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Long-Term Immune Reconstitution and Infection Burden after Mismatched Hematopoietic Stem Cell Transplantation

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Mismatched unrelated donor (MMUD) or umbilical cord blood (UCB) can be chosen as alternative donors for allogeneic stem cell transplantation but might be associated with long-lasting immune deficiency. Sixty-six patients who underwent a first transplantation from either UCB (n = 30) or 9/10 MMUD (n = 36) and who survived beyond 3 months were evaluated. Immune reconstitution was prospectively assessed at sequential time points after transplantation. NK, B, $CD4^+$, and $CD8^+$ T cells and their naïve and memory subsets, as well as regulatory T cells (Treg), were studied. Detailed analyses on infections occurring after 3 months were also assessed. The 18-month cumulative incidences of infection-related death were 8% and 3%, and of infections were 72% and 57% after MMUD and UCB transplantation, respectively. Rates of infection per 12 patient-month were roughly 2 overall (1 for bacterial, .9 for viral, and .3 for fungal infections). Memory, naïve $CD4^+$ and $CD8^+$T cells, naïve B cells, and Treg cells reconstitution between the 2 sources were roughly similar. Absolute $CD4^+$T cells hardly reached 500 per μL by 1 year after transplantation and most B cells were of naïve phenotype. Correlations between immune reconstitution and infection were then performed by multivariate analyses. Low $CD4^+$ and high $CD8^+$T cells absolute counts at 3 months were linked to increased risks of overall and viral (but not bacterial) infections. When assessing for the naïve/memory phenotypes at 3 months among the $CD4^+$ T cell compartment, higher percentages of memory subsets were protective against late infections. Central memory $CD4^+$T cells protected against overall and bacterial infections; late effector memory $CD4^+$T cells protected against overall, bacterial, and viral infections. To the contrary, high percentage of effector- and late effector-memory subsets at 3 months among the $CD8^+$ T cell compartment predicted higher risks for viral infections. Patients who underwent transplantation from alternative donors represent a population with very high risk of infection. Detailed phenotypic analysis of immune reconstitution may help to evaluate infection risk and to adjust infection prophylaxis.

INTRODUCTION

Only 30% of patients who require an allogeneic hematopoietic stem cell transplantation (HSCT) will have an HLA-matched sibling donor. A search for an unrelated donor will be undertaken for patients without a matched family donor. However, for many patients, particularly patients of diverse racial and ethnic backgrounds, it may not be possible to rapidly identify a suitably matched unrelated donor. Three alternative graft sources, umbilical cord blood (UCB), haploidentical related donor, and mismatched unrelated donor (MMUD) are available. UCB is associated with delayed hematologic recovery and immune reconstitution. Haploidentical transplantation is characterized by donor availability for transplantation but may be complicated by a high risk of graft failure and relapse. A MMUD transplantation may also be an option, but graft-versus-host disease (GVHD) and immune deficiency may be of greater concern. Phase 2 studies have documented advances in HLA typing, GVHD prophylaxis, and infection prevention, which have improved survival (reviewed by Ballen et al. [1]). The same patient evaluated in different transplantation centers may be offered MMUD, UCB, or Haploidentical HSCT depending on center preference. At the Hospital Saint Louis, Paris, France, our policy has been to restrict our indication to either MMUD or UCB, depending on the emergency to transplantation and on technical aspects. However, after both UCB and MMUD transplantations, delayed

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immune reconstitution and infection risk are of clear clinical concern [1-4].

Despite advances in antimicrobial therapy, infections remain a major cause of death after alternative donor HSCT, particularly in older patients. UCB contains fewer T cells than other stem cell sources, and UCB lymphocytes have specific immunologic characteristics, such as a different response pattern to cytokines and a greater proportion of naive T cells [5,6]. In a prospective analysis of immune reconstitution in UCB recipients and HLA-matched unrelated donor (MUD) recipients from the Dana Farber Cancer Institute, Jacobson et al. found that $CD3^+$ T cells recovery was significantly delayed in the UCB group compared with the MUD group for as long as 6 months after HSCT, including naive and memory $CD4^+$ T cells, regulatory T cells (Treg), and $CD8^+$ T cells [7]. These unique properties of UCB may contribute to the high risk of infection reported in some studies. However, this study compared UCB to MUD and not to MMUD, the most clinically relevant comparator. So far, no study has compared a cohort of patients who underwent transplantation either from UCB or from MMUD with regard to infectious complications and long-term immune recovery that prompted the report herein.

PATIENTS AND METHODS

Patients

Patients who underwent first allogeneic HSCT from either UCB or MMUD at Saint-Louis Hospital (Paris, France) between January 2005 and December 2010 were considered for this study. Patients with hematological malignancies and aplastic anemia were included. Patients were eligible for HSCT from 1 of these 2 alternative stem cell sources if they had no available HLA-matched related or unrelated donor (10/10 HLA-matched with the recipient, at the allelic level for HLA-A, -B, -C, -DRB1, and -DQB1 loci). According to institutional guidelines, the algorithm for alternative donor selection was MMUD first, followed by UCB for adult patients and UCB first, followed by MMUD for pediatric patients. HSCT from MMUD was performed either with peripheral blood stem cells or with bone marrow as graft source. For MMUD selection, donor/recipient HLA-match was based on the results of high-resolution molecular typing for HLA-A, -B, -C, -DRB1, and -DQB1 loci and only 1 mismatch was permitted (9/10 HLA-matched donor). For UCB HSCT, double-UCB HSCT was performed in patients for whom no single unit of adequate cell dose (total nucleated cell $<1 \times 10^7$/kg) was available. For UCB unit selection, donor/recipient HLA-match was determined by low-resolution generic oligotyping for HLA-A and -B loci and high-resolution molecular typing for HLA-DRB1. According to institutional guidelines, UCB units were 3/6 to 6/6 HLA-matched with the recipients and with each other, for double UCB HSCT. No graft was ex vivo T cell depleted. To study immune reconstitution and infectious outcomes after alternative HSCT, patients who experienced primary or secondary graft failure were excluded from this analysis.

A total of 105 consecutive patients who underwent transplantation from MMUD ($n = 56$) or from UCB ($n = 49$) met inclusion criteria. To assess late infectious outcomes (>3 months after HSCT) and immune reconstitution from 3 months after HSCT, 11 patients who died or relapsed in the early period after HSCT (0 to 3 months after HSCT) were secondarily excluded from the analysis (MMUD recipients, $n = 6$; UCB recipients, $n = 5$). An additional 28 patients were not evaluable because of lack of immune recovery data (MMUD recipients, $n = 14$; UCB recipients, $n = 14$). As a result, 66 patients were considered for late infectious outcomes and immune reconstitution analyses. Baseline characteristics of assessable and excluded patients are compared in Supplementary Table 1. The 2 cohorts were comparable for pre-HSCT characteristics with the exception of recipient gender and recipient age.

Data concerning pretransplantation characteristics and most transplantation outcomes were prospectively collected in our transplantation database and extracted for this study. Precise data about late infectious events and the cumulative dose of corticosteroids administered were retrospectively collected by a review of all medical records. Immune cell subsets reconstitution was assessed prospectively. All patients provided written informed consent for use of protected health data for research and for blood sample collection, in accordance with the Declaration of Helsinki. The study was approved by the institutional review board.

Transplantation Modalities and Outcomes: Definitions

Myeloablative conditioning regimens and reduced-intensity conditioning were defined as previously described [8]. All patients were treated in laminar airflow rooms and received oral amoxicillin, ofloxacine, fluconazole, and acyclovir as prophylaxis. According to local policy, fluconazole was stopped when patients had an absolute neutrophil count above 1×10^9/L and were off corticosteroids. Amoxicillin was continued for at least 5 years after HSCT. Prophylaxis against Pneumocystis Carinii and Toxoplasmosis was started after neutrophils recovery and was withdrawn when immunosuppressive treatment was discontinued and CD4 count was ≥.4 109/L. Patients were vaccinated according to European recommendations. While in hospital, patients were tested twice each week by real time PCR for cytomegalovirus and once a week for herpes virus (HSV1, HSV2, HHV6, and EBV), adenovirus, aspergillus, and toxoplasma until discharge. After patients were discharged from hospital, the same infection screening was performed once each week until day + 100 and at least once each month until 6 months after hematopoietic stem cell transplantation.

Chronic GVHD was diagnosed according to the 2005 National Institutes of Health Consensus Criteria [9].

Infectious Events: Definitions and Monitoring

This study focused on late infectious events, defined as those occurring beyond 3 months after transplantation. Infections were diagnosed according to standard criteria [10]. Only serious infections potentially associated with clinical compromise were considered in this analysis, as previously described [11]. These include bacterial infections of any organ site requiring i.v. therapy and/or hospitalization. Other infections not requiring therapy or those requiring only oral antibiotics on an outpatient basis were excluded from this study. Bacterial infections were either proven or presumed based on the combination of clinical presentation and response to treatment with antibiotics. For example, all microbiologically undocumented pneumonias that resolved after empirical antibiotics were considered as of bacterial origin. Bacteremia by coagulase-negative staphylococci, Micrococcus spp, and saprophytic Corynebacterium spp were not included in this analysis. Uncomplicated fevers of unknown origin were also excluded because of potential for reporting bias. Also included are viral infections including cytomegalovirus infection and disease, Epstein-Barr virus reactivation requiring treatment, and other documented severe invasive viral infections requiring i.v. therapy and/or hospitalization (such as disseminated form or visceral involvement of herpes family virus infections, adenovirus disease, viral B or C hepatitis, and lower respiratory tract infection by respiratory viruses). We did not include benign presumably viral upper airway infections and bronchitis, or BK-virus cystitis. CMV infection was defined as the presence of 1 or more positive quantitative PCR result (more than 1000 CMV DNA copies/mL) that prompted clinicians to initiate pre-emptive treatment. CMV disease was defined as the demonstration of CMV by histology in biopsy or autopsy specimens from clinically involved visceral sites or if CMV was detected in samples from clinically defined sites of disease. Invasive fungal infections involving lung, sinus, central nervous system or all other organs were also included. Invasive aspergillosis was defined as proven, probable or possible according to previously reported criteria (reviewed in [4]). Finally, arasitical infections includes toxoplasmosis and other invasive parasitical infections were included. Infections were considered as life threatening if they necessitated intensive care with vasopressors or mechanical ventilation. Infection-related mortality was defined as death from infection as primary cause of death, basing on the Copelan hierachical scheme for causes of death assignment [12].

Immunological Analyses of Lymphocytes Subsets

Immune cell subsets reconstitution was prospectively assessed (as described in Corre et al. [13]) by flow cytometry on fresh peripheral blood samples collected at approximately 1 month before HSCT and then at 3, 6, and 12 months after HSCT. Blood cells were characterized using 4-color flow cytometry after treatment with a red blood cell lyzing solution. Measurements of forward and side scatter were combined with CD45 and CD14 to identify lymphocytes and to exclude monocytes. A minimum of 10,000 lymphocytes were analyzed to ensure adequate subset analysis. Antibodies used were CD45-FITC, CD14-PE, CD3-FITC and CD3-PerCP, CD4-APC-Cy7, CD8-PerCP, CD45RA-APC and CD45RA-PE-Cy7, CCR7-PE, CD25-PE and CD25-APC, CD127-PE, CD56-PE, CD19-PE-Cy7 and CD27-PE (all from BD Biosciences, Le Pont-De-Claix Cedex, France). Appropriate isotype-matched controls were carried out simultaneously on each sample. The analyzed cell subsets were T cells ($CD3^+$), $CD4^+$ T cells ($CD3^+CD4^+$), $CD8^+$ T cells ($CD3^+CD8^+$), NK cells ($CD3^-CD56^+$) and B cells ($CD19^+$). More detailed T and B cell phenotypes analyses were also performed if the number of collected cells was sufficient. The different populations were defined as follows: naïve $CD4^+$ T cells ($CD4^+CD45RA^+CCR7^+$); central memory $CD4^+$ T cells (CD4+ CD45RA-CCR7+); effector memory $CD4^+$ T cells ($CD4^+CD45RA^-CCR7^-$); late effector memory $CD4^+$ T cells ($CD4^+CD45RA^+CCR7^-$); Treg ($CD4^+CD25^+$ CD127low); naïve $CD8^+$ T cells ($CD8^+CD45RA^+CCR7^+$); central memory $CD8^+$ T cells ($CD8^+CD45RA^-CCR7^+$); effector memory $CD8^+$ T cells ($CD8^+$ $CD45RA^-CCR7^-$); late effector memory $CD8^+$ T cells ($CD8^+CD45RA^+CCR7^-$);