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CORRESPONDENCE

Anti-HLA donor-specific antibodies in allogeneic stem cell transplantation: management and desensitization protocol

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The role of antibodies directed against the human leukocyte antigen (HLA) system has been well analyzed in rejection of solid organ transplantations [1, 2] and in transfusion medicine [3]. In the setting of allogeneic hematopoietic stem cells transplantation (HSCT), only in the recent years their importance has been better defined, even though anti-HLA antibodies are frequently detectable in hematologic patients, due to sensitization from multiple transfusions, usually before the introduction of online universal leukoreduction, previous transplantations, and pregnancies in female patients.

In 1989, Anasetti et al. [4] showed that the incompatibility for HLA-B and HLA-DR, and a positive complement-dependent cytotoxicity assay for anti-donor lymphocytotoxic antibody were independent risk factors associated with graft failure (GF) after an allo-HSCT. Since then, different experiences have suggested the role of antibodies directed against donor-specific HLA loci (DSA) as a risk factor for GF and graft rejection [5–10]. In case of DSAs detection, the selection of an alternative donor, if available and feasible, or the planning of an immunosuppressive strategy, to decrease the DSA levels, are required. So far, there is no standardized immunosuppression method to manage the presence of DSAs in HSCT [11]. We hereby report the incidence of anti-HLA antibodies and DSAs, and the results of the desensitization strategy employed at the Hematology Center and Immunohematology and Transfusion Medicine Unit of the "Sapienza" University in Rome. From August 2014 to January 2017, we prospectively screened for anti-HLA antibodies 65 consecutive patients who were candidates for a mismatched HSCT and 9 donors who had had immunologic events. The search for anti-HLA antibodies was carried out using the multianalyte bead assay, with the Luminex platform (Luminex, Austin, TX), including Lifecode Screen and LSA I/II (Immucor, Inc., Norcross, Georgia). The results were expressed as mean fluorescence intensity (MFI); a MFI > 1000 was considered positive. The analysis was performed 40–30 days before the transplant date. In case of a mismatched related donor, a flow cytometric cross-match test was performed.

If the patient had DSAs and more than one available donor, a donor without the corresponding HLA antigen was selected; if there was only one available donor, a desensitization protocol was employed, including the following:

- anti-CD20 monoclonal antibody (rituximab) administration on day − 15, to inhibit antibody production by CD20 + B-lymphocytes
- two single-volume plasmapheresis (PP), to remove preformed anti-HLA antibodies, on days − 9 and − 8, if the anti-thymocyte globuline (ATG) was employed as graft-vs.-host disease prophylaxis, (day − 4 to day − 2), to avoid its removal; when ATG was not used, PP were performed on days − 1 and 0
- intravenous immunoglobulins (IVIGs) infusion on day − 7 (800 mg/kg), to limit antibody rebound
- infusion of HLA-selected platelets for DSA absorption in case of persistence of antibodies directed against class I HLA antigens.

In case of still persistent DSA levels before the administration of ATG (day − 4), we employed additional single-volume PP procedures with immune-adsorption techniques (plasma treatment, PT), using columns to selectively remove

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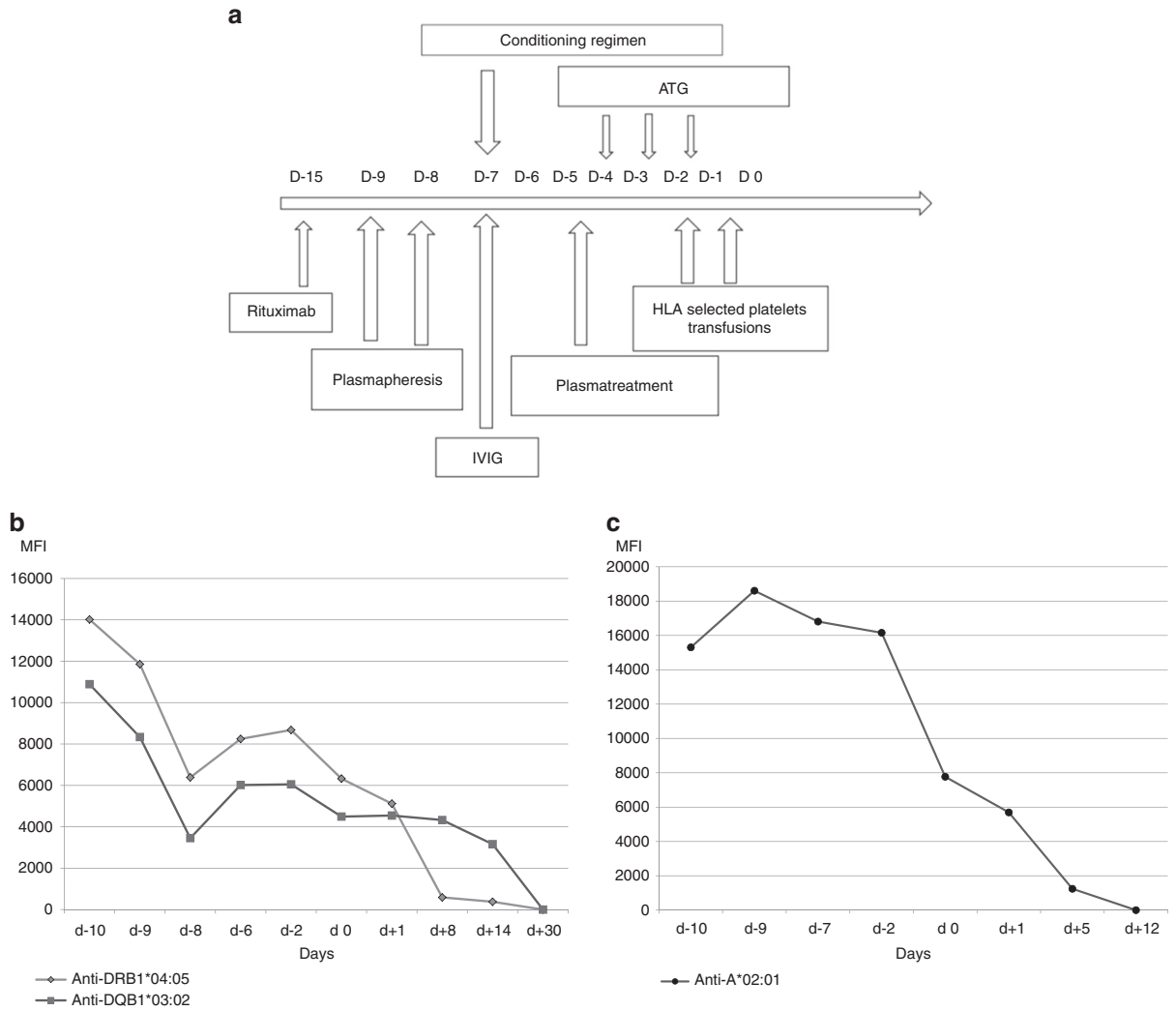

Fig. 1 **a** Desensitization strategy. ATG, anti-thymocyte globuline; D, day; IVIG, intravenous immunoglobulins. **b** MFI monitoring in the first case of desensitization. **c** MFI monitoring in the second case of desensitization

IgG antibodies, avoiding the removal of chemotherapy (Fig. 1.a).

Informed consents were obtained from all patients, or their parents or legal guardians in case of minors, both for the search for anti-HLA antibodies and for the desensitization protocol.

Non-parametric test were used to evaluate differences among groups (Fisher's exact test and Wilcoxon's test for categorical and continuous variables, respectively). Confidence intervals were reported at 95% level and all tests were two-tailed, accepting $p \leq 0.05$ as statistically significant. All analyses were performed using the R software. Thirty-nine patients were males (60%), 26 were females (40%), and median age was 41 years (range 2–65). All patients had hematologic malignancies. Fifty-nine patients (90.8%) had received blood transfusions. Twelve female patients had had previous pregnancies and blood transfusions. We studied seven female donors who had had pregnancies and two male donors who had previously received blood transfusions, finding anti-HLA antibodies in two female donors. In both cases, plasma reduction of the graft was carried out. Twenty out of 65 patients (30.8%) showed anti-HLA antibodies: 4 of them showed DSAs (6.2%). We did not find differences in the risk of developing anti-HLA antibodies, between patients with or without immunologic events, such as pregnancies/abortions and/or previous transfusions ($p = 1$). In two out of four cases with DSAs (respectively, anti-DP*04:01, MFI 2228, and anti-A24, MFI 1852), an alternative donor was selected. Two patients were treated with the desensitization strategy. The first patient, a 7-year-old male with myelodysplasia, candidate for a haploidentical transplant, had DSAs against class II paternal antigens DRB1*04:05 and DQB1*03:02, with

MFI of 14019 and 10892, respectively, and a positive cross-match test. DSA levels, monitored during the desensitization treatment and after the infusion of the paternal HSC (Total Nucleated Cell 13.42×10^8/kg, 5.5×10^6 CD34 + /kg), showed a decreasing profile with a final negativity (Fig. 1.b). Donor neutrophil and platelet engraftment were reached on days + 21 and + 50, respectively. On day + 28, a full donor chimerism was reached. Two years after the transplant, the child is in good clinical conditions, in complete remission, with a full donor chimerism. The second case, a 52-year-old female with FLT3-positive acute myeloid leukemia, candidate for an unrelated mismatched HSCT, showed DSAs against class I antigen A*02:01, with a MFI of 16807. She underwent the desensitization treatment. Due to the persistence of DSAs, on day − 4, she received a PT procedure as well, followed by three infusions of selected platelets (on days − 2, − 1, and 0), before the HSC infusion (11.4×10^6 CD34 + /kg), according to the protocol. The analysis of DSAs showed a slowly decreasing profile, with a final negativity on day + 12 (Fig. 1.c). Donor neutrophil and platelet engraftments were reached on days + 22 and + 27, respectively. The patient died on day + 48 post transplant for a multi-drug-resistant bacteria Gram-negative septic shock. In our prospective unicentric study, we confirm the importance to search DSAs before the donor selection. In different experiences, the prevalence of anti-HLA antibodies in patients who underwent a HSCT varies from about 20% to 40% [12]. According to these data, we observed 30.8% of patients with anti-HLA, without statistically significant differences in alloimmunization, between transfused or not transfused patients, thanks to the introduction of online universal leukoreduction [13]. The European Society for Blood and Marrow Transplantation has published consensus guidelines [14] for the management of DSAs in haploidentical transplant, suggesting to desensitize patients with DSAs before HSCT, if another suitable donor is not available, without suggesting a defined schedule. The most frequent strategies employ B-lymphocyte depletion with rituximab and plasma exchanges, combined or not with high-dose IVIG [5, 9, 12, 14–17], other strategies use transfusion of platelets bearing HLA antigens corresponding to DSAs [5, 12, 14, 18], or the infusion of donor-irradiated lymphocytes [17]. Taking into account the previous experiences [5, 9, 12, 14–18], we planned a desensitization schedule obtaining DSAs removal, without observing antibodies rebound and avoiding any interference with the pharmacokinetics of the chemotherapy of the conditioning regimen and ATG administration. Even if only two patients were treated, both obtained a stable engraftment without late effects. The schedule of desensitization hereby employed appears to be safe and effective to obtain a stable engraftment. We can speculate that the availability of a safe and efficacy desensitization strategy might allow to avoid the change of the first selected donor, even in the case of DSA detection, if the immunogenetic and clinical characteristics are better, compared with other donors. Prospective multicentric studies are required to conclusively define the role of DSAs in relation to each of the HLA loci, the cutoff of MFI associated with a higher risk of graft rejection, and the efficacy of a desensitization strategy. A strong collaboration between transplant physicians, transfusion specialists, and immunogeneticists is required.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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