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Recent Thymic Emigrants: A New Method for Thymus Function Evaluation

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ABSTRACT

The factors affecting T cells reconstitution post haematopoietic stem cell transplantation (HSCT) are not well characterised. We carried out a prospective analysis of naive T cell reconstitution in 26 HSCT recipients before and during the first six months post transplantation. We analysed the recent thymic emigrants (RTEs) and thereby monitored thymic output and evaluated the thymus function using a new and easy method in comparing to the previous used methods. We found that the thymic-dependent pathway for the T cells reconstitution was activated from the second month in the majority of patients with increasing the numbers of naive T cells. We also compared the RTEs values between the patients with and without adenovirus reactivation, and we found that the patients with adenovirus reactivation had higher numbers of naive T cells on the six month post HSCT.

Keywords: ***Adenovirus- Hematopoietic stem cell transplantation -Thymus function- Recent Thymic Emigrants - Immune reconstitution.***

INTRODUCTION

The transplantation of allogeneic hematopoietic stem cells (HSCT) provides a potentially curative treatment for a variety of immunodeficiency and metabolic disorders, a plastic anaemia and haematopoietic malignancies. Through this procedure, thousands of subjects have been cured from their original disease.

The success of hematopoietic stem cells transplantation (HSCT) is determined by many parameters [1] include the type of haematological disorder [2,3], stage of disease at the time of transplant [4], human leucocytes antigens (HLA)-matching of donor and patient and whether the donor and patient are related or unrelated [5-8], pretransplant conditioning [9-12], T cell depletion [13-15], graft versus host disease (GVHD) prophylaxis [16], incidence and severity of GVHD [8,16,17], post transplant infections [8,16,24] patient age [25] and stem cell source [26]. While each of these parameters may have mutually exclusive effects on the outcome of the transplant, it is the combination of all of these parameters that will determine the ultimate success of the transplant.

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Furthermore, the effects of each of these parameters on immune reconstitution post HSCT are unclear. The immediate post transplant period is followed by a severe and often prolonged immune deficiency that results in prolonged susceptibility to infection [8, 17, 19-22]. Although infections that occur in the first month after engraftment probably result from deficiencies in both granulocytes and other mononuclear cell subsets, the more prolonged immune deficiency arises from deficiencies in effective CD4+ T cell and B cell reconstitution and immunosuppressive therapy [23, 27-29].

Following hematopoietic stem cell transplantation (HSCT), there is a prolonged period of profound immune deficiency, which includes defects in thymopoiesis [30].

This immune deficiency contributes to the high incidence of opportunistic infection, which continues for years after HSCT [22,32]. The etiology of the immune defect is multifactorial.

Thymopoietic defects resulting in decreased ability to generate new T cells after HSCT are important since complete immune reconstitution ultimately depends on

the generation of new T cells from hematopoietic stem cell (HSC), just as long-term myeloid and erythroid reconstitution depends on HSC engraftment. Transfer of committed progenitors or mature donor-derived T cells may permit short-term immune function. Analyses of patients after HSCT have demonstrated that the presence of immune function at one year or later was correlated with the number of CD4+CD45RA+ naive T cells, suggesting that immune function at later time points is dependent on the ability to generate new T cells [27,32].

One of the opportunistic pathogens after transplantation is adenovirus (AdV) that have emerged in the hematopoietic stem cell transplant (HSCT) recipient population as new techniques have reduced the risk of graft-versus-host disease (GVHD) but resulted in more profound and prolonged immunosuppression.

HSCT recipients are susceptible to invasive infections caused by adenovirus (AdV), including pneumonitis, hepatitis, colitis, and hemorrhagic cystitis [33-36]. AdV infections occur more frequently in paediatric than in adult HSCT recipients [34,37,38]. Patients who receive an allogeneic transplant, in particular with a T cell depleted or CD34 + (stem cell) selected graft, and patients who develop graft- versus -host disease (GVHD) have a higher incidence of AdV infection [39].

T cell recovery, as a multifaceted process including recovery of the thymic function and that of the regulatory T cell compartment, plays a key role in the clinical recuperation of patients post hematopoietic stem cell transplantation (HSCT) [23, 29,32]. Immunodeficiency post allogeneic stem cell transplantation is, on its part, associated with significant morbidity and mortality [22,41]. T lymphocytes are generated through two different pathways: thymus dependent and independent [42–46]. Particularly in the hematopoietic stem cell transplantation setting, Peripheral expansion of T cells can contribute significantly to the composition of the T cell compartment post HSCT [47].

As additional factors, radiotherapy [48] and graft-versus-host disease (GVHD) [49–52] post HSCT and thymic involution as a part of ageing have a negative impact on thymic function [51,53,54]. Thymic function has been assessed by imaging and analysis of T cell subtypes in blood but more recently also by measuring TRECs (T cell receptor excision circles). Quantitative measurement of TRECs using PCR is assumed to reflect thymic output. However, persistence of naive T cells and TREC dilution in peripheral blood by cell division complicate the interpretation of TREC data as a measure of recent thymic output [50,53].

Previously published studies report on a correlation between TRECs and the frequency of naive CD4+ T cells in blood among pediatric and adult recipients of HSCT suggesting that most naive T cells are processed in thymus [52]. Lewin [51] reports on a faster thymic recovery post HSCT among children indicating that the high residual thymic activity of early childhood might allow for a rapid regeneration of T cells. The level of TRECs correlates negatively with chronic GVHD (cGVHD) in most studies, [49–52,55] but conflicting results have also been reported [54,56]. Data is also indicative of an association between low TREC levels and post transplant infections [50,51,55]. As alternative for the TRECs estimation, we analysed the CD4+ recent thymic emigrants recovery by Flow cytometric Assay of CD4+CD31+ CD62L+CD45RO- T-cells. Naive CD4+ T cells that express CD31 antigen on their surfaces have high levels of TRECs.

METHODOLOGY

Patients

26 patients (median age 6 years, range 0.8-25 years) underwent HSCT. Median Age of donors 30 years, range 7-50 years. 69% of patients were male, 31% were female. Twenty –one received their grafts from matched unrelated donors (MUD) and five patients from matched related donors (MRD).All the donors and 22 patients were sero-positive for the adenovirus. All the patients received Aciclovir as Antiviral prophylaxis. The key characteristics of allogeneic patients are given in table 1.

Table 1 Clinical characteristic of the patients

Patients characteristic	Patiens Group (26)	
ALL/NHL	7	27 %
AML/CML/MDS	9	35 %
Fanconi Anaemia	3	12 %
Metabolic disorders	2	8 %
Primary Immune defect	5	19 %
MRD	5	19 %

MUD	21	81 %
BMT	19	73 %
PBSCT	7	27 %

***Abbreviations**: ALL = Acute lymphoblastic Leukaemia, AML = Acute myeloid Leukaemia CML = Chronic myeloid Leukaemia, MDS = Myelodysplastic Syndrome, MRD = matched related donor, MUD = matched unrelated donor, BMT = Bone marrow transplantation, PBSCT = Peripheral Blood stem cell transplantation.*

The viruses' reactivity during the study period was as follow: nine patients (35%) for cytomegalovirus (CMV), eleven patients (42%) for Epstein-Barr virus (EBV),eight patients(31%) for adenovirus (ADV) and three patients (12%) for the herpes human 6 (HH6). Five patients (19%) had no virus reactivity, twelve patients (46%) had one virus infection, eight patients (31%) had two virus infections, and one patient (4%) had three virus infections.

All eight patients who had adenovirus infection were positive for PCR in blood, nose fluids, stool and urine. Two of them had adenovirus infection before the transplantation.

Twenty-one of the patients (81%) had acute graft versus host disease, range from grade I – III, three patients (12%) had grade II of chronic graft versus host disease.

Sample preparation

Blood samples were collected once pretransplant and once every month after transplantation during the first six months post transplantation. Informed consent was obtained from all patients or their parents. The Study was done on whole blood.

Materials

CD4+ Recent Thymic Emigrant Enumeration Kit: Miltenyi Biotec, Germany, Lot N°: 5080729096.

Method

The principle of the estimation of CD4+ Recent Thymic Emigrants (RTE) kit is the detection of naive CD4+ T cells on the basis of the expression of CD4, CD31, CD62L and the absence of the expression of CD45RO. The whole blood was incubated with the RTE kit-reagents for ten minutes, then the erythrocytes were lysed using the erythrocytes-lysis reagent according to manufacturer's instructions. After centrifugation and fixation, the RTEs were analysed using flow cytometry.

Flow cytometry

The cells were analyzed on FACSCalibur using the Cell Quest software (Becton Dickinson, San Jose, CA, USA). Naive CD4+ cells were measured and expressed as the absolute number of CD4+CD31+CD62L+CD45RO- T cells in whole blood.

Statistical analysis

The data was analyzed using the Excel program and the statistical package for social scientist (SPSS), version 18.0 for windows. Metric data (absolute cell numbers) were calculated as median, minimal and maximal values and by Box- und Whisker-Plots shown. Values of $P<0.05$ were considered significant.

RESULTS

By all patients was the RTEs analysis done before the Radio-chemotherapy. The absolute count of RTEs (CD4+CD31+CD62L+CD45RO) in allogeneic recipients before transplant (a median of 93.055/ml) was low comparing with healthy controls because of the immunologic- haematologic effects of the underlying disease and the previous chemotherapy of the malignant disease on the thymus function. The Recent Thymic Emigrants were estimated before and every month after transplantation during the first six months of transplantation. The regeneration of RTEs starts slowly after the second month of transplantation increased slowly but not reaches the normal count after the sixth month.

We compared the RTEs values per ml between the patients with adenovirus reactivation, and we found that the patients with adenovirus reactivation had higher numbers of RTEs on the sixth month in compare to the third month.

Table 2 RTEs per ml in peripheral Blood of patients before and after allogeneic HSCT (Median, Minimal-/ Maximal values und Interquartile area).

Time of RTEs estimating	Median	Minimal	Maximal	Interquartile area
Before HSCT	93.055	6.930	691.980	436.273
1 Month after HSCT	390	0	2.960	1.500

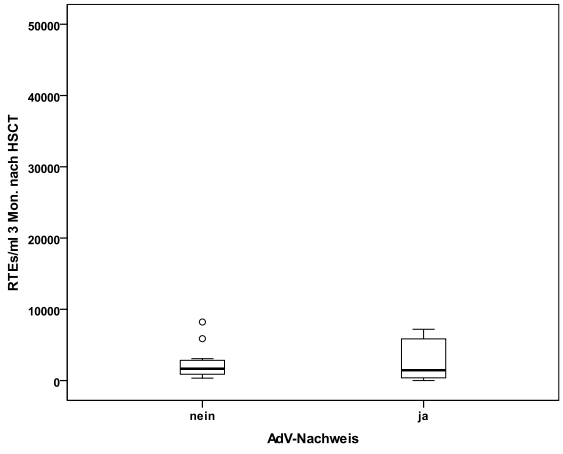
2 Month after HSCT	620	0	5.880	2.025
3 Month after HSCT	1.640	0	8.220	3.811
4 Month afte HSCT	1.230	0	120.080	5.218
5 Month after HSCT	2.700	0	147.060	25.780
6 Month after HSCT	3.930	0	196.240	62.005

Clinically significant adenovirus infection (positive –PCR) was associated with high RTEs/ ml values at sixth month (p=.047).

Table 3 RTEs /ml (median values-comparison) in the patients with adenovirus infection after third and sixth month of transplantation (significance limit)

Patients with adenovirus infection (AdV)	**RTEs/ml**	
Time after Transplantation	3 Months	6 Months
AdV-positive PCR	.752	.047

We found also, that the regeneration of the Recent Thymic Emigrants was faster in the patients with adenovirus reactivation (Positive Adenovirus –PCR) than those without adenovirus infection.



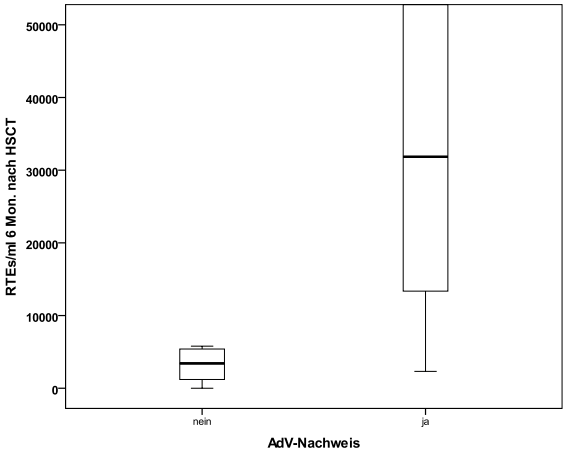


Figure 1 RTEs/ml in patients with and without molecular ADV evidence 3 Months (left) and 6 Months (right) after allogeneic HSCT (median, minimal-/maximal values)

DISCUSSION

In this prospective study focusing on pediatric recipients of allogeneic stem cell grafts we show that the regeneration of naive T lymphocytes and thymic recovery are slow. The delayed immune reconstitution is associated with an increased risk of clinical complications such as extensive cGVHD and a higher mortality.

Our study and those previously published indicate that thymic dysfunction exists even before transplant among stem cell graft recipients with a malignant disease and following a conventional chemotherapy [55]. In our material the thymic output of naive T cells (RTEs) levels were lower just before transplant among children with a malignant disease. This may be a result of the underlying, and in many cases malignant lymphoid, disease and its therapy [57].

We emphasize that in the interpretation of the RTEs results an important bias has to be recognized. Peripheral expansion of the T cell pool in the recipient during for example infection(s) may have diluting effect on the RTEs levels.

The kinetics of thymic recovery (Recent Thymic Emigrants) post HSCT demonstrated that the initiation of regeneration of naive T lymphocytes and thymic function appeared by 6 months post transplant among the allogeneic recipients [58].

The regeneration of the Recent Thymic Emigrants (RTEs) is increased used as prediction parameter for the

infections-risk by the immune suppressed patients [46,53,59]. Our work demonstrates that the RTEs analysis was a good marker for the antigen-specific T cells regeneration after allogeneic HSCT, because our result demonstrate that the patients with adenovirus reactivation have higher levels of RTEs after the six months than those without adenovirus infection.

Our study demonstrates the key effect of recuperation of the T cell compartment on a variety of factors influencing the outcome in pediatric stem cell transplantation. Importantly, the impact of therapy administered before transplant may have to be considered in for example designing the conditioning regimen, pre-emptive therapy of viral infections post transplant etc. For post-transplant follow-up of T cell reconstitution flow cytometry appears more readily employable, but studies on the potential use of a pre- and/ or post transplant 'immunological profile' in tailoring the therapy of an individual patient post transplant are warranted.

Conflict of interst: Not declared.

REFERENCES

1. Devine SM, Adkins DR, Khoury H et al. Recent advances in allogeneic hematopoietic stem-cell transplantation. J Lab Clin Med 2003; 141: 7–32.
2. Burnett AK, Eden OB. The treatment of acute leukaemia. Lancet 1997; 349: 270–275.
3. Clift RA, Appelbaum FR, Thomas ED. Treatment of chronic myeloid leukemia by marrow transplantation. Blood 1993; 82: 1954–1956.
4. Horowitz MM, Bortin MM. The role of registries in evaluating the results of bone marrow transplantation. In: Treleaven J (ed). Bone Marrow Transplantation in Practice 1992. Churchill Livingstone: Edinburgh, pp 367–377.
5. Davies SM, Wagner JE, Shu XO et al. Unrelated donor bone marrow transplantation for children with acute leukemia. J Clin Oncol 1997; 15: 557–565.
6. Gustafsson A, Remberger M, Winiarski J, Ringden O. Unrelated bone marrow transplantation in children: outcome and a comparison with sibling donor grafting. Bone Marrow Transplant 2000; 25: 1059–1065.
7. Hows JM, Yin JL, Marsh J et al. Histocompatible unrelated volunteer donors compared with HLA nonidentical family donors in marrow transplantation for aplastic anemia and leukemia. Blood 1986; 68: 1322–1328.
8. Marks DI, Cullis JO, Ward KN et al. Allogeneic bone marrow transplantation for chronic myeloid leukemia using sibling and volunteer unrelated donors. A comparison of complications in the first 2 years. Ann Intern Med 1993; 119: 207–214.
9. Feinstein L, Sandmaier B, Maloney D et al. Non myeloablative hematopoietic cell transplantation. Replacing high dose cytotoxic therapy by the graft-versus-tumor effect. Ann N Y Acad Sci 2001; 938: 328–337.
10. Michallet M, Bilger K, Garban F et al. Allogeneic hematopoietic stem-cell transplantation after nonmyeloablative preparative regimens: impact of pretransplantation and post transplantation factors on outcome. J Clin Oncol 2001; 19: 3340–3349.
11. Morecki S, Gelfand Y, Nagler A et al. Immune reconstitution following allogeneic stem cell transplantation in recipients conditioned by low intensity vs myeloablative regimen. Bone Marrow Transplant 2001; 28: 243–249.
12. Nagler A, Aker M, Or R et al. Low-intensity conditioning is sufficient to ensure engraftment in matched unrelated bone marrow transplantation. Exp Hematol 2001; 29: 362–370.
13. Ash RC, Horowitz MM, Gale RP et al. Bone marrow transplantation from related donors other than HLA-identical siblings: effect of T cell depletion. Bone Marrow Transplant 1991; 7: 443–452.
14. Gilmore MJ, Patterson J, Ivory K et al. Standardization of T-cell depletion in HLA matched bone marrow transplantation. Br J Haematol 1986; 64: 69–75.
15. Prentice HG, Blacklock HA, Janossy G et al. Depletion of T lymphocytes in donor marrow prevents significant graftversus-host disease in matched allogeneic leukaemic marrow transplant recipients. Lancet 1984; 1: 472–476.
16. McGlave P, Bartsch G, Anasetti C et al. Unrelated donor marrow transplantation therapy for chronic myelogenous leukemia: initial experience of the National Marrow Donor Program. Blood 1993; 81: 543–550.
17. Hansen JA, Gooley TA, Martin PJ et al. Bone marrow transplants from unrelated donors for patients with chronic myeloid leukemia. N Engl J

Med 1998; 338: 962–968.
18. Davies SM, Shu XO, Blazar BR et al. Unrelated donor bone marrow transplantation: influence of HLA A and HLA B incompatability on outcome. Blood 1995; 86: 1636–1642.
19. Hongeng S, Krance RA, Bowman LC et al. Outcomes of transplantation with matched-sibling and unrelated-donor bone marrow in children with leukaemia. Lancet 1997; 350: 767–771.
20. Kernan NA, Bartsch G, Ash RC et al. Analysis of 462 transplantations from unrelated donors facilitated by the National Marrow Donor Program. N Engl J Med 1993; 328: 593–602.
21. Oakhill A, Pamphilon DH, Potter MN et al. Unrelated donor marrow transplanation for children with relapsed acute lymphoblastic leukaemia in second complete remmision. Br J Haematol 1996; 94: 574–578.
22. Ochs L, Shu XO, Miller J et al. Late infections after allogeneic bone marrow transplantations: comparison of incidence in related and unrelated donor transplant recipients. Blood 1995; 86: 3979–3986.
23. Small TN, Avigan D, Dupont B et al. Immune reconstitution following T-cell depleted bone marrow transplantation: effect of age and posttransplant graft rejection prophylaxis. Biol Blood Marrow Transplant 1997; 3: 65–75.
24. Snyder DS, Chao NJ, Amylon MD et al. Fractionated total body irradiation and high-dose etoposide as a preparatory regimen for bone marrow transplantation for 99 patients with acute leukemia in first complete remission. Blood 1993; 82: 2920–2928.
25. Madrigal JA, Scott I, Arguello R et al. Factors influencing the outcome of bone marrow transplants using unrelated donors. Immunol Rev 1997; 157: 153.
26. Korbling M, Anderlini P, Hematology TA. Peripheral blood stem cell versus bone marrow allotransplantation: does the source of hematopoietic stem cells matter? Blood 2001; 98: 2900–2908.
27. Small TN, Papadopoulos EB, Boulad F et al. Comparison of immune reconstitution after unrelated and related T-cell depleted bone marrow transplantation: effect of patient age and donor leukocyte infusions. Blood 1999; 93: 467–480.
28. Storek J, Espino G, Dawson MA et al. Low B-cell and monocyte counts on day 80 are associated with high infection rates between days 100 and 365 after allogeneic marrow transplantation. Blood 2000; 96: 3290–3293.
29. Storek J, Gooley T, Witherspoon RP et al. Infectious morbidity in long-term survivors of allogeneic marrow transplantation is associated with low CD4 T cell counts. Am J Hematol 1997; 54: 131–138.
30. Parkman R, Weinberg K. Immunological reconstitution following hematopoietic stem cell transplantation. In: Thomas ED, Blume KG, Forman SJ, eds. Hematopoietic Cell Transplantation. 2nd ed. Oxford, England: Blackwell Science; 1999:704-711.
31. Socie´ G, Stone JV, Wingard JR, et al. Long-term survival and late deaths after allogeneic bone marrow transplantation. Late Effects Working Committee of the International Bone Marrow Transplant Registry. N Engl J Med. 1999; 341: 14-21.
32. Weinberg K, Annett G, Kashyap A, Lenarsky C, Forman SJ, Parkman R. The effect of thymic function on immunocompetence following bone marrow transplant. Biol Blood Marrow Transplant. 1995; 1:18-23.
33. Bruno B, Gooley T, Hackman RC, Davis C, Corey L, Boeckh M. Adenovirus infection in hematopoietic stem cell transplantation: effect of ganciclovir and impact on survival. Biol Blood Marrow Transplant. 2003; 9:341-352.
34. Flomenberg P, Babbitt J, Drobyski WR, et al. Increasing incidence of adenovirus disease in bone marrow transplant recipients. J Infect Dis. 1994;169:775-781.
35. Lion T, Baumgartinger R, Watzinger F, et al. Molecular monitoring of adenovirus in peripheral blood after allogeneic bone marrow transplantation permits early diagnosis of disseminated disease. Blood. 2003; 102:1114-1120.
36. Shields AF, Hackman RC, Fife KH, Corey L, Meyers JD. Adenovirus infections in patients undergoing bone-marrow transplantation. N Engl J Med. 1985; 312:529-533.
37. Howard DS, Phillips GL II, Reece DE, et al. Adenovirus infections in hematopoietic stem cell transplant recipients. Clin Infect Dis. 1999; 29:1494-1501.
38. Baldwin A, Kingman H, Darville M, et al. Outcome and clinical course of 100 patients with adenovirus infection following bone marrow transplantation. Bone Marrow Transplant. 2000; 26: 1333-1338.

39. Suparno C, Milligan DW, Moss PA, Mautner V. Adenovirus infections in stem cell transplant recipients: recent developments in understanding of pathogenesis, diagnosis and management. Leuk Lymphoma. 2004; 45: 873-885.
40. Storek J, et al. Immunological reconstitution after hematopoietic cell transplantation -its relation to the contents of the graft. Expert Opin Biol Ther 2008; 8:583–597
41. Balduzzi A, Gooley T, Anasetti C, Sanders JE, Martin PJ, Petersdorf EW et al. Unrelated donor marrow transplantation in children. Blood 1995; 86: 3247–3256.
42. Mackall CL, Granger L, Sheard MA, Cepeda R, Gress RE. T cell regeneration after bone marrow transplantation: differential CD45 isoform expression on thymic-derived versus thymic-independent progeny. Blood 1993; 82: 2585–2594.
43. Mackall CL, Fleisher TA, Brown MR, Andrich MP, Chen CC, Feuerstein IM et al. Age, thymopoiesis, and CD4+ T-lymphocyte regeneration after intensive chemotherapy. N Engl J Med 1995; 332: 143–149.
44. Mackall CL, Hakim FT, Gress RE. T cell regeneration: all repertoires are not created equal. Immunol Today 1997; 18: 245–251.
45. Mackall CL, Gress RE. Pathways of T cell regeneration in mice and humans: implications for bone marrow transplantation and immunotherapy. Immunol Rev 1997; 157: 61–72.
46. Heitger A, Neu N, Kern H, Panzer-Grumayer ER, Greinix H, Nachbaur D et al. Essential role of the thymus to reconstitute naive (CD45RA+) T-helper cells after human allogeneic bone marrow transplantation. Blood 1997; 90: 850–857.
47. Mackall CL, Bare CV, Granger LA, Sharrow SO, Titus JA, Gress RE. Thymic-independent T cell regeneration occurs via antigen-driven expansion of peripheral T cells resulting in a repertoire that is limited in diversity and prone to skewing. J Immunol 1996; 156: 4609–4616.
48. Chung B, Barbara-Burnham L, Barsky L, Weinberg K. Radiosensitivity of thymic interleukin-7 production and thymopoiesis after bone marrow transplantation. Blood 2001; 98: 1601–1606.
49. Clark FJ, Gregg R, Piper K, Dunnion D, Freeman L, Griffiths M et al. Chronic graft-versus-host disease is associated with increased numbers of peripheral blood CD4+CD25 high regulatory T cells. Blood 2004; 103: 2410–2416.
50. Hazenberg MD, Otto SA, de Pauw ES, Roelofs H, Fibbe WE, Hamann D et al. T cell receptor excision circle and T cell dynamics after allogeneic stem cell transplantation are related to clinical events. Blood 2002; 99: 3449–3453.
51. Lewin SR, Heller G, Zhang L, Rodrigues E, Skulsky E, van den Brink MR et al. Direct evidence for new T cell generation by patients after either T cell-depleted or unmodified allogeneic hematopoietic stem cell transplantations. Blood 2002; 100: 2235–2242.
52. Weinberg K, Blazar BR, Wagner JE, Agura E, Hill BJ, Smogorzewska M et al. Factors affecting thymic function after allogeneic hematopoietic stem cell transplantation. Blood 2001; 97: 1458–1466.
53. Douek DC, McFarland RD, Keiser PH, Gage EA, Massey JM, Haynes BF et al. Changes in thymic function with age and during the treatment of HIV infection. Nature 1998; 396: 690–695.
54. Storek J, Joseph A, Dawson MA, Douek DC, Storer B, Maloney DG. Factors influencing T-lymphopoiesis after allogeneic hematopoietic cell transplantation. Transplantation 2002; 73: 1154–1158.
55. Clave E, Rocha V, Talvensaari K, Busson M, Douay C, Appert ML et al. Prognostic value of pretransplantation host thymic function in HLA-identical sibling hematopoietic stem cell transplantation. Blood 2005; 105: 2608–2613.
56. Zorn E, Kim HT, Lee SJ, Floyd BH, Litsa D, Arumugarajah S et al. Reduced frequency of FOXP3+ CD4+CD25+ regulatory T cells in patients with chronic graft-versus-host disease. Blood 2005; 106: 2903–2911.
57. Petridou E, Klimentopoulou AE, Moustaki M, Kostrikis LG, Hatzakis A, Trichopoulos D. Recent thymic emigrants and prognosis in T-and B-cell childhood hematopoietic malignancies. Int J Cancer 2002; 101: 74–77.
58. Eyrich M, Wollny G, Tzaribaschev N, Dietz K, Brugger D, Bader P et al. Onset of thymic recovery and plateau of thymic output are differentially regulated after stem cell transplantation in children. Biol Blood Marrow Transplant 2005; 11: 194–205.
59. Douek DC, Vescio RA, Betts MR, et al (2000) Assessment of thymic output in adults after haematopoietic stem-cell transplantation and prediction of T-cell reconstitution. Lancet 355: 1875-81