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LETTER TO EDITOR

Different Clonal T-Large Granular Lymphocyte Proliferations in SCID

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Abbreviations

CMV	Cytomegalovirus
EBV	Epstein-Barr virus
GvHD	Graft versus host disease
IVIG	Intravenous immunoglobulin
JAK3	Janus Kinase 3
LGL	Large granular lymphocytes
SCID	Severe combined immunodeficiency
STAT	Signal transducer and activator of transcription 3
STR	short tandem repeat
T-LGL	T-large granular lymphocyte
TCR	T cell receptor
UCBT	Umbilical cord blood transplantation

To The Editor:

Large granular lymphocytes (LGLs) are morphologically defined cells containing small numbers of discretely recognizable granules when examined under the microscope. They can belong to the natural killer or T cell subtypes and are implicated in innate and adaptive immunity, respectively. Their proliferations in peripheral blood and tissues have been described; some of those proliferations are clonal and can represent either malignant or reactive processes [1]. The discovery of signal transducer and activator of transcription 5, *STAT3* mutations, in LGL leukemia was thought to be a potential marker of malignancy; however, they have been reported in some patients with Felty syndrome as well [2].

Proliferations of T cell LGL (T-LGL)—also known as T cell large granular lymphocytosis—are described in association with many different entities: myelodysplastic syndromes, autoimmune cytopenias, rheumatologic conditions, following transplantation, and with dasatinib therapy for leukemia. Decreased CD5 expression is a feature of T-LGL [1]. In this letter, we describe a case of severe combined immunodeficiency (SCID) due to a homozygous Janus Kinase 3 (*JAK3*) mutation, which was associated with the development of three different clonal T-LGL proliferations: the first with significant maternal engraftment, the second following unrelated mismatched umbilical cord blood transplantation (UCBT) during cytomegalovirus (CMV) infection, and the third during an acute Epstein-Barr virus (EBV) infection after discontinuation of immunosuppression.

The Hispanic female patient was born at term to distantly related parents, and immune deficiency was identified by T cell receptor (TCR) excision circle testing as part of newborn screen. Lymphocyte subset analysis was consistent with T-B+NK+ SCID. She was started on intravenous immunoglobulin (IVIG) supplementation and anti-microbial prophylaxis; prior to supplementation, her IgA was low, and IgM was within normal limits. She was breastfed for the first several days of life, which was halted once newborn screen results became available. Additional genetic testing subsequently identified a homozygous deletion of JAK3 exons 18–23 (GeneDx, Gaithersburg, MD).

Due to rising lymphocyte counts and T cells in particular, short tandem repeat (STR) analysis was performed and showed maternal engraftment, up to 97% of T cells at day 38 of life. Due to a further increase in number of T cells, predominantly of CD8+ type, she was started on tacrolimus for the prevention of symptomatic engraftment syndrome (Table 1). Flow cytometric analysis revealed increased presence of CD5-dim T cells with several LGL observed on the peripheral blood smear by light microscopy, together indicating increased T-LGL (Fig. 1a and b). Furthermore, TCR rearrangement studies showed clonality, which was described as the presence of one or two peaks at least three times the height of the polyclonal background without any clonality in B cells

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Table 1 Laboratory findings observed over time

Time	Age	CD3+	CD4+	CD8+	CD19+	NK	C-M	C-L	CMV	EBV	T-LGL-P	T-LGL-C	TCR	IgG/A/M
Pre-UCBT	4d	0.012 Low	0.01 Low	0.002 Low	0.397 Low	0.035 WNL	–	–	–	–	–	–	–	–
	32d	1.564 Low	0.361 Low	1.206 WNL	0.862 WNL	0.053 WNL	–	–	–	–	–	–	–	
	38d	4.840 WNL	0.280 Low	4.594 High	0.782 WNL	0.086 WNL	M-0	M-97	–	–	30	1.452	Clonal	526/< 8/57 mg/dayL All WNL
	58d	8.026 High	0.572 Low	7.037 High	2.426 WNL	0.173 WNL	–	–	ND	–	–	–	Clonal	721/< 8/38 mg/dL All WNL
	99d	1.491 Low	0.238 Low	1.168 WNL	0.917 WNL	0.146 WNL	M-5	M-68	ND	–	43	0.641	–	795/NA/NA WNL
Post-UCBT	+26d	–	–	–	–	–	D-Only	D-Only	11,949	ND	–	–	–	1080/NA/NA High
	+38	–	–	–	–	–	–	–	380, 458	ND	57	–	Clonal	1740/NA/NA High
	+74	0.891 Low	0.152 Low	0.688 WNL	1.128 WNL	0.371 High	D-Only	D-Only	1577	ND	–	–	–	1160/NA/NA High
	+135	–	–	–	–	–	–	–	1464	ND	–	–	Clonal	
	+211	2.319 Low	1.228 Low	1.090 WNL	0.997 WNL	0.455 High	D-Only	D-Only	< 137	ND	–	–	Clonal	1020/NA/NA High
	+284	–	–	–	–	–	D-Only	D-Only	ND	ND	38	–	Clonal	
	+365	5.344 High	2.538 WNL	2.595 High	1.869 WNL	1.302 High	–	–	ND	ND	16	0.855	–	1426/55/174 High/WNL/High
	+395	7324 High	3111 High	4195 High	0.606 WNL	0.997 High	92%	D-Only	ND	10,825	18	1318	Clonal	1491/162/239 High/High/High

UCBT, umbilical cord blood transplantation; *d*, days; *NKC*, natural killer; CD3, CD4, CD19, NK: cell counts ($\times 10^9$ /L); *WNL*, within normal limits for the age; *C-M*, myeloid chimerism (%); *C-L*, lymphoid chimerism (%); *M*, maternal; *D*, donor; *CMV*, cytomegalovirus copy number by PCR (IU/mL); *EBV*, Epstein-Barr virus copy number by PCR (IU/mL); *NA*, not assessed; *T-LGL-P*, T-large granular lymphocyte percent of T cells; *T-LGL-C*, T-large granular lymphocyte count ($\times 10^9$ /L); *TCR*, T cell receptor rearrangement status; *ND*, not detected

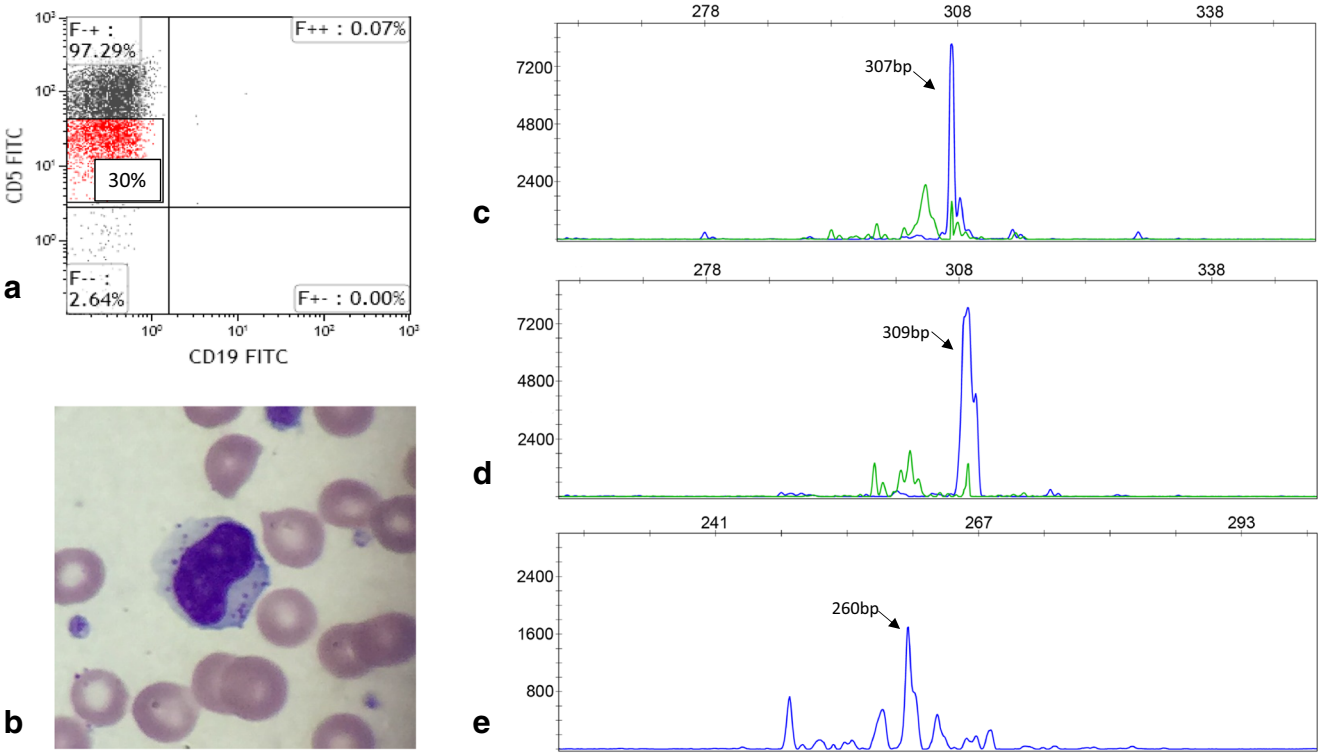

Fig. 1 Flow cytometric detection of T-large granular lymphocytes (T-LGL) characterized by CD5-dim staining in CD3-backgated lymphocyte population (**a**). Large granular lymphocytes with distinct, small in number, and easily identifiable cytoplasmic granules on Wright-Giemza-stained peripheral blood slides (**b**). Representative TCR clonality profiles from peripheral whole blood collected pre-UCBT (**c**), during post-UCBT CMV infection (**d**) and with acute EBV infection (**e**). Clonal peaks are indicated by arrows (**c**–**e**, x axis: base pairs, y-axis: relative fluorescence units). GeneScan results of the Dβ-Jβ1/2 gene rearrangements detected with the Euroclonality/BIOMED-2 *TCRB* tube C (**c**, **d**). GeneScan results of the Vβ-Jβ1/2 gene rearrangements detected with the Euroclonality/BIOMED-2 *TCRB* tube A (**e**)

by immunoglobulin gene rearrangement evaluation. Lymphoid maternal chimerism was 68%, 99% in separated CD3+ lymphocytes, and 33% in CD56+CD16+ cells, and B cells were of patient origin on day 99 of life. Her mother was sero-positive for cytomegalovirus (CMV); no circulating CMV was detected in the patient likely due to anti-CMV T cell immunity secondary to maternal engraftment. She never developed any clinical symptoms or signs of maternal engraftment while on therapeutic tacrolimus. The same clonal T-LGL proliferation was persistent on repeated testing until UCBT (Fig. 1c; Table 1).

She received a molecularly 4/6-matched unrelated UCBT following fludarabine 1.2 mg/kg/dose on days −8 to −3, busulfan 55 mg/L × h equating to an area under the curve (AUC) of 4466 μmol × min/L per dose on days −5 to −3 for a cumulative AUC of 13,398 μmol × min/L and thymoglobulin 2.5 mg/kg/dose on days −4 to −2 conditioning with a total nucleated cell (TNC) e.g. mononuclear cell dose of 14.3×10^7/kg and CD34+ cell dose of 8.0×10^5/kg when she was 126 days old. Tacrolimus and mycophenolate were used for graft versus host disease (GvHD) prophylaxis. She had neutrophil engraftment on post-transplant day 25 and has never developed GvHD. She experienced CMV viremia starting on day 26 following transplantation likely due to the presence of CMV in lymphocytes transferred from the patient's mother. Despite sequential use of intravenous ganciclovir and foscarnet along with IVIG and anti-CMV globulin, she continued to have viremia. Surprisingly, both CD4+ and CD8+ proliferations to CMV pp65 antigen were within normal limits by commercial Elispot testing while she was viremic. No known mutations (UL97 and UL54) for resistance to ganciclovir, foscarnet, or cidofovir were detected in culture-grown CMV. She never manifested CMV disease and oral valganciclovir was later used for treatment of the CMV infection.

At post-transplant day 38, she was found to have increased T-LGL in peripheral blood by flow cytometry along with detection of a clonal population by TCR rearrangement studies. Interestingly, the clonal TCR rearrangements were different than the ones detected prior to UCBT. This was demonstrated by a different clonal rearrangement detected in TCR Dβ1-Jβ2 (Fig. 1d), and a new clonal rearrangement detected in TCR Vβ-Jβ2 that was not present prior to UCBT (data not shown). She had full donor chimerism at the time. Repeated studies showed continued presence of clonal T-LGL proliferation with a clonal TCR Dβ1-Jβ2 rearrangement that persisted over several months of follow-up (Fig. 1d; Table 1). Blood CMV became negative and she was tapered off immunosuppression completely by the end of 11 months following UCBT. Her

IgG was elevated and IgA was within normal limits without supplementation.

One year post-UCBT, she continued to display full donor chimerism with normal immune functions and was otherwise doing well without any evidence of GvHD, and flow cytometric analysis showed a dramatically lower T-LGL population. A few weeks after her 1-year post-transplant visit, she presented with bilateral periorbital edema and decreased activity without fever following an episode of acute gastroenteritis the week prior. She did not have other physical examination findings; several activated lymphocytes were observed on peripheral blood. Her EBV PCR showed 10, 825 copies per milliliter and later development of EBV IgM antibody indicating acute infection [3]. Investigations including renal function and urinalysis were reassuring, and features resolved within several days. No therapeutic intervention was necessary. At this time, a different clonal Vβ-Jβ1/Jβ2 rearrangement was detected (Fig. 1e), but no clonal TCR Dβ1-Jβ2 rearrangements were detected (data not shown).

Throughout the tests, peripheral blood T cells were predominantly of CD8+ type and CD3+CD8+CD57+ populations correlated with CD3+CD5-dim cells. Expansion of clonal maternal T cells has been rarely reported in SCID cases with maternal engraftment [4]. However, clonal cells were not identified as T-LGL in those cases. The target of proliferating clonal cells was not reported, either. In our case, three episodes of T-LGL proliferation with three different clonal TCR immunoclones were observed, one prior to and the others following UCBT. Interestingly, expanding clonal T-LGL was maternal, since all T cells were of maternal origin before UCBT. Passage of maternal lymphocytes during delivery and later preferential expansion of a certain clone, likely targeting a widely expressed patient-specific antigen, is possible in this case.

One of the lessons from this case was the possibility of obfuscation of the diagnosis of SCID due to maternally engrafted T cells, where assessment of the CBC with differential to occur in the absence of (a) T/B/NK subset STR analysis and/or (b) newborn screening assessment for TCR excision circles. In our case, absolute lymphocyte count and lymphocyte subset counts were within normal limits in the pre-transplant period. However, all T cells and a proportion of NK cells were of maternal origin as determined by STR analysis (Table 1). Furthermore, TCR excision circles were absent on the newborn screen. This lends credence to the value of routine newborn screening as such a patient as this could easily have been "missed" until the patient presented with severe infectious complications and/or symptoms of maternal engraftment. Our observation of clonally expanded T-LGL population may provide an additional clue to the diagnosis of maternal engraftment.

The two different immunoclones detected post-transplant were due to expansion of different donor-derived T-LGL populations—as the patient had full donor lymphoid chimerism at those time points—likely targeting the CMV infection and later the EBV infection. Expansion of T-LGL has been reported in association with CMV infection following allogeneic hematopoietic stem cell transplantation [5]. Nevertheless, in our patient, the reduction in T-LGL population and the specific clonotype happened along with immune suppression withdrawal and eradication of CMV viremia in the first post-UBCT expansion.

In this case, the common denominator for the first two clonal T-LGL proliferations before and after UCBT is SCID, additional immune suppression due to tacrolimus use, and post-transplant immune deficiency/tacrolimus use, respectively. The last clonal T-LGL development was associated with acute EBV infection, and also occurred in the context of an infection-related immunosuppression status. However, the question of why the proliferating T cells are T-LGL type remains a mystery; underlying immune deficiency and relative resistance of T-LGL to immunosuppression may constitute the predisposing factors. As such, tacrolimus use did not reverse or prevent clonal T-LGL proliferation prior to UCBT and the first expansion post-UCBT.

The patient has not developed any clinical or laboratory evidence of leukemia. We therefore, conclude that the clonal T-LGL proliferations in this case were reactive in nature and likely represented an example of the more widespread presence of this phenomenon in different immune dysregulation conditions under certain stressors as a compensatory/protective mechanism. Future studies identifying the specific targets of clonally expanded T-LGL in different settings may shed light on the pathophysiology of this entity.

Compliance with Ethical Standards

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

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