Long-term immune deficiency after allogeneic stem cell transplantation: B-cell deficiency is associated with late infections

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ABSTRACT

Immune reconstitution was analyzed in 140 consecutive patients who were 2-year disease-free and who underwent myeloablative allogeneic transplantation. A CD4 and CD8 defect was observed involving naive, terminally differentiated, memory and competent cells and above limits values for activated subsets. Natural killer cells normalize at six months while we observed expansion of $CD19^+/CD5^+$ B cells after three months and a persisting defect of memory B cells. Chronic graft-versus-host disease did not influence significantly those parameters for CD8 subsets while the naïve and competent CD4 subsets were strongly affected. But the most profound impact of chronic graft-versus-host disease was on B-cell subsets, especially on the memory B population. The cumulative incidence of late severe infections was low (14% at four years). Using Cox's models, only low B-cell counts at 12 (P=0.02) and 24 (P=0.001) months were associated with the hazard of developing late infection, in particular if patients did not develop chronic graft-versus-host disease.

Key words: immune deficiency, allogeneic transplantation, B cell.

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Introduction

Immune reconstitution has emerged as a general concern in hematopoietic stem cell transplantation. After resolution of peri-transplantation neutropenia infections ranged from 40% to nearly 80%.[1] In contrast to the relatively early recovery of innate immunity, all, but especially adult, recipients of an allogeneic hematopoietic stem cell transplantation experience post-transplant deficiencies in B- and T-cell reconstitution. This delay in function recovery may persist for more than one year.[1-4] Although the T-cell component of the immune defect has been relatively well defined, the long-term B-cell component is less well known. The majority of current studies focus on immune parameters measured sequentially to one year post-transplant.[3, 5-17] We previously reported a study aimed at evaluating the long-term immune reconstitution after allogeneic hematopoietic stem cell transplantation using standard immune parameters (CD3, CD4, CD8 and NK cells).[18] However, since the early 2000s, significant progress has been made in molecular HLA-typing and in the treatment of viral and fungal infections. Furthermore, advances in basic immunology have made the definition of phenotypes more complex with the introduction of naïve and memory subtypes. This new study considers these clinical and biological advances in a reassessment of long-term immune deficiency after allogeneic hematopoietic stem cell transplantation.

Design and Methods

One hundred and forty consecutive patients are included in this study. All underwent transplantation at the Hospital Saint Louis, Paris between 1995 and 2005. The selection criterion was being alive, without relapse, for more than two years after a myeloablative conditioning with available sequential routine immune-reconstitution analyses. This is thus a highly selected population of patients who did not die during the first two years of either graft-versus-host disease and/or of infections. Patients who received cord blood cell transplantation were excluded. Median follow-up was 48 months. Median age was 27 years at transplant with 39 children and 101 adults. Patients-, disease- and transplant-characteristics of the patients are summarized in Table 1. Our policy concerning microbial prophylaxis has been previously reported in detail,[19] and is summarized in the Online Supplementary Appendix.

The study was performed in accordance with the Helsinki declaration as part of routine immunological follow-up, after approval by the institutional review board of the Hospital St Louis.

T CD3, CD4 and CD8, B and NK lymphocytes were enumerated in fresh whole blood EDTA samples by direct 3 or 4-color immunofluorescence with a Facscalibur analyser (Becton Dickinson, San José, California, USA) as previously described.[18] Percentages and absolute counts were determined. 104 lymphocytes were gated and competent (CD28+), naïve ($CD45RA^+CD62L^+$), memory (CD45RO) CD4 and CD8 T cells, $CD5^+CD19^+$, naïve ($CD27^-$) and memory ($CD27^+$) $CD19^+$

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B cells were analyzed. Isotypic controls were performed simultaneously. Antibodies used were CD45-PerCP or -FITC, CD14-PE, CD3-FITC or -PerCP, CD4- APC, CD8-PerCP or –PE, CD28-PE, CD45RO-PE, CD16+56-PE, CD19-FITC, from Becton Dickinson, CD45RA-APC, CD27 PE (from Pharmingen, San Diego, CA, USA); CD5-PE-Cy5 (Immunotech Coulter Beckman, Fullerton, CA, USA). Lymphocyte subset populations for each patient were quantified three, six, 12, and 24 months after transplant.

The main clinical outcome post transplant studied was severe late infection. Definition of severe infections was as previously described[19] and is summarized in the Online Supplementary Appendix.

Univariate and multivariate statistical methods were used to analyze the process of reconstitution of lymphocyte subsets according to clinical parameters in the post-transplant period. A logarithmic transformation was performed to normalize quantitative data. Non-parametric and parametric tests were used where appropriate. To compare lymphocyte subset populations according to clinical parameters, Kruskal-Wallis test was used for crude data and ANOVA for transformed data. Due to multiple comparisons and low numbers in some subsets only *P* values under 0.01 were deemed significant. Differences for categorical variables between 2 groups were evaluated by c^2 (with Yates correction if needed) or Fisher's test. Cumulative incidence using competing risk method was used to assess prognostic factors of severe late infections with death or relapse as a competing event. Cox's proportional hazard model was used in multivariate analyses of risk factors for late infections. A stepwise backward procedure was used to construct a set of independent predictors of each end point. All predictors achieving a *P* value below 0.15 were considered and sequentially removed if the *P* value in the multiple model was above 0.05. The proportional hazard assumption was checked by graphical method. Chronic graft-versus-host disease was always introduced in the models as a time dependant variable. Other variables were age, donor related or not, total body irradiation, malignant disease and source of cells. All tests were two-sided, with type I error rate fixed at 0.01 except for Cox's analysis (*P*=0.05). Statistical analyses were performed with SPSS 15 and Stata® 10 software. The R package: "cmprsk" developed by Fine and Gray was used to evaluate competing risks.

Table 1.

	Number (%)
Male sex	73 (52.1)
Donor/recipient gender	
Male/Male	47 (33.6)
Male/Female	32 (22.9)
Female/Male	26 (18.6)
Female/Female	35 (25)
Source	
Bone marrow	113 (80.7)
PBSC	25 (17.9)
Bone marrow+ PBSC	2 (1.4)
Diagnoses	
Acute myeloblastic leukemia	31 (22.1)
Acute lymphoblastic leukemia	26 (18.1)
Chronic myelogenous leukemia	22 (15.7)
Aplastic anemia	30 (21.4)
Myelodysplastic syndrome	11 (7.9)
Myeloproliferative syndrome	2 (1.4)
Non-Hodgkin's lymphoma	4 (2.9)
Hodgkin's disease	8 (5.7)
Multiple myeloma	3 (2.1)
Chronic lymphocytic leukemia	1 (0.7)
Hemoglobinopathy	2 (1.4)
Donor Type	
Matched sibling	92 (65.7)
Mismatched related	4 (2.9)
Unrelated	44 (31.4)
Donor/recipient cytomegalovirus serological status	
Positive/positive	36 (25.7)
Positive/negative	16 (11.4)
Negative/positive	36 (25.7)
Negative/negative	52 (37.1)
Conditioning regimen	
Cyclophosphamide-TBI (with or without other drugs)	42 (30)
Cyclophosphamide-ATG	16 (12)
Busulfan-cyclophosphamide (with or without other drugs)	60 (43)
Other	22 (15)
Graft-versus-host disease prophylaxis	
Methotrexate/cyclosporine	118 (84)
Cyclosporine alone	9 (6.4)
Cyclosporine + other	12 (8.6)
Acute graft-versus-host disease	
No	37 (26.4)
Grade I	32 (22.9)
Grade II	61 (43.6)
Grade III	8 (5.7)
Grade IV	2 (1.4)
Chronic graft-versus-host disease	
No	71 (50.7)
Limited	28 (20)
Extensive	41 (29.3)

Results and discussion

Long-term analyses of lymphocyte subset reconstitution

Patients had long-lasting lymphopenia. T lymphocytes ($CD3^+$) normalized only by 24 months overall with an earlier recovery of $CD8^+$ than $CD4^+$ T cells. In contrast, patients exhibited an early natural killer cell recovery and an early expansion of the $CD19^+CD5^+$ subset within $CD19^+$ B cells (*Online Supplementary Figure S1*). The analysis of CD4 and CD8 subsets revealed a yet poorly described pattern of recovery in the long-term. Naïve, terminally differentiated and memory CD8 cells did not normalize till 24 months post-transplantation (Figure 1). In contrast, $HLA\text{-}DR^+$ activated $CD8^+$ T cells expanded early after transplant with values above our normal range from three months till two years post transplantation (Figure 1). Similarly naïve, terminally differentiated and memory CD4 T cells did not normalize till 24 months post-transplantation (Figure 1) while activated $CD4^+$ $HLADR^+$ T cells normalized by nine months and remained above normal value till 24 months. Finally, naive B cells ($CD19^+$ $CD27^-$) were within the normal range by six months and then above normal value till 24 months, while memory B cells remained under normal range during the follow-up time (Figure 1).

Our prospective series involved patients who were alive and disease free more than two years after transplantation using myeloablative conditioning. Patients who underwent transplantation after a reduced-intensity conditioning regimen or who were grafted with cord blood were excluded because both situations display different and

specific immune reconstitution timing. Of note, patients with the worst immune reconstitution post transplant have thus been virtually excluded from this study because of potential death from early infection/graft-versus-host disease or later disease relapse/progression. Furthermore, it's impossible to know if late infections or anti-microbial prophylaxes, chronic graft-versus-host disease or its treatment by immunosuppressive therapy could influence (and if so to what extent) some of the biological parameters we measured because these parameters are confounding factors. Other authors have reported that CD8 T cells early post transplant consist largely of memory/effector cells with slow recovery of naïve CD8 cells.[20,21] The expansion of the $CD8^+DR^+$ subset is rather suggestive of activated $CD8^+$ cells primed toward allogeneic and infectious antigen. Other authors have reported that early CD4 regeneration is associated with expression of CD45RO, and HLA-DR and less frequent expression of CD28, CD45RA.[1-3] However, although classically recovering by one year, our results do show that, with the exception of activated DR+ cells, other subsets remain profoundly affected even two years post transplant. We especially focused our analysis on B-cell reconstitution in this cohort of patients. Storek and co-workers have previously reported data suggesting that recapitulation of normal lymphoid ontogeny occurs during reconstitution of B lymphocytes.[11,14,15] These authors reported that a relatively rapid initial rise in naive B cells is followed by a slow recovery of memory B cells.

Impact of graft-versus-host disease on long-term reconstitution

In this selected population, acute graft-versus-host disease severity did not influence significantly T-lymphocyte subsets (*data not shown*). However, total B cells were significantly lower at three (P=0.001) and six months (P=0.004) in patients who previously developed grade II-IV acute graft-versus-host disease. The same correlation between acute graft-versus-host disease and $CD19^+CD5^+$ (P=0.003 at three months), and naïve B cells (P=0.02 and P=0.007 at three and six months) was found. We then studied the influence of the extensive form of chronic graft-versus-host disease on the long-term immune recovery. A long-lasting lymphopenia (till 24 months) was observed in patients who developed extensive chronic graft-versus-host disease. CD4 and CD8 T cells were affected by chronic graft-versus-host disease. This was particularly

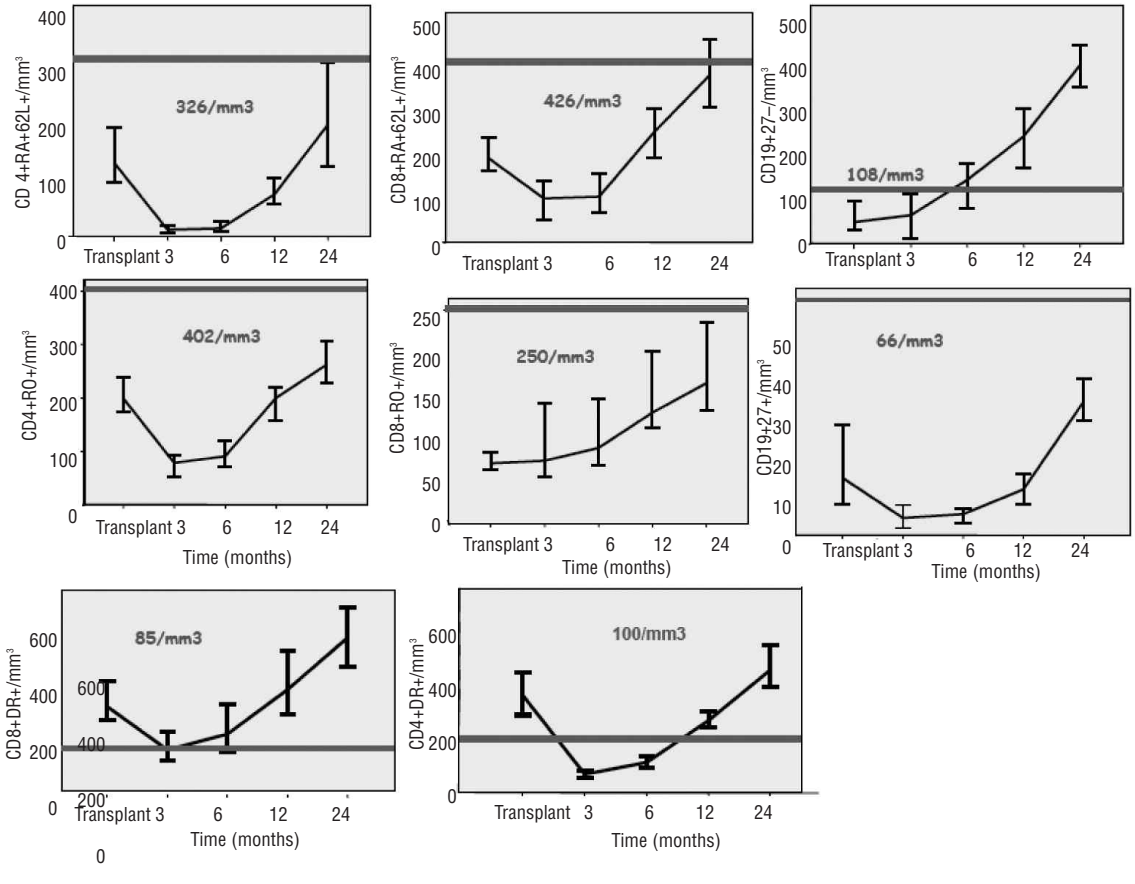
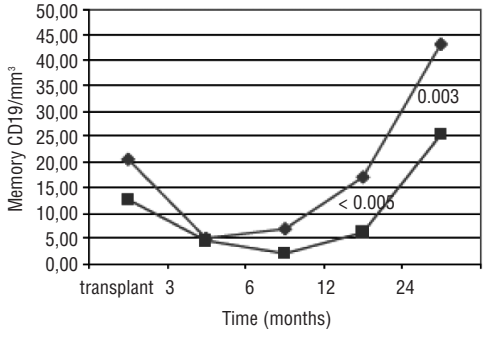
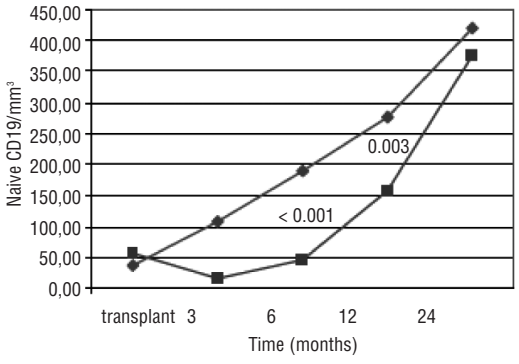


Figure 1. Reconstitution of T- and B-cell subsets after Allogeneic Stem Cell Transplantation (ASCT) assessed by flow cytometry in long-term survivors. Median values (±SD) of T- and B-cell subsets (naïve $CD45RA^+CD4^+$ lymphocytes, upper left panel; naïve $CD45RA^+CD8^+$ lymphocytes, upper middle panel, naive $CD19^+CD27^-$ lymphocytes, upper right panel; memory $CD45RO^+CD4^+$ lymphocytes, middle left panel; memory $CD45RO^+CD8^+$ lymphocytes, middle panel, memory $CD19^+CD27^+$ lymphocytes, middle right panel; activated $HLADR^+CD4^+$ lymphocytes, lower left panel; activated $HLADR^+CD8^+$ lymphocytes, lower right panel) are represented at the indicated time (months) following ASCT in the overall population. The y-axis show cell count/mm^3. Normal values (healthy controls) are represented by a line in each panel.





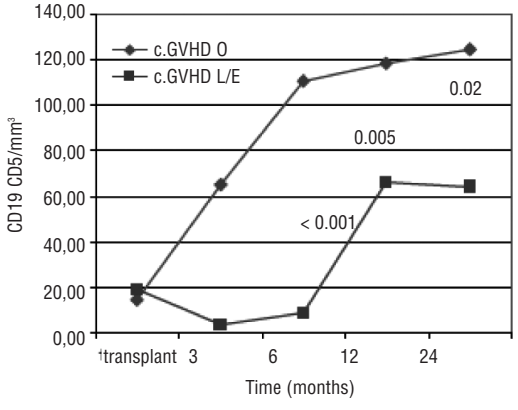


Figure 2. Influence of chronic graft-versus-host disease (GvHD) on the reconstitution of the B-cell compartment after Allogeneic Stem Cell Transplantation (ASCT) assessed by flow cytometry in long-term survivors. Median values of B lymphocyte subsets (memory $CD19^+CD27^-$ lymphocytes, upper left panel; naïve $CD27^+CD19^+$ lymphocytes, upper right panel, $CD19^+CD5^+$; lymphocytes, lower panel) are represented for patients with GvHD (square) or without (diamond) at the indicated time following ASCT in the overall population. The y-axis show cell count/mm^3. Significant *P* values are represented at the indicated time (months) after ASCT.

true for memory and competent CD8 cells. The CD4 T-cell subsets were most affected, with significant decrease of naïve and competent $CD4^+$ cells at 12 and 24 months (*data not shown*). But the biggest impact of extensive chronic graft-versus-host disease was found on B cells and B-cell subsets (Figure 2). CD 19^+ and $CD19^+/CD5^+$ B cells were significantly decreased at six (P=0.001), 12 (P=0.005) and 24 months (P=0.02); memory B-cell counts were decreased at 12 (P=0.005) and 24 months (P=0.003) (Figure 2) while naïve B cells remained lower till 12 months (P=0.003).

The magnitude of the impact of chronic graft-versus-host disease on B-cell subset recovery has not yet been well described. Impact on both naive CD4 and B-cell subsets could explain defaults in T-B cooperation and consequently in B-cell switch, maturation and Ig production. Recently, Greinix and co-workers[22] have reported elevated numbers of immature/transitional $CD19^+/CD21^-$ B cells in association with the occurrence of severe infections. The numbers of memory B cells were significantly lower in patients with active chronic graft-versus-host disease. Such a subset ($CD19^+/CD21^-$ B cells) was not included in our panel, but clearly warrants further confirmatory studies.

Impact of the immune biological defect on late infections rate

Finally, we focused our attention on late infections (occurring after three months post transplantation). Twenty-eight infections were recorded among 22 patients from three months to two years post transplantation. During the first year, 13 severe bacterial infections, 3 fungal infections and 3 cytomegalovirus-reactivation were recorded, while between the first and the second year, only 4 severe bacterial infections developed. The cumulative incidence of first late infection was 14% at three years (*Online Supplementary Appendix Figure S2A*). By univariate analysis, chronic graft-versus-host disease and the source of cells marginally affected this cumulative incidence: 10 *vs.* 18% at 6OO days in patients without as compared to patients with chronic graft-versus-host disease (P=0.06). Other tested factors included the source of cell (11% with bone marrow *vs.* 22% with peripheral blood; P=0.08). Age (older *vs.* younger than 25 years median age), donor type (sibling *vs.* others), and total body irradiation did not significantly influence the cumulative incidence of infections. None of these factors remained significant by multivariate analyses.

The relation, if any, between any cell subsets at different time points post transplant and their association with late infections, were tested using Cox's modeling to take into account few infectious events and the large number of analyzed cell subsets. In univariate analyses made at three, six, 12 and 24 months, low natural killer cell count at three and six months marginally accounted for increased risk of infection (P=0.08 and P=0.01, respectively). These results remained significant at six months (P=0.01, HR=4.96, CI95%: 1.45-17.0) in multivariate analyses, including age, source of cells and chronic graft-versus-host disease as covariates. However, increased infection was linked with the use of peripheral blood grafts three months (P=0.01, HR=3.52, CI95%: 1.32-9.15) 12 months (P =0.05, HR=2.5, CI95%: 1.01-6.21) and 24

months (P=0.03, HR= 2.97, CI95%: 1.1-8.0). None of the $CD4^+$ or $CD8^+$ T-cell subsets was linked to increased risk of infections. Only B-lymphocyte counts at 12 and 24 months were associated with late infections. These results were found by univariate (P=0.02 and 0.001, respectively) and multivariate (P =0.03, HR=0.41, CI95%: 0.19-0.92 and P=0.001, HR=0.20, CI95%: 0.08-0.54, respectively) analyses including the same covariates. Interestingly, in these models, source of cells was not significant. Of note, this association with low B-cell counts and infection was essentially found in patients who did not develop chronic graft-versus-host disease (*Online Supplementary Appendix Figure S2B*).

The spectrum of late infection (beyond a year) mainly includes bacterial infections.[19] The B-cell defects we describe, in addition to low gamma globulin levels in patients with chronic graft-versus-host disease, both explain these long-term bacterial infections. As stated in the methods section, IV-Ig prophylaxis is not our policy since we previously reported in a double blinded randomized study their lack of efficacy.[23] However, Ig supplementation in patients with low Ig levels and recurrent infections was systematically given.

Authorship and Disclosures

EC executed the research plan, collected and analyzed the data and wrote the paper; MC, CR and AT designed the study, collected and analyzed the data and wrote the paper; MB did the statistical analysis; RPL, MR, PR collected data; GS designed the study, supervised the study and wrote the manuscript.

The authors reported no potential conflicts of interest.

References

1. Zakrzewski JL, Goldberg GL, Smith OM, van den Brink MR. Enhancing T cell reconstitution after hematopoietic stem cell transplantation: a brief update of the latest trends. Blood Cells Mol Dis. 2008;40(1):44-7.
2. Geddes M, Storek J. Immune reconstitution following hematopoietic stem-cell transplantation. Best Pract Res Clin Haematol. 2007;20(2):329-48.
3. Small TN, Papadopoulos EB, Boulad F, Black P, Castro-Malaspina H, Childs BH, 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(2):467-80.
4. Parkman R, Weinberg KI. Immunological reconstitution following bone marrow transplantation. Immunol Rev. 1997;157:73-8.
5. Storek J, Dawson MA, Storer B, Stevens-Ayers T, Maloney DG, Marr KA, et al. Immune reconstitution after allogeneic marrow transplantation compared with blood stem cell transplantation. Blood. 2001;97(11):3380-9.
6. Abrahamsen IW, Somme S, Heldal D, Egeland T, Kvale D, Tjonnfjord GE. Immune reconstitution after allogeneic stem cell transplantation: the impact of stem cell source and graft-versus-host disease. Haematologica. 2005;90(1):86-93.
7. D'Sa S, Peggs K, Pizzey A, Verfuerth S, Thuraisundaram D, Watts M, et al. T- and B-cell immune reconstitution and clinical outcome in patients with multiple myeloma receiving T-cell-depleted, reduced-intensity allogeneic stem cell transplantation with an alemtuzumab-containing conditioning regimen followed by escalated donor lymphocyte infusions. Br J Haematol. 2003;123(2):309-22.
8. Heining C, Spyridonidis A, Bernhardt E, Schulte-Monting J, Behringer D, Grullich C, et al. Lymphocyte reconstitution following allogeneic hematopoietic stem cell transplantation: a retrospective study including 148 patients. Bone Marrow Transplant. 2007;39(10):613-22.
9. Maris M, Boeckh M, Storer B, Dawson M, White K, Keng M, et al. Immunologic recovery after hematopoietic cell transplantation with nonmyeloablative conditioning. Exp Hematol. 2003;31(10):941-52.
10. Sanchez-Garcia J, Serrano J, Gomez P, Martinez F, Martin C, Roman-Gomez J, et al. The impact of acute and chronic graft-versus-host disease on normal and malignant B-lymphoid precursors after allogeneic stem cell transplantation for B-lineage acute lymphoblastic leukemia. Haematologica. 2006;91(3):340-7.
11. Storek J, Ferrara S, Ku N, Giorgi JV, Champlin RE, Saxon A. B cell reconstitution after human bone marrow transplantation: recapitulation of ontogeny? Bone Marrow Transplant. 1993;12(4):387-98.
12. Storek J, Gooley T, Witherspoon RP, Sullivan KM, Storb R. Infectious morbidity in long-term survivors of allogeneic marrow transplantation is associated with low CD4 T cell counts. Am J Hematol. 1997;54(2):131-8.
13. Storek J, Joseph A, Espino G, Dawson MA, Douek DC, Sullivan KM, et al. Immunity of patients surviving 20 to 30 years after allogeneic or syngeneic bone marrow transplantation. Blood. 2001;98(13):3505-12.
14. Storek J, Saxon A. Reconstitution of B cell immunity following bone marrow transplantation. Bone Marrow Transplant. 1992;9(6):395-408.
15. Storek J, Wells D, Dawson MA, Storer B, Maloney DG. Factors influencing B lymphopoiesis after allogeneic hematopoietic cell transplantation. Blood. 2001;15;98(2):489-91.
16. Storek J, Witherspoon RP, Storb R. T cell reconstitution after bone marrow transplantation into adult patients does not resemble T cell development in early life. Bone Marrow Transplant. 1995;16(3):413-25.
17. Tayebi H, Kuttler F, Saas P, Lienard A, Petracca B, Lapierre V, et al. Effect of granulocyte colony-stimulating factor mobilization on phenotypical and functional properties of immune cells. Exp Hematol. 2001;29(4):458-70.
18. Maury S, Mary JY, Rabian C, Schwarzinger M, Toubert A, Scieux C, et al. Prolonged immune deficiency following allogeneic stem cell transplantation: risk factors and complications in adult patients. Br J Haematol. 2001;115(3):630-41.
19. Robin M, Porcher R, De Castro Araujo R, de Latour RP, Devergie A, Rocha V, et al. Risk factors for late infections after allogeneic hematopoietic stem cell transplantation from a matched related donor. Biol Blood Marrow Transplant. 2007;13(11):1304-12.
20. Storek J, Joseph A, Dawson MA, Douek DC, Storer B, Maloney DG. Factors influencing T-lymphopoiesis after allogeneic hematopoietic cell transplantation. Transplantation. 2002;73(7):1154-8.
21. 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(5):1458-66.
22. Greinix HT, Pohlreich D, Kouba M, Kormoczi U, Lohmann I, Feldmann K, et al. Elevated numbers of immature/transitional CD21- B lymphocytes and deficiency of memory CD27+ B cells identify patients with active chronic graft-versus-host disease. Biol Blood Marrow Transplant. 2008;14(2):208-19.
23. Cordonnier C, Chevret S, Legrand M, Rafi H, Dhedin N, Lehmann B, et al. Should immunoglobulin therapy be used in allogeneic stem-cell transplantation? A randomized, double-blind, dose effect, placebo-controlled, multicenter trial. Ann Intern Med. 2003;139(1):8-18.