Impact of acute and chronic graft-versus-host disease on human B-cell generation and replication

Salomé Glauzzy,1,2,3 Juliette Soret,1,2,3 Isabelle Fournier,2,3 Corinne Douay,1,2 Hélène Moins-Teisserenc,1,2,3 Régis Peffault de Latour,1,4 Guillaume Makl,1,2,3 Marie Robin,1,4 Gérard Socié,1,2,4 Antoine Toubert,1,2,3 and Emmanuel Clave1,2,3

1Université Paris Diderot, Sorbonne Paris Cité, Institut Universitaire d’Hématologie, Paris, France; 2Institut National de la Santé et de la Recherche Médicale UMR1160, Paris, France; 3Laboratoire d’Immunologie et d’Histocompatibilité and 4Service d’Hématologie-Greffe de Moelle, Hôpital Saint-Louis, Assistance Publique–Hôpitaux de Paris, Paris, France

Key Points

- B-cell neogenesis is decreased independently by both aGVHD and cGVHD.
- B cells during GVHD undergo a higher number of cell divisions related, in the chronic form, to a higher BAFF/CD19 ratio.

Using B-cell rearrangement excision circle measurements, we analyzed B-cell reconstitution in a cohort of 243 patients who underwent allogeneic stem cell transplantation. Acute and chronic graft-versus-host disease (aGVHD and cGVHD, respectively) transiently increased B-cell replication but decreased overall B-cell neogenesis with a clear difference in terms of kinetics. Moreover, the impact of aGVHD in the absence of cGVHD was transient, recovering at month 6 similar values as in patients who did not suffer from GVHD. Conversely, impact of cGVHD at month 12 in multivariate analysis was independent of the previous aGVHD effect on B-cell output. Finally, we showed in patients affected with cGVHD a higher B-cell division rate that correlates with an elevated BAFF/CD19-1 B-cell ratio, supporting a B-cell hyperactivation state in vivo. (Blood. 2014;124(15):2459-2462)

Introduction

One of the main of concerns in allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the profound and long-lasting immunodeficiency leaving patients highly susceptible to multiple infections. Accordingly, a low B-cell recovery after allo-HSCT has been associated with high infection rate.1,2 B-cell reconstitution is a slow process characterized by a relative increase of the percentage of naïve B cells and a recovery of memory B-cell counts that lags long behind. Several factors, such as stem cell source and composition, can affect post-HSCT B-cell recovery with graft-versus-host disease (GVHD) having the strongest influence.3-5 Indeed, B-cell lymphopoiesis is highly dependent on the marrow microenvironment, susceptible to be clarified whether acute GVHD (aGVHD) may have a persistent impact on B-cell neogenesis. It is now possible to measure the bone marrow (BM) B-cell output using real-time polymerase chain reaction (PCR) for quantification of k-deleting recombination excision circles (KRECs), which are generated in the BM during B-cell development.12-14 The coding joint (Cj) of this rearrangement is duplicated during each cell division, whereas the signal joint KREC (sjKREC) remains stable as episomal DNA. Measuring the ratio between Cj and sjKREC directly reflects the mean number of divisions achieved by B cells after the occurrence of the recombination event, thus providing a unique way to quantify the in vivo replicative history of mature B lymphocytes.12

However, the direct evidence in vivo of an increased B-cell replication in patients with cGVHD is still lacking, and it remains to be clarified whether acute GVHD (aGVHD) may have a persistent impact on B-cell neogenesis. It is now possible to measure the bone marrow (BM) B-cell output using real-time polymerase chain reaction (PCR) for quantification of k-deleting recombination excision circles (KRECs), which are generated in the BM during B-cell development.12-14 The coding joint (Cj) of this rearrangement is duplicated during each cell division, whereas the signal joint KREC (sjKREC) remains stable as episomal DNA. Measuring the ratio between Cj and sjKREC directly reflects the mean number of divisions achieved by B cells after the occurrence of the recombination event, thus providing a unique way to quantify the in vivo replicative history of mature B lymphocytes.12

Study design

All patients (n = 243) received a non–T-cell–depleted allo-HSCT at the Hôpital Saint-Louis (Paris, France) between January 2006 and December 2008 (supplemental Table 1, available on the Blood Web site). Patients having received cord blood graft or anti-CD20 treatments were excluded. GVHD was graded according to published criteria.15 The investigation was approved by the Medical Ethic Committee of the Hôpital Saint-Louis, and written informed consent was obtained from all participants in accordance with the Declaration of Helsinki.

Absolute lymphocyte count was calculated from freshly collected blood using the TruCount system (Becton Dickinson, le Pont-de-Clai, France).


S.G. and J.S. contributed equally to this study.
A.T. and E.C. contributed equally to this study.

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B-lymphocyte subpopulations were labeled with the following monoclonal antibodies (all from BD Bioscience): CD45-PercP, CD5-FITC, CD27-PE, and CD19-APC. Data were analyzed using FACSDiva (BD Biosciences). Cells were separated on lymphocyte separation medium, lysed, and stored in TriReagent. DNA was then extracted, and Cj and sjKREC were quantified by real-time PCR (ABI PRISM7700; Applied Biosystems, Foster City, CA) as described.12 Plasma was collected from EDTA blood samples. Soluble BAFF concentration was determined using Quantikine ELISA human BAFF (R&D System). Plates were read on a Biochrom Anthos Zenyth 340s.

**Results and discussion**

In this study, we followed the B-cell reconstitution consecutively at 3, 6, 12, and 24 months in a large monocentric cohort of allo-HSCT adult patients. Patients who developed aGVHD had lower counts of CD19−CD27−, CD19−CD27+, and CD19−CD5+ cells than patients without aGVHD. In CD27+ naïve B cells, the major subpopulation after transplantation, we observed the same effect of aGVHD as in the global CD19+ population. The impact of aGVHD was delayed in “memory” CD27+ B cells, being significant at months 6 and 12. CD19+CD5+ cell counts were markedly decreased in aGVHD patients at months 3 and 6. cGVHD reduced the recovery of all B-cell subsets at months 12 and 24 (supplemental Figure 1). Of note, the number of CD19+CD27− or CD19+CD5+ cells was also significant lower before transplantation for GVHD patients. In agreement with previous studies,2,3,16 we observed an early impact (months 3 to 12) of aGVHD and a late impact (months 12 to 24) of cGVHD on B-cell reconstitution.

In 43 healthy donors, Cj and sjKREC mean (± standard error of the mean) normal values were 339 (± 123) and 59 (± 34) by blood mm3, respectively, independently from gender and age. At every time point after allo-HSCT, patients’ Cj and sjKREC correlated significantly with the number of CD19+ and CD19+CD27+ B cells, respectively (supplemental Figure 2). Fewer Cj’s and sjKRECs were found in patients with severe aGVHD (grade 2 to 4) from months 3 to 12. At month 3, the number of Cj’s and sjKRECs decreased with the aGVHD grade (data not shown). From month 6 onward, Cj and sjKREC values recovered similarly in patients with 0 to 1 aGVHD. The mean number of B-cell divisions was increased in patients with cGVHD at months 6 and 12 compared with

![Figure 1. aGVHD and cGVHD delay the B-cell reconstitution but increase B-cell divisions.](image-url)

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patients without cGVHD who evidenced a lower than normal division rate, especially in the CD27^1 subset, maybe reflecting a more continuous and effective supply of quiescent B cells (Figure 1 and supplemental Figure 3). In aGVHD as well as in cGVHD, there was a reduction of naïve B-cell output measured by sjKREC together with a higher division rate, consistent with a compensatory mechanism in response to GVHD-induced lymphopenia. When we looked at both forms of GVHD at months 3 and 6, only aGVHD had an impact on B-cell production regardless of the occurrence of a further cGVHD. Conversely, patients who developed cGVHD had a lower recovery at month 12 regardless of their aGVHD status (Figure 1). Factors involved in GVHD and/or lymphoid reconstitution, such as malignant vs nonmalignant diseases, myeloablative conditioning vs reduced intensity conditioning, the source of graft (BM vs mobilized peripheral blood cells), the use of antilymphocyte or antithymocyte globulins, the recipient age, HLA identical siblings vs unrelated donors, and finally, gender mismatch were also found to have a significant impact on B-cell reconstitution in univariate analysis (supplemental Table 2). So, they were included in a multivariate analysis including aGVHD (grade 0-1 vs 2-4) and cGVHD, which confirmed that aGVHD impaired B-cell reconstitution at months 3 and 6 and cGVHD at months 12 and 24 independently of other factors (supplemental Table 2).

Given the importance of BAFF in cGVHD pathogenesis,8,10,18 we tested BAFF levels at 1 year after HSCT in a subgroup of 60 patients treated for a hematologic malignancy with no other difference in graft parameters (supplemental Table 1). All patients had significantly higher BAFF levels compared with healthy individuals. As patients with cGVHD had significantly lower CD19^1 B-cell counts, they had an increased BAFF/CD19^1 B-cell ratio compared with patients without cGVHD (Figure 2A). Notably, in patients with cGVHD, the number of B-cell divisions correlated with the level of BAFF (Figure 2B) and could correspond to the replication of cells involved in the pathogenesis of this disease.8

These data point out in a large cohort of patients some salient features of B-cell defects in cGVHD patients: a prolonged lymphopenia lasting more than 2 years after graft, compensatory mechanisms leading to sustained B-cell divisions up to 24 months, and elevated BAFF/CD19^1 B-cell ratios. Finally, this study enabled us to analyze B-cell divisions in comparison with the elevated BAFF/CD19^1 B-cell ratio, evidencing that B cells in cGVHD patients appear more responsive to BAFF than patients without cGVHD, consistently with a higher B-cell receptor activation threshold.11

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**Authorship**

Contribution: S.G. and J.S. performed research, analyzed and interpreted data, and wrote the manuscript. G.S. contributed analytical tools and collected data; R.P.d.L. and M.R. collected data; G.S. interpreted data and critically reviewed the manuscript; and E.C. and A.T. designed research, analyzed and interpreted data, and wrote the manuscript.

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Correspondence: Emmanuel Clave, Laboratoire d’Immunologie et d’Histocompatibilité AP-HP, INSERM UMRS-1160, Institut Universitaire d’Hématologie, Hôpital Saint-Louis, 1, avenue Claude Vellefaux, F-75475 Paris, CEDEX 10, France; e-mail: emmanuel.clave@univ-paris-diderot.fr; and Antoine Toubert, Laboratoire d’Immunologie et d’Histocompatibilité AP-HP, INSERM UMRS-1160, Institut Universitaire d’Hématologie, Hôpital Saint-Louis, 1, avenue Claude Vellefaux, F-75475 Paris, CEDEX 10, France; e-mail: antoine.toubert@univ-paris-diderot.fr.

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