A case of severe combined immunodeficiency with sticky myeloid cells

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Background

Severe Combined Immunodeficiency (SCID) represents a group of rare diseases characterized by severe disruption of T cell development and function which leads to impaired cellular and humoral immunity[1]. Our patient is a 10-month-old Caucasian male with T- B+ NK+ X-linked SCID phenotype due to an interleukin 2 receptor γ chain (*IL2RG*) gene hemizygous mutation c.710G>A (p.Trp237*) identified at newborn screen[2]. A few weeks after birth, he presented with a mild to moderate case of diaper dermatitis and preseptal cellulitis. Otherwise, the patient has been doing well: he is afebrile and exhibited no other signs of infection pre-transplant. The patient received an allogeneic unrelated peripheral blood stem cell transplant (PBSCT) with α/β positive T cell and CD19+ B cell depletion ~3.5 months after birth. A moderate but consistent increase in T cell count was observed from ~1 to 10 months post-transplant. The patient is developing well post-transplant without any complications.

Materials and Methods

Engraftment Monitoring (EMO) by Short Tandem Repeat (STR)

EMO analyses is used to determine the level of donor cells engrafted in a recipient post allogeneic HSCT. This test exploits allelic differences between patient and donor to quantitate the level of donor engraftment. To identify informative markers pre-transplant, patient and donor DNA samples were amplified by PCR using fluorescently labelled forward and unlabelled reverse primers for 24-locus short tandem repeats (21 autosomal and 3 sex-determining loci) and the amelogenin gene (GlobalFiler PCR Amplification Kit, Thermo Fisher Scientific, Waltham, MA). Amplicons were separated by size using capillary electrophoresis and are identified by fluorescence detection. The peak area of the informative loci is used to determine the overall % donor engraftment using the formula shown below:

$$\% \text{ Donor Engraftment} = \frac{\text{Donor Allele 1} + \text{Donor Allele 2}}{\text{Donor Allele 1} + \text{Donor Allele 2} + \text{Recipient Allele 1} + \text{Recipient Allele 2}} \times 100$$

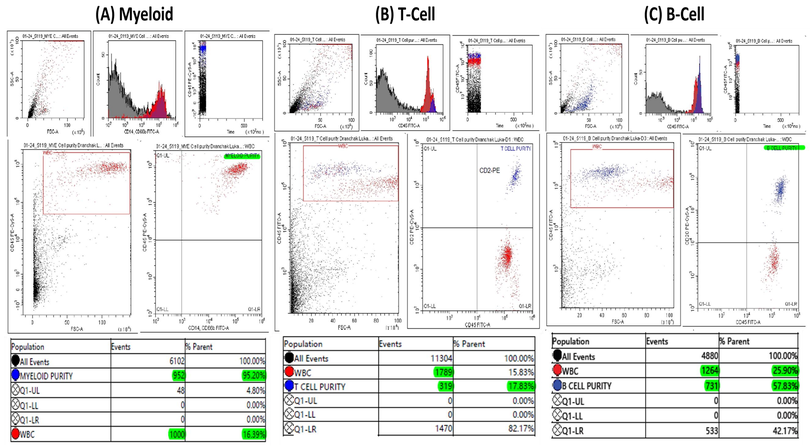
An average of all the informative loci is used to determine the overall % donor engraftment.

Lineage Specific Cell isolation

During the post-transplant period, lineage specific cell isolation from the recipient peripheral blood samples was performed using immunomagnetic beads to positively select for T, B, and myeloid cells using anti-CD3, -CD19, and -CD33/66b respectively (EasySep Positive Selection kits, STEMCELL Technologies, Cambridge, MA). The cell isolates were then stained with cell-specific fluorescently conjugated anti-human antibodies (Beckman Coulter Life Sciences, Brea, CA) and analyzed using flow cytometry. The purity of each cell fraction is calculated as a percentage of gated CD45 positive cells (pan-leukocyte marker) that are also positive for anti-CD2 (T cells), -CD20 (B cells), -CD14/66b (myeloid cells). DNA from each cell isolate was extracted and subjected to chimerism STR testing as described.

Results

Donor EMO began on day +28, and serial testing continued monthly. Donor T cells at 100% engraftment were first detected on day +124 and continued to be detected above 90% until day +205. On day +229, abnormally low T (17.8%) and B cell (57.8%) purities were measured with an unexpected donor T cell engraftment of 83% (Table 1). In Figure 1, the gated WBCs in the CD45+ FITC vs. FSC plot shows two distinct cell populations one measuring ~20 to 60 x10^4 FSC units (blue dots) and the other at ~60 to 100 x10^4 FSC units (red dots). The presence of these larger cells were negatively impacting purity assessments of T and B cells. Based on the size, increased granularity, and inherent cellular properties, the events captured in Figure 1 Q1-LR are most likely to be myeloid cells. To rectify this, myeloid cells were first depleted, then the remaining supernatant was used to isolate T and B cells. Selected T and B cells were then stained with a combination of anti-CD45/CD2 and anti-CD45/CD20 respectively for flow cytometric analyses. The results showed that, in the absence of interfering myeloid cells, the T and B cell purities were 93.6% and 99.6%, respectively (Figure 2). Also, the donor T cell engraftment of 98% was as expected (Table 1). There was no effect of myeloid depletion on donor B cell engraftment. For this patient myeloid cell removal is recommended for all samples used for EMO.

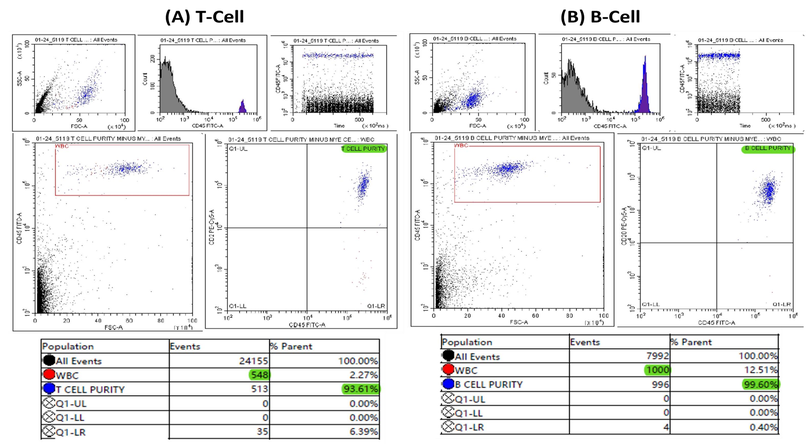


Population	Events	% Parent
All Events	6102	100.00%
MYELOID PURITY	853	95.20%
Q1-UL	48	4.80%
Q1-LL	0	0.00%
Q1-LR	0	0.00%
WBC	1000	6.39%

Population	Events	% Parent
All Events	11304	100.00%
WBC	1789	15.83%
T CELL PURITY	319	17.83%
Q1-UL	0	0.00%
Q1-LL	0	0.00%
Q1-LR	1470	82.17%

Population	Events	% Parent
All Events	4880	100.00%
WBC	1264	25.90%
B CELL PURITY	731	57.83%
Q1-UL	0	0.00%
Q1-LL	0	0.00%
Q1-LR	533	42.17%

Figure 1. Flow cytometry plots showing (**A**) myeloid, (**B**) T cell, and (**C**) B cell subsets from recipient blood collected on day +229. The T cell purity percentage is abnormally low at 17.83%.



Population	Events	% Parent
All Events	24155	100.00%
WBC	548	2.27%
T CELL PURITY	513	93.61%
Q1-UL	0	0.00%
Q1-LL	0	0.00%
Q1-LR	35	6.39%

Population	Events	% Parent
All Events	7992	100.00%
WBC	1000	12.51%
B CELL PURITY	996	99.60%
Q1-UL	0	0.00%
Q1-LL	0	0.00%
Q1-LR	4	0.40%

Figure 2. Flow cytometry plots of (**A**) T and (**B**) B cells after removal of myeloid cells from recipient blood collected on day +229.

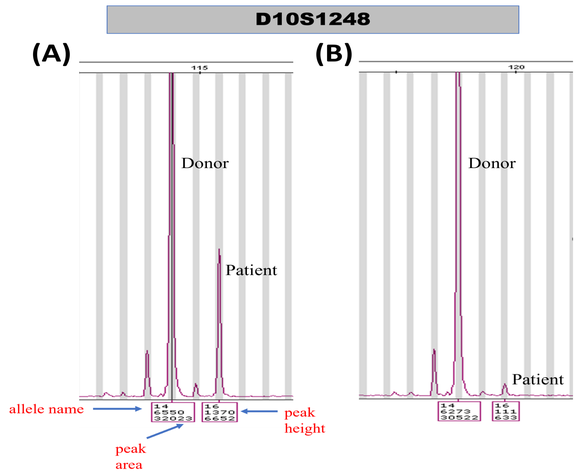


Figure 3. Engraftment analysis of recipient T cells post-HSCT on day +229 before (**A**) and after (**B**) myeloid depletion

Table 1. Donor Engraftment Results

Day after Transplant	Sample Type	Non-Enriched	Myeloid	T Cell	B Cell
+376	Peripheral Blood	69%	35%	100%	70%
+257	Peripheral Blood	75%	63%	100%	81%
+229 (*)	Peripheral Blood	78%	70%	98% (*)	81% (*)
+229 (^)	Peripheral Blood	78%	70%	83% (^)	81% (^)
+205	Peripheral Blood	83%	68%	100%	85%
+180	Peripheral Blood	82%	78%	98%	80%
+152	Peripheral Blood	88%	82%	100%	85%
+124	Peripheral Blood	93%	92%	100%	91%

(*) Corrected results for T and B cell engraftment performed on peripheral blood collected on day +229. Engraftment testing and purity assessments of T and B cells were conducted following the depletion of myeloid cells from the sample. The purity of T and B cells was 93.6% and 99.6%, respectively. (^) Original non-corrected engraftment results collected on day +229.

Conclusions

Myeloid cells express various cell-adhesion molecules on their surface which enable them to bind to specific ligands on other cells and the intercellular matrix[3]. Consequently, myeloid cells tend to be inherently 'sticky' and may adhere to other cell populations whereby they are co-selected even after lineage specific isolation of T cells where myeloid cells and other cell types presumably are discarded prior to staining. Why this happens in blood samples from some patient's and not others is not well understood. This case represents an atypical example of donor cell engraftment results in a SCID patient. We have not observed this phenomenon in other SCID patients. Our case highlights the importance of correlating the patient's clinical history with the findings generated by engraftment monitoring testing. Also, purity assessments of isolated cell populations are essential for accurate lineage-specific chimerism because contamination from non-target cells can distort results, making them unreliable. Furthermore, an understanding of the patient's condition helped to inform the necessary troubleshooting experiments.

Contact

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