

Letter to the Editor

Persistence of natural killer cells with expansion of a hypofunctional CD56⁺CD16⁺KIR⁺ NKG2C⁺ subset in a patient with atypical Janus kinase 3-deficient severe combined immunodeficiency

To the Editor:

Janus kinase 3 (JAK3) is an intracellular tyrosine kinase expressed in hematopoietic cells required for signal transduction by the γ chain (γc), which is a subunit of multiple cytokine receptors. JAK3-deficient severe combined immunodeficiency (SCID) is typically diagnosed within the first few months of life; if not, then the mortality rate is very high during the first 2 years of life. Patients with typical JAK3-deficient SCID exhibited a T⁺B⁺ natural killer (NK) cell-negative phenotype.¹ Herein we report an unusual persistence of NK cells in a patient with JAK3-deficient SCID with a mild clinical and immunologic presentation resulting in leaky SCID associated with an expanded, unusual CD56⁺CD16⁺ NK cell subset, a subset previously described in patients with chronic HIV and others viremia,² with atypical phenotypic and functional characteristics.

The patient was the second son of Algerian consanguineous parents. SCID was diagnosed at 27 months because of a lymphopenia discovered in the context of bronchopneumonia responding well to antibiotics. Additional clinical features associated with SCID were a prolonged episode of fever of unknown origin, mouth ulcers, and 2 episodes of severe diarrhea, with low weight (10th percentile) and height (25th percentile).

The patient's initial immune studies revealed profound CD3⁺T-cell deficiency and normal CD19⁺ and high CD3⁺CD56⁺ lymphocyte counts (see Table E1 in this article's Online Repository at www.jacionline.org). NK cells accounted for 58% of total lymphocytes. There were no CD4⁺CD45RA⁺ naive cells. Imaging confirmed the absence of thymic tissue. Functional investigations of T lymphocytes demonstrated no proliferative response to mitogen and a very weak positive response reproducible to vaccine antigens (proliferative index, 3.7 and 3.5 for tetanus toxoid and purified protein derivative antigens, respectively [positive if >3]). Serum immunoglobulins of all major classes were detected in standard or increased amounts, and IgG to some vaccine antigens (tetanus toxoid and poliomyelitis) was also present. In the context of T⁺B⁺NK⁺ SCID, α subunit of the IL-7 receptor (IL-7R α) protein deficiency was first suspected. IL-7R α protein expression was low, as detected in flow cytometric analysis (5%; data not shown). Nevertheless, genomic sequencing of *IL7R* did not demonstrate mutations. Persistence of maternal lymphocytes was excluded by using fluorescence *in situ* hybridization (XY: 100% of cells). Genomic sequencing of the *JAK3* gene revealed a homozygous *JAK3* R117C missense mutation in exon 3 that has never been described previously. The mutation was localized in the proximity of the *JAK3* protein region associated with the γc . Although *JAK3* protein was present, as detected in flow cytometric analysis, *JAK3* mutation impaired *JAK3* function (see Fig E1 in this article's Online Repository at www.jacionline.org).

Unusually, as shown in Table E1, NK cell counts were normal or increased. Fig 1 shows the phenotypic characteristics of the patient's NK cells. The CD56⁺CD16⁺ subset accounted for 61%

versus 10.7% ($\pm 2.7\%$) of the control group (Fig 1, A) and displayed very high expression of KIR2DL2/DL3 (CD158b: 89.2% vs 5.7% [median] in the control group) and low levels of NKp30 but high levels of NKp46 and perforin. Expression of NKG2C-activating receptor was high (>80% of NK cells), whereas that of NKG2A-inhibitory receptor was low (Fig 1, B and C), a phenotype that has been previously associated with cytomegalovirus (CMV) infection.^{3,4} Moreover, similar to patients with HIV,⁵ JAK3 NK cells displayed strongly reduced expression of Siglec-7 on most CD56^{dim}CD16⁺ and CD56⁺CD16⁺ cells (Fig 1, D). NK cells displayed a poor functional response to the K562 cell line (expressing most of the ligand of their natural cytotoxicity receptors, including NKp30 and NKp46, and NKG2D), as expressed by the low percentage of CD107 degranulation and IFN- γ secretion compared with healthy control subjects. Nevertheless, NK cells degranulate and secrete IFN- γ after anti-CD16 (natural cytotoxicity receptor- and NKG2D-independent response) and anti-NKG2C redirected lysis assay with P815 (Fig 2).

The patient underwent an HLA bone marrow transplantation from his mother 9 months after diagnosis. At 4 years after transplantation, the CD56⁺CD16⁺ subset accounted for 25% of total NK cells. NK cell chimerism showed 15% persistence in the patient's autologous NK cells, whereas 100% of T and B cells were from donor origin.

Our patient presented with 2 atypical features compared with the usual patients with JAK3-deficient SCID: (1) mild and late onset of clinical manifestations and (2) persistence of NK cells. Actually, little is known about the mechanisms by which homozygous *JAK3* R117C missense mutations impair *JAK3* signaling in lymphocyte ontogeny to result in the persistence of dysfunctional NK cells, although a hypomorphic mutation could be possible. Indeed, in favor of this hypothesis is the persistence of a small amount of T cells with a weak but positive response to some vaccine antigen *in vitro* and the presence of specific IgG to some vaccine peptide antigens at 27 months of age, suggesting that functional B-cell/T-cell cooperation should have existed within the first 2 years of the patient's life. The predominance of CD56⁺CD16⁺ cells, although previously described in active chronic viral infections,² was unexpected because it has not been described in the context of JAK3-deficient SCID. Of note, Roberts et al⁶ described a patient who exhibited an unusual CD56⁺CD16⁺ NK cell population in a case of SCID linked to complete deficiency of the CD3 ϵ subunit during a CMV infection. Interestingly, in our patient this expansion persisted during the 9 months of follow-up until transplantation, although no viral infection was detected during this period (including CMV PCR). Also of interest, more than 80% of CD56⁺CD16⁺ cells expressed NKG2C, which has been associated with lysis of CMV-infected cells.^{3,4} Therefore the absence of detecting CMV DNA in this CMV⁺ patient could be the result of successful control of CMV infection by the patient's NK cells. The persistence of mixed NK cell chimerism after transplantation supported the important functional specificity of this NK contingent. The CD56⁺CD16⁺ subset shared most phenotypic and functional features with those described in HIV-viremic patients, who have frequent coinfection with CMV, except for NKp46, which is barely detectable in patients with HIV but

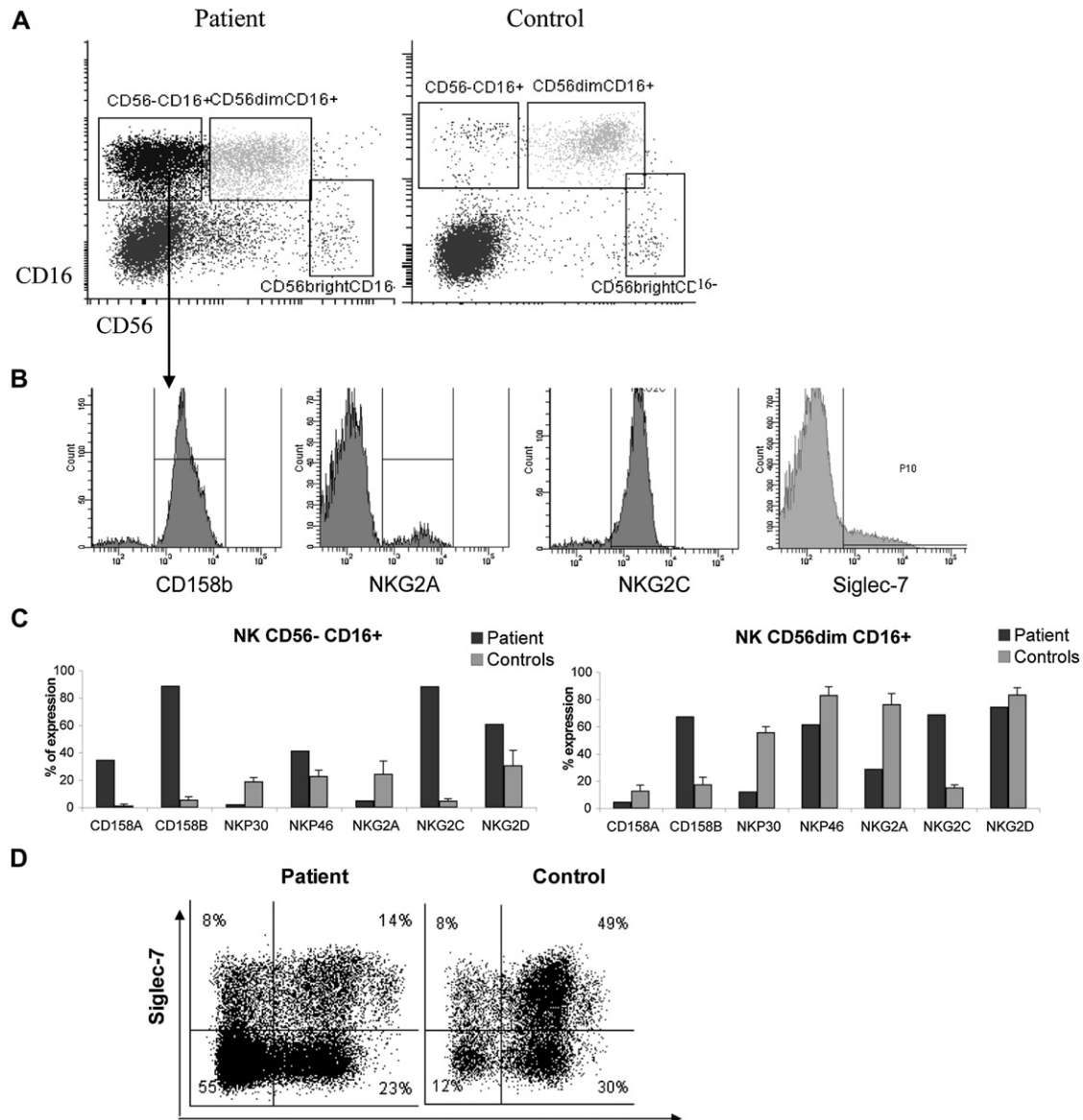


FIG 1. Phenotypic characteristics of NK cell subsets. **A**, Dot plots show NK cell subsets as defined by CD56 and CD16 expression in our patient and in representative age-matched control subjects. The represented cells were gated on CD3⁺ lymphocytes. **B**, Histograms of CD158b, NKG2A, NKG2C, and Siglec-7 expression on the CD56⁻CD16⁺ NK cell subset of patient. **C**, Graphs summarizing the expression of various markers in CD56⁻CD16⁺ and CD56^{dim}CD16⁺ NK cell subsets of the patient (black bars) and control subjects (white bars; n = 4 age-matched healthy volunteers). **D**, Dot plot showing the expression of CD56 and Siglec-7 in NK cells of our patient and of a representative control subject. Cells were gated on Siglec-7-aminocytinomycin D-negative, CD19⁻, CD14⁻, and CD3⁺ lymphocytes.

similar or even higher than seen in control subjects in our patient (see above). Nevertheless, despite a poor functional response against K562, the CD56⁻CD16⁺ cells had a positive response to P815 CD16⁺ cells in the CD107 assay, which has not been described in HIV-viremic patients. Moreover, NKG2C⁺ NK cells, including CD56⁻CD16⁺ cells, degranulate and secrete IFN- γ in response to stimulation through the NKG2C receptor.

In conclusion, the present study describes a case of T⁻B⁺NK⁺ SCID caused by a homozygous *JAK3* missense mutation, resulting in leaky SCID. It is the first description of the expansion of a CD56⁻CD16⁺KIR⁺NKG2C⁺ NK cell subset in the

setting of SCID without evidence of active chronic viral infection.

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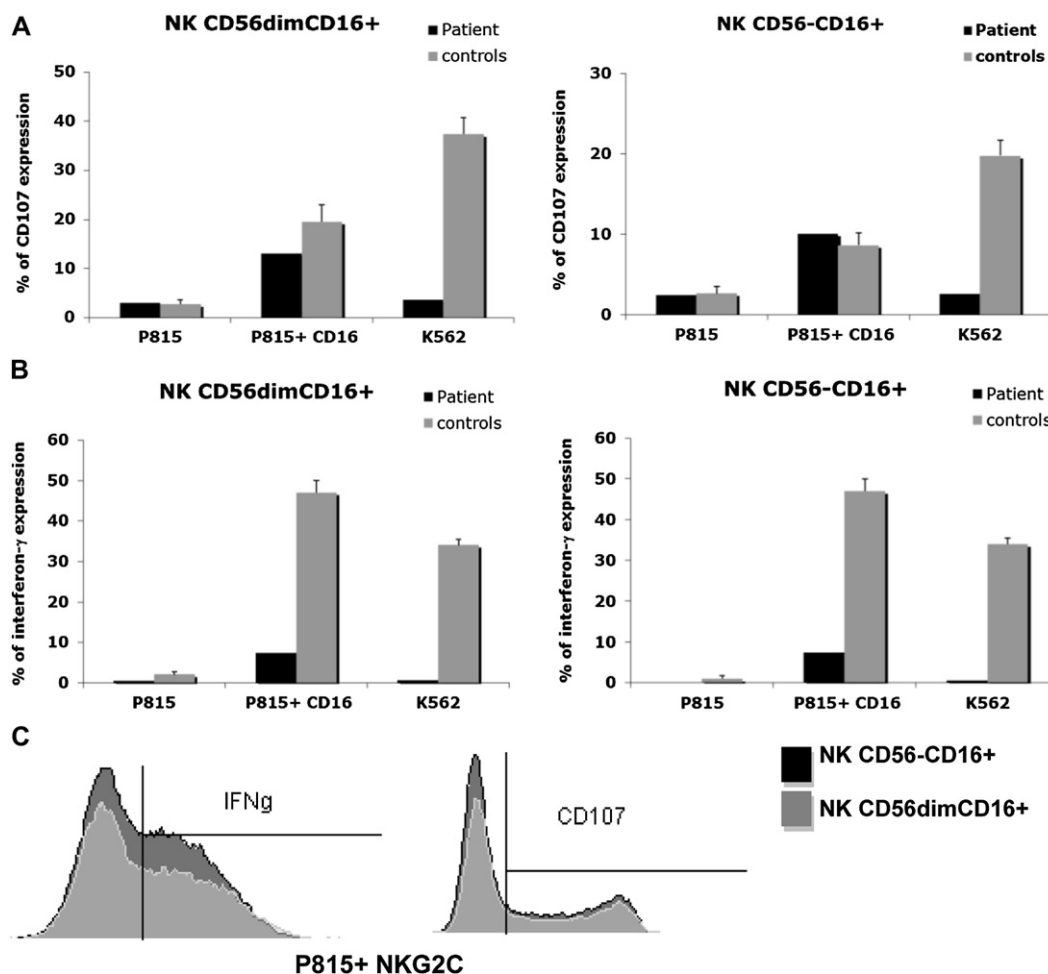


FIG 2. Functional capacity of CD56^{dim}CD16⁺ and CD56⁻CD16⁺ NK cell subsets. The degranulation activity (**A**) and cytokine secretion capacity (**B**) of CD56^{dim}CD16⁺ and CD56⁻CD16⁺ NK cell subsets of the patient (black bars) and the control group (n = 3, gray bars) are shown. The graph represents the percentage of degranulation measured by using CD107 expression of NK subsets after stimulation with the negative control P815, K562, and P815 coated with anti-CD16 mAb. **C**, Histograms of IFN-γ secretion and CD107 expression by CD56⁻CD16⁺ and CD56^{dim}CD16⁺ NK cell subsets after stimulation with P815 coated with anti-NKG2C mAb.

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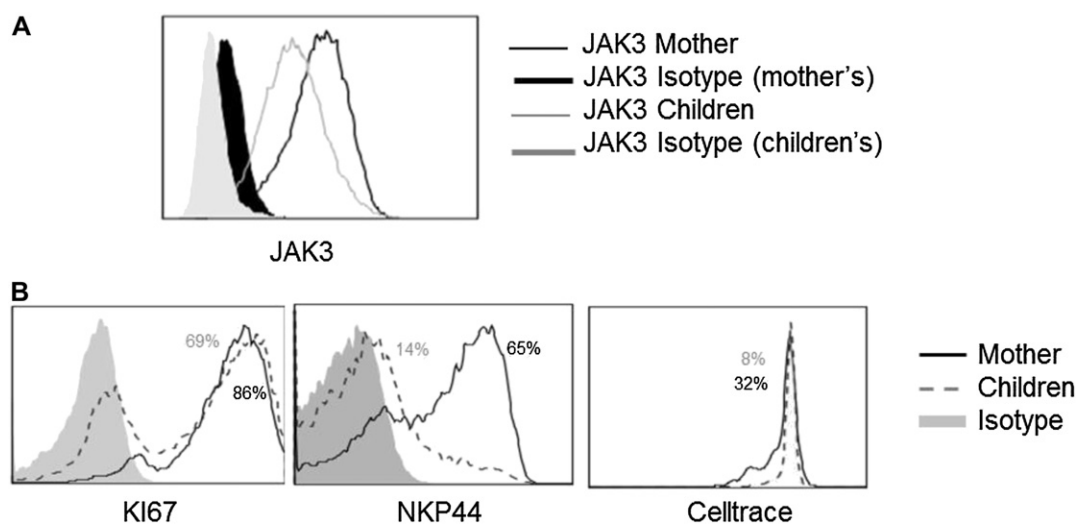


FIG E1. Functionality of JAK3 protein. **A**, Histogram of JAK3 protein expression on NK cells of the patient and his mother. **B**, NK cell activation through IL-2 and IL-15 stimulation through the JAK3 and subsequently signal transducer and activator of transcription 5 pathway. Histograms show increased expression of the proliferation-associated molecule Ki67, upregulation of Nkp44, and increased cell division evaluated with Cell Trace (Invitrogen, Carlsbad, Calif) in the patient's mother's NK cells conversely to the patient's NK cells, which exhibited only weak or no response to IL-2 plus IL-15 stimulation.

TABLE E1. Patient's immunologic phenotype from diagnosis to transplantation

Lymphocyte subset	Lymphocytes/ μ L	CD3 ⁺ cells/ μ L	CD3 ⁺ CD4 ⁺ cells/ μ L	CD3 ⁺ CD8 ⁺ cells/ μ L	CD19 ⁺ cells/ μ L	CD3 ⁺ CD16 ⁺ cells/ μ L	CD3 ⁺ CD56 ⁺ cells/ μ L	IgG (g/L)	IgM (g/L)	IgA (g/L)	IgE (g/L)
Patient, median (range)	1315 (780-1914)	235 (149-268)	105 (79-114)	109 (55-232)	406 (231-625)	657 (285-942)	188 (84-241)	11.8	1.7	2	8.5
Age-matched control subjects,* median (range)	3600 (2300-5400)	2390 (1400-3700)	1380 (700-2200)	840 (490-1300)	750 (390-1400)	320 (140-780)	333 (140-790)	4.8-9	0.3-0.8	0.3-1	1-30

*From Shearer WT, Rosenblatt HM, Gelman RS, Oyomopito R, Plaeger S, Stiehm ER, et al. Lymphocyte subsets in healthy children from birth through 18 years of age: the Pediatric AIDS Clinical Trials Group P1009 study. J Allergy Clin Immunol 2003;112:973-80.