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De novo production of granulocyte-macrophage colony-stimulating factor antibodies following hematopoietic stem cell transplantation from HLA-matched unrelated donors is associated with acute graft-versus-host disease

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Objective

The aim was to examine any associations between the presence of non-HLA antibodies and the occurrence of acute graft-versus-host disease (aGVHD) after unrelated donor hematopoietic stem cell transplantation (UD HSCT).

Patients

HLA matching status was used to classify 58 adult patients who survived three months after UD HSCT. Group A contained 16 patients with full 9-loci HLA match (HLA-A, -B, -C, -DRB1, -DRB3/4/5, -DQB1, and -DPB1). Group B comprised 24 patients who had a single, permissive HLA-DPB1 allele mismatch, which was identified as having the lowest risk of developing aGVHD using T-cell epitope and HLA-DP expression models. Group C consisted of 18 patients with any non-permissive HLA mismatch.

Methods

Serum samples were collected from patients before the conditioning regimen (S0), one month (S1), two months (S2), and three months (S3) after HSCT. A panel of 60 non-HLA antigens in the LIFECODES® Non-HLA Antibody Kit (Werfen) was used to test serum samples for antibodies using Luminex technology. Statistical analysis was conducted using the Fisher exact test. Statistical significance was defined as a *p*-value of < 0.05.

Results

Analysis of S3 samples showed that 47 of 58 patients (81.0%) had varying non-HLA antibody patterns. Data of S0 samples indicated that 23 of 58 patients (39.7%) produced *de novo* non-HLA antibodies 3 months after HSCT. Post-HSCT and *de novo*-only antibody patterns indicated GM-CSF antibodies were the most prevalent. 15 of 58 patients (25.9%) had GM-SCF antibodies and 8 of 58 patients (13.8%) produced them *de novo* (Figure 1). The Fisher exact test showed statistically significant associations between either all post-HSCT or *de novo*-only produced GM-CSF antibodies with aGVHD in the whole cohort (p = 0.034 and p = 0.021, respectively). The subsequent examinations revealed that only patients in Group A are accountable for the prior reported associations. *De novo* GM-CSF antibody production was linked with aGVHD (p = 0.001) (Table 1).

Examination of the S1 and S2 samples of patients, who had aGVHD or were *de novo* GM-CSF antibody-positive in their S3 samples, revealed that two S2 samples of Group B patients who developed aGVHD within two months of HSCT produced *de novo* GM-CSF antibodies. Their medical records indicated that aGVHD symptoms resolved rapidly. This clarified the negative GM-CSF antibody findings from their S3 sample testing. The Fisher exact tests have been repeated, including the data mentioned above. In Group B, the presence of *de novo* GM-CSF antibodies became substantially linked with aGVHD (Table 1). No *de novo* GM-CSF antibody generation was identified among Group C patients who received non-permissively HLA-mismatched UD transplants.

Time points of collection of S1, S2, and S3 samples with *de novo* GM-CSF antibodies and the first records of aGVHD in patients' medical histories showed that aGVHD triggers the generation of de novo GM-CSF antibodies..

No significant results were found for any other non-HLA antibodies.

Conclusions

To our knowledge, the presented study is the first to identify a correlation between the production of *de novo* GM-CSF antibodies and occurrence of the grade II-IV aGVHD. Based on our results, we hypothesize that the aGVHD initiation and development process after the HLA-matched HSCT may differ from that seen after the non-permissively HLA-mismatched HSCT. Our results should be further examined in larger patient cohorts. Thus, more research is required to get a deeper understanding of the biological connections between GM-CSF secretion, development of aGVHD, and *de novo* GM-CSF antibody production following the HLA-matched, UD HSCT.

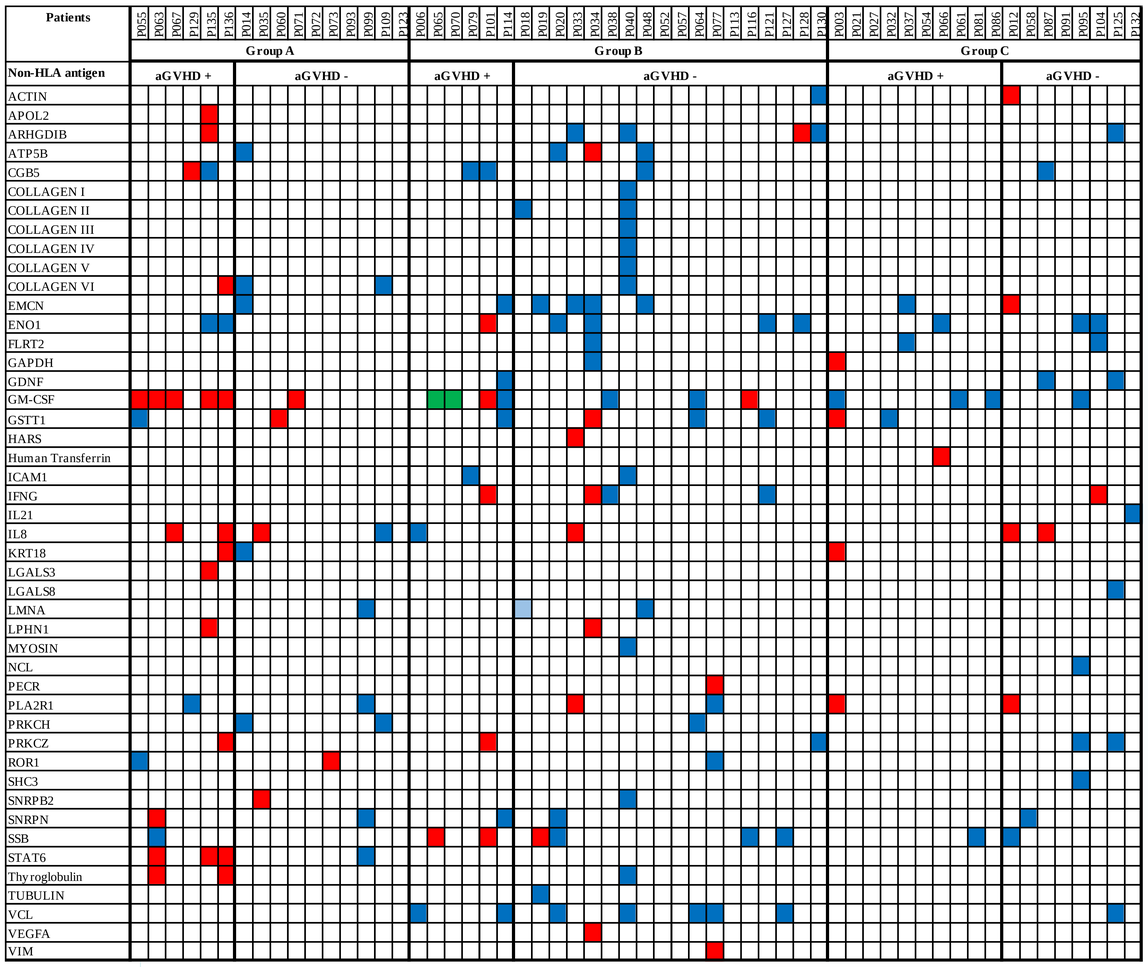

Figure 1. Non-HLA antibody patterns three months after HSCT. Only reactive non-HLA antigens are shown. Blue – non-HLA antibodies detected before and after HCT. Red – *de novo* non-HLA antibodies. Green – *de novo* GM-CSF antibodies detected after S2 sample testing..

Table 1. Fisher exact test results.

Patient groups		Cohort		Group A		Group B		Group C	
		58		16		24		18	
		aGVHD		aGVHD		aGVHD		aGVHD	
		Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive
post-HSCT non-HLA antibodies	Negative	6	5	3	0	2	2	1	3
	Positive	31	16	8	5	16	4	7	7
		p = 0.504		p = 0.509		p = 0.251		p = 0.588	
de novo non-HLA antibodies	Negative	24	11	6	0	13	3	5	8
	Positive	13	10	5	5	5	3	3	2
		p = 0.409		p = 0.093		p = 0.362		p = 0.608	
post-HSCT GM-CSF antibodies	Negative	31	12 (10*)	10	0	14	5 (3*)	7	7
	Positive	6	9 (11*)	1	5	4	1 (3*)	1	3
		p = **0.034** (**0.006***)		p = **0.001**		p = 1.000 (0.307*)		p = 0.588	
de novo GM-CSF antibodies	Negative	35	15 (13*)	10	0	17	5 (3*)	8	10
	Positive	2	6 (8*)	1	5	1	1 (3*)	0	0
		p = **0.021** (**0.003***)		p = **0.001**		p = 0.446 (**0.035***)		–	

Statistical significance is marked in **bold**.

* recalculated numbers of patients and results of statistical analysis after testing S1 and S2 samples**.**