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CHARACTERIZATION OF HLA ANTIBODIES SPECIFIC TO TWO RECIPIENTS OF HEMATOPOIETIC STEM CELLS GRAFTS FROM THEIR HAPLOIDENTICAL MOTHER

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Two brothers with dyskeratosis congenita were referred to the lab for hematopoietic stem cells donors search. The haploidentical mother was the donor because no compatible donors were found. As brothers inherited the same paternal haplotype (A*24:02/C*03:04/B*40:02/ DRB1*16:02/DQB1*03:01/DPB1*17:01), the mother could had been sensitized by the paternal HLA molecules. Search for recipient-specific antibodies (RSA) in maternal serum was assessed by solid phase assays using isolated HLA antigens and phenotypes panels (LABScreen SAB/PRA) (Cutoff: MFI≥1000). Epitope reactivity analysis was done with EpVix and HLA Fusion software. Results showed that mother had RSAs against HLA incompatible molecules A24, B61 and DP17 expressed by her children; Epitope specificities were Eplet 62EE/TerEp 28 shared by A23, A24 and A80 (Average MFI=3,250); Eplet 163EW+73TE/TerEp 223 present in B7 CREG including B7, B13, B27, B47, B48, B60, B61, B73 and B81 (Average MFI=10,114); and Eplet 84DEAV/TerEp 4001 shared by DP1, DP3, DP5, DP6, DP9, DP10, DP11, DP13, DP14, DP17, DP19 and DP20 (Average MFI=1,303). Investigation continued with crossmatches (XMs) and adsorption/elution experiments in order to confirm this pattern of reactivity in silico. XMs were performed with mother's serum and reference cells expressing RSAs target HLA molecules. Flow XMs using A24 cells showed weak positive reactions with B+pronase (MCF=325; Cutoff=275.5) and T lymphocytes (MCF=308; Cutoff=269.5); B61 cells showed strong positive reactions with B+pronase (MCF=592; Cutoff=251.25) and Tcells (MCF=425; Cutoff=224). CDC-XMs with T, T+DTT, T+AGH, T+AGH+DTT, B and B+DTT were negative for A24 and B61 cells. Adsorption/elution experiments were done with maternal serum and children T/B lymphocytes, and SAB assay was repeated with eluates. The in silico analysis was compared to the eluate epitope reactivity, and results confirmed the RSA anti-A24 against Eplet 62EE/TerEp 28. However, RSA anti-B61 identified in the eluate showed a different pattern of epitope reactivity than that inferred by in silico analysis. Besides reactions with B7, B13, B27, B47, B48, B60, B61, B73 and B81, it also showed positive reactions with A*66:02, B73 and Cw2. The configuration 163EW+self73T explains this unexpected reactivity, despite not being described yet in Epvix and HLA Fusion software nor in HLA Epitope Registry. In regard to RSA anti-DP, eluate analysis confirmed the anti-84DEAV antibody and revealed another type of RSA not detected by the in silico analysis. This second RSA is specific to 57D Eplet which is shared by DP3, DP6, DP9, DP14, DP17 and DP20. This study shows that only the adsorption/elution experiments were able to evidence with accuracy the epitope reactivity of RSAs present in the maternal serum. And it also emphasizes the importance of careful epitope analysis when searching for RSAs in the sera of any HLA incompatible donor, because these antibodies are associated with increased risk of GVHD development.