%)	$p<0.05$
Chronic GVHD	165 (84%)	32 (38%)	$p<0.05$
Relapse	32 (16%)	6 (7%)	$p<0.05$
Deaths	80 (40%)	18 (21%)	$p<0.05$
Patients alive	117 (59%)	66 (79%)	$p<0.05$

n=84). The conditioning regimen consisted of cyclophosphamide (120 mg/ kg body weight [BW]) in combination with total body irradiation (TBI) in 4 daily fractions of 2.5 Gy (n=239), or oral busulfan (BU) (1 mg/ kg BW every 6 hours over 4 days) in combination with cyclophosphamide (120 mg/ kg BW) (n=23). Treosulfan (Medac, Hamburg, Germany), 12-14 g/ kg for 3 days was used with subsequent cyclophosphamide (120 mg/ kg BW) application in 19 patients. Inclusion criteria for CD34-PBSCT is as previously described.[8] Patients scheduled for CD34-PBSCT received a conditioning regimen with fractioned TBI, cyclophosphamide (120 mg/ kg), and thiotepa (10 mg/ kg BW) with anti-thymocyte globuline (ATG) (ATG-S, Fresenius, Bad Homburg, Germany) 10 mg/kg of BW for 4 days (n=70). GVHD prophylaxis consisted of a short course of methotrexate on days 1, 3, 6, and 11 in combination with continuous intravenous cyclosporine in group 1 patients (n=197). Patients with CD34-PBSCT received no further GVHD prophylaxis. For *in vivo* T-cell depletion alemtuzumab (Campath 1-H, 10 or 20 mg) was administered for 5 days followed by continuous intravenous cyclosporine application. Post-transplant T-cell add-backs were performed as previously described.[8] Patient demographics and treatment characteristics are summarized in Table 1. $CD34^+$ cell selection was performed using the CliniMACS device (Milteny Biotech, Bergisch Gladbach, Germany), as previously described.[8] Pre-transplantation histocompatibility testing of recipients and related donors generally consisted of low- and intermediate-resolution HLA-A, -B, -C, and high resolution HLA-DRB1 and DQB1 DNA-based typing as previously reported.[9] The diagnosis of BO and BOOP was based on clinical presentation, chest radiography, computed tomography (CT) or high-resolution CT (HRCT), pulmonary function tests (PFT) and pathological findings according to Palmas *et al.*[3] Patients were classified as having BO if a decline in the forced expiratory volume of 1 s (FEV1) to less than 80% of the predicted value and FEV1/ vital capacity (VC) less than 70% was observed. If lung biopsies could not be obtained for a definitive histological confirmation of BOOP, the diagnosis was assumed by correlating PFT findings (restrictive defect and normal expiratory flow) with CT and HRCT findings (peripherally distributed patchy air space consolidation, ground-glass attenuation, nodular opacities) in conjunction with clinical features after exclusion of an infectious etiology by bronchoalveolar lavages (BAL). Acute and chronic GVHD was graded according to standard criteria.[10,11] Single nucleotide polymorphisms 8, 12, and 13 of the NOD2/CARD15 gene were determined by a Taqman® protocol as published earlier by Hampe *et al.*[12] and Holler *et al.*[13] Genotyping for SNPs of TLR-4 were analyzed by a Lightcycler™ protocol using hybridization probes as published earlier by Hamann and co-worker.[14] PCR and subsequent melting curve analyses were performed using the Lightcycler device and related software.

Statistics

Cumulative estimates were calculated with the use of the Kaplan-Meier method. A proportional hazards Cox regression model was used to assess the independent effect of several covariates on the clinical endpoint development of BOOP.

Differences between time-to-event distribution functions were compared by a log-rank test (Mantel-Haenszel). Differences between the groups concerning disease stage, relapse, death, acute and chronic GVHD were evaluated with the use of the Fisher´s exact or χ^2 test.

Results and Discussion

The clinical features, demographic profile and transplant related data of patients are summarized in Table 1. Both patient groups were observed over the same time period with a median follow up of 30 months after transplantation. Patients of group one showed a significantly higher portion of advanced disease stages and HLA-nonidentical donors. This correspond to criteria used to assign patients to treatment groups. Bone marrow was transplanted more frequently in the conventional transplant group (group 1) compared to the T-cell depletion group (group 2). Group 1 patients presented a higher incidence of acute as well as chronic GVHD ($p<0.001$). Treatment related mortality in group 1 was 33.7 %±3.5%, significantly higher than the 17.2%±4.2% seen in group 2 ($p<0.0025$).

Among all hematopoietic stem cell recipients the incidence of BOOP was 3.6 % and of BO 2.1 %. BOOP occurred a median of 365 days after HSCT, and a BO median of 330 days post-transplant. Ten of the patients with a disease-free survival of more than 3 months after transplantation fulfilled the diagnostic criteria for BOOP and 6 for BO. The most common symptoms associated with BO and BOOP were dyspnea, dry cough and fever. Lung biopsies with consecutive histological verification of clinically suspected BOOP were obtained in 5 patients. All cases of BO and BOOP were observed in group 1 ($p=0.036$). The estimated probability for the development of BOOP 690 days post-transplant was 8.9%±2.7%, and for BO 3.3%±1.3%.

Clinical characteristic of the patients who developed BOOP or BO after HSCT are shown in Table 2. While the overall incidence of grades I and II acute GVHD was 46% for all transplant recipients, 90%-100% of the patients with BOOP or BO had grade I-II acute GVHD ($p<0.01$). Nine of the 10 patients with BOOP were male and all 10 BOOP patients received allografts from female donors. Although chronic GVHD was more common in BOOP and BO compared to the overall incidence (70%), differences did not reach statistical significance. BOOP and BO resolved or remained stable in 81% of all cases under treatment with bronchodilators, corticosteroids and immunosuppression. Two patients. one with BOOP and one with BO, died of respiratory failure due to pulmonary disease progression and one BO patient died of severe sepsis. All but one BOOP patient and all of the BO patients received peripheral blood stem cells as transplants.

Among the patients with BOOP and BO, heterozygous NOD2 mutations occurred with a frequency of 6.3% in recipients for SNP[11] and 6.3% in donors for SNP[12] respectively. Calculated haplotype frequencies did not differ from controls of an earlier reported study.[15] This resulted in an overall percentage of 88% pairs with unmutated SNPs at the patient and donor side. For TLR-4, only SNP Thr399Ile was shown to influence GVHD.[15] Here no differences in the mutation rate were observed for patients with or without BO or BOOP, neither on patient nor donor side (*data not shown*).

Table 2. Clinical and demographic profile of the patients who developed BO and BOOP after transplant.

	BOOP n=10	*BO n=6*
Median patient age [years] (range)	39 (26-59)	50 (33-54)
Median donor age [years] (range)	37 (18-62)	42 (33-54)
Occurrence [days] post transplant (median; range)	365 (250-500)	333 (85-450)
Male/female ratio (patients)	9/ 1	4/ 2
Male/female ratio (donors)	0/ 10	3/ 3
Advanced disease stages	4 (40%)	1 (17%)
HLA-nonidentical donor	2 (20%)	1 (17%)
Acute GVHD [grade I-II]	9	5
Acute GVHD [grade III-IV]	0	0
Chronic GVHD	9	6
Relapse	1	1
Patients alive	9	4
Median follow up [months] post transplant	47	24

Although the pathogenetic mechanisms for the development of LONIPCs are unclear there is some evidence that alloreactive T-cells have an impact since non-infectious lung complications were observed in the context of donor lymphocyte infusions.[4] Cooke *et al*. suggested an association between lung injury and alloreactive donor lymphocytes in a murine model[16] while Majeski et al. reported that respiratory reovirus1/L induction of intraluminal fibrosis used as an animal model of BOOP is dependent on T lymphocytes.[17] In our present study, BO as well as BOOP only developed among patients transplanted following conventional myeloablative conditioning combined with standard immunprophylaxis while T-cell depletion apparently seemed to protect against late pulmonary complications which corroborates the assumption of alloreactive donor T-cells to be involved in the inflammatory processes of BO and BOOP.[2,4,6] Clinical Improvement of BOOP following extracorporeal photopheresis was reported to implicate some clinical relationship between BOOP and chronic GVHD.[18] Although we could not identify acute or chronic GVHD as independent risk factors for BO or BOOP in multivariate analysis, acute as well as chronic GVHD were observed significantly less frequent in the T-cell depletion group.

Because of the recently reported association of single nucleotide polymorphisms (SNP) of the NOD2/CARD15 and TLR-4 gene with increased acute GVHD severity and TRM,[13,15] it was assumed that NOD2/CARD15 or TLR-4 variants may contribute to changes in host immunity, predisposing for BO or BOOP.[7] Despite the small number of cases, our results suggest BO and BOOP to develop independently from mutations of the NOD2 and TLR-4 (Thr399Ile) genes.

It is interesting to note that BOOP occurred almost exclusively in male recipients who received PBSCT from female donors. All patients except one who developed BO and BOOP were transplanted with G-CSF mobilized and

peripherally collected stem cells. This might imply that besides donor gender, the graft composition or graft type could also be relevant for the induction of late pulmonary complications. The influence of the graft origin on immunologic reactions after allogeneic transplantation was confirmed by the fact that, when compared with BMT, PBSCT is associated with a significantly increased risk for high grade acute GVHD and extensive chronic GVHD.[19] In addition to GVHD, conditioning-related toxicity as a major contributing factor for BO and BOOP has also to be taken into consideration.[20,21] Although in the present evaluation there is an apparent imbalance between the clinical features of the patient groups, T-cell depletion resulted in significantly less TRM. This was probably due to both a reduction in applied conditioning-related toxicity and effective GVHD-prophylaxis. So diminished acute toxicity in combination with delayed T-cell immune reconstitution[8] might be the cause for the significantly decreased occurrence of late noninfectious pulmonary complications in patients treated with T-cell depleted transplant procedures. Further clinical and experimental studies are needed to identify risk factors for LONIPCs and to substantiate T-cell mediated immune mechanisms in BO and BOOP to improve clinical outcome after allogeneic SCT.

Authors Contributions

MD wrote the manuscript and designed the study; DWB designed and supervised the study; AHE and RT performed statistical analyses; MK, RP, CS contributed in data collection.

Conflicts of Interest

The authors reported no potential conflicts of interest.

References

1. Chan CK, Hyland RH, Hutcheon MA. Pulmonary complications following bone marrow transplantation. Clin Chest Med 1990;11:323-32.
2. Sakaida E, Nakaseko C, Harima A, Yokota A, Cho R, Saito Y, et al. Late-onset noninfectious pulmonary complications after allogeneic stem cell transplantation are significantly associated with chronic graft-versus-host disease and with the graft-versus-leukemia effect. Blood 2003; 102: 4236-42.
3. Palmas A, Tefferi A, Myers JL, Scott JP, Swensen SJ, Chen MG, et al. Late-onset noninfectious pulmonary complications after allogeneic bone marrow transplantation. Br J Haematol 1998;100:680-7.
4. Duncker C, Dohr D, Harsdorf S, Duyster J, Stefanic M, Martini C, et al. Non-infectious lung complications are closely associated with chronic graft-versus-host disease: a single center study of incidence, risk factors and outcome. Bone Marrow Transplant 2000; 25:1263-8.
5. Afessa B, Litzow MR, Tefferi A. Bronchiolitis obliterans and other late onset non-infectious pulmonary complications in hematopoietic stem cell transplantation. Bone Marrow Transplant 2001;28:425-34.
6. Freudenberger TD, Madtes DK, Curtis JR, Cummings P, Storer BE, Hackman RC, et al. Association between acute and chronic graft-versus-host disease and bronchiolitis obliterans organizing pneumonia in recipients of hematopoietic stem cell transplants. Blood 2003; 102: 3822-8.
7. Hildebrandt G, Wolff D, Hertenstein B, Greinix HT, Rogler G, Brenmoehl J, et al. NOD/CARD15 variants of donor and recipient as critical risk factors for the development of bronchiolitis obliterans syndrome after allogeneic stem cell transplantation. Blood 2005;106:144.
8. Elmaagacli AH, Peceny R, Steckel N, Trenschel R, Ottinger H, Grosse-Wilde H, et al. Outcome of transplantation of highly purified peripheral blood CD34+ cells with T-cell add-back compared with unmanipulated bone marrow or peripheral blood stem cells from HLA-identical sibling donors in patients with first chronic phase chronic myeloid leukemia. Blood 2003;101:446-53.
9. Ottinger HD, Ferencik S, Beelen DW, Lindemann M, Peceny R, Elmaagacli AH, et al. Hematopoietic stem cell transplantation: contrasting the outcome of transplantations from HLA-identical siblings, partially HLA-mismatched related donors, and HLA-matched unrelated donors. Blood 2003;102:1131-7.
10. Glucksberg H, Storb R, Fefer A, Buckner CD, Neiman PE, Clift RA, et al. Clinical manifestations of graft-versus-host disease in human recipient of marrow from HLA-matched sibling donors. Transplantation 1974; 18:295-304.
11. Thomas E, Storb R, Clift RA, Fefer A, Johnson FL, Neiman PE et al. Bone-marrow transplantation. N Engl J Med 1975; 292:832-43.
12. Hampe J, Grebe J, Nikolaus S, Solberg C, Croucher PJ, Mascheretti S, et al. Association of NOD2 (CARD 15) genotype with clinical course of Crohn's disease: a cohort study. Lancet 2002; 359:1661-5.
13. Holler E, Rogler G, Herfarth H, Brenmoehl J, Wild PJ, Hahn J, et al. Both donor and patient NOD2/CARD15 mutations associate with transplant-related mortality and GvHD following allogeneic stem cell transplantation. Blood 2004;104:889-94.
14. Hamann L, Hamprecht A, Gomma A, Schumann RR. Rapid and inexpensive real-time PCR for genotyping functional polymorphisms within the Toll-like receptor -2, -4, and -9 genes. J Immunol Methods 2004; 285:281-91.
15. Elmaagacli AH, Koldehoff M, Hindal H, Steckel NK, Trenschel R, Peceny R, et al. Mutations in innate immune system NOD2/CARD 15 and TLR-4 (Thr399Ile) genes influence the risk for severe acute graft-versus-host disease in patients who underwent an allogeneic transplantation. Transplantation 2006;81:247-54.
16. Cooke KR, Krenger W, Hill G, Martin TR, Kobzik L, Brewer J, et al. Host reactive donor T cells are associated with lung injury after experimental allogeneic bone marrow transplantation. Blood 1998;92:2571-80.
17. Majeski EI, Paintlia MK, Lopez AD, Harley RA, London SD, London L. Respiratory reovirus 1/L induction of intraluminal fibrosis, a model of bronchiolitis obliterans organizing pneumonia, is dependent on T lymphocytes. Am J Pathol 2003;163:1467-79.
18. Oyan B, Koc Y, Emri S, Kansu E. Improvement of chronic pulmonary graft-vs-host disease manifesting as bronchiolitis obliterans organizing pneumonia following extracorporeal photopheresis. Med Oncol 2006;23: 125-9.
19. Stem Cell Trialists' Collaborative Group. Allogeneic peripheral blood stem-cell compared with bone marrow transplantation in the management of hematologic malignancies: an individual patient data meta-analysis of nine randomized trials. J Clin Oncol 2005; 23:5074-87.
20. Watkins TR, Chien JW, Crawford SW. Graft versus host-associated pulmonary disease and other idiopathic pulmonary complications after hematopoietic Stem cell transplant. Semin Respir Crit Care Med 2005;26:482-9.
21. Yoshihara S, Tateishi U, Ando T, Kunitoh H, Suyama H, Onishi Y, et al. Lower incidence of Bronchiolitis obliterans in allogeneic heamtopoietic stem cell transplantation with reduced-intensity conditioning compared with myeloablative conditioning. Bone Marrow Transplantation 2005;35:1195-200.