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To the editor:

Massive expansion of maternal T cells in response to EBV infection in a patient with SCID-XI

X-linked severe combined immunodeficiency (SCID-Xl) is caused by defects in *IL2RG*, the gene encoding the IL-2 receptor γ chain. Accounting for 50% to 60% of cases of SCID,[1] it SCID-XI is typically characterized by an absence of mature T and natural killer (NK) lymphocytes, whereas native B cells are detectable and are present in increased numbers. Viral infection caused by Epstein-Barr virus (EBV) in SCID patients can lead to fulminant and often fatal B-cell lymphoproliferative disease, similar to those occurring in immunosuppressed organ-transplant recipients.[2-4]

A 3-month-old boy, born to nonconsanguineous parents, was referred to our center for investigation of a rapidly progressive hepato-splenomegaly without peripheral lymphadenopathy. Chest x-rays revealed an absence of thymic shadow. Liver and spleen were found homogeneously enlarged by ultrasound examination. Whole blood count showed a marked lymphocytosis (up to 100×10^9/L) that consisted of $CD3^+CD8^+TCR\alpha\beta$ $HLA\text{-}DR^+$ activated cells with a complete absence of $CCR7^+CD45RA^+CD8^+$ and $CD4^+CD45RA^+CD31^+$ naive T cells (Figure 1A). The T-cell repertoire, as evaluated by immunoscope,[5] showed an increase in Vβ5,

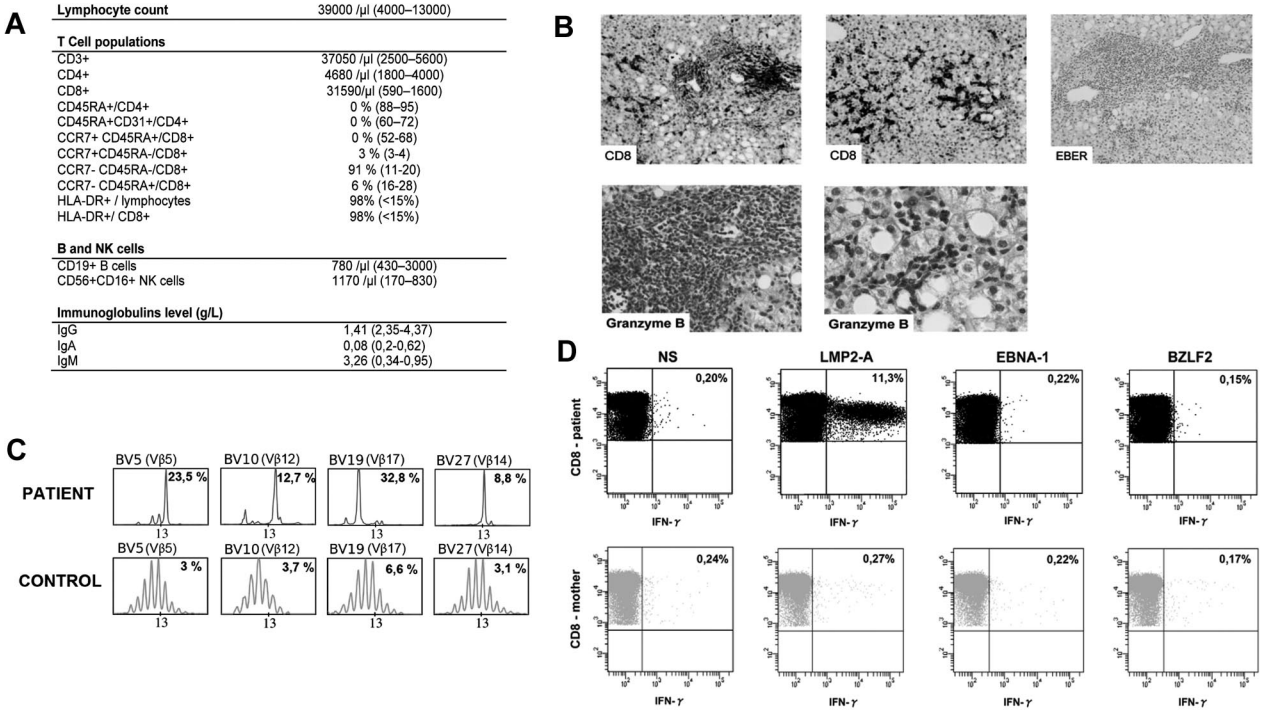

Figure 1. Immunological features of the patient. (A) Lymphocytes subpopulations. Serum immunoglobulin levels. (B) Liver histopathology. Immunohistochemistry was performed on fixed tissues with a peroxidase-based method (Dako). Antibodies used were raised against CD20, CD3, CD8, CD4 and granzyme B (Dako). EBV-encoded RNA (EBER) was probed on some specimen with the use of in situ hybridization technique. Slides were observed using a Leica DM LB microscope with ×20, ×40, and ×100 objectives and a 10× eyepiece. Aquisition of images was with IM50 software (Leica Microsystems). First line: $CD8^+$ lymphocytic infiltrates in lobular (left) and portal (middle) area. Negative EBER staining (right). Second line: positive granzyme B staining in lobular and portal area (left and right panels, respectively). These infiltration could result of the trapping of the activated $CD8^+$ T cells in liver during the immune response.[10] (C) Immunoscope quantitative T-cell repertoire analysis. Most significant specific T-cell clonal expansion is shown. The x-axis indicates CDR3 length (in amino acid), and the y-axis displays the fluorescence intensity of the run-off products (in arbitrary units). Percentages indicate the frequency of occurrence for each Vβ family. (D) $CD8^+$ maternal engrafted T cells express IFN-γ in response to EBV latent antigen LMP-2A antigen. Freshly isolated mononuclear cells of the patient and his mother were incubated without stimulation (NS) or in the presence of latent antigen *LMP-2A,* latent antigen *EBNA-1*, and lytic antigen *BZLF-2*, then stained for the expression of IFN-γ, CD3 and CD8. Numbers are the percentage of cells in the lymphoid gate expressing the indicated surface markers.

Vβ12, Vβ14, and Vβ17 TCR usage among $CD8^+$ cells (Figure 1C). Those features led us to investigate for the existence of a SCID. The maternal origin of the circulating T and NK cells was confirmed by FISH analysis of the $CD3^+$ and $CD56^+$ cell fraction, respectively, which were obtained by cell sorting. There was no engraftment of maternal stem cells, as verified by FISH analysis of the polymorphonuclear neutrophils. SCID-Xl was confirmed by gene sequencing of *IL2RG* on the patient's genomic DNA that revealed a previously described R226C mutation. The mother carried the mutation.

An EBV infection was diagnosed by amplification of the viral DNA in blood samples by polymerase chain reaction with a whole blood viral load of 6 $\log_{10}$ DNA copies/mL. The child's mother displayed a serologic profile of past EBV infection (ie, IgG anti-VCA and IgG anti-EBNA positive). We investigated the possible role of this ongoing viral infection as a trigger for the extreme lymphocytosis, the latter being reminiscent of the one observed during infectious mononucleosis.[6-8] Interestingly, in vitro stimulation of lymphocytes with LMP2-A, but neither with BZLF-2 nor EBNA-1, induced a significant activation of $CD8^+$ T cells as shown by detection of intracytoplasmic interferon γ (IFN-γ) by flow cytometry. The same test, performed on the mother's circulating T cells, did not detect LMP2-A specific in vivo activated T cells, a result that is not surprising in the absence of active EBV infection (Figure 1D). This result indicates a major expansion of LMP2-A specific maternal T cells in the patient's blood secondary to EBV infection.

The EBV infection was treated by rituximab infusions until the EBV viral load became undetectable by PCR. The hepatosplenomegaly gradually regressed secondary to this therapy associated with a short course of steroids. A liver biopsy, performed 4 weeks after initiation of therapy, showed an infiltration of the portal and lobular area by T lymphocytes that were mostly $CD8^+$ with a granzyme B–positive staining (Figure 1B). The Epstein-Barr virus–encoded small RNA (EBER) staining was negative.

Transplacental-acquired maternal T cells have already been reported to cause allograft rejection and immune cytopenias.[9] To the best of our knowledge, this is the first report of "natural" adoptive immunity toward EBV with a massive in vivo expansion of maternal engrafted T cell conferring specific immunity against this virus that may account for the patient's survival and relative control of EBV-driven B-cell proliferation.

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Acknowledgments: This work was supported by grants from Inserm and European Research Council (ERC).

Contribution: F.T. designed the research, collected the data, and wrote the manuscript; L.D.-C. performed experiments and critically read the manuscript; V.V., A.L., and S.K. performed experiments; A.C.-A. collected the data and participated in the clinical care of the patients; D.M. participated in the clinical care of the patient, critically read the manuscript, and participated in writing the manuscript; P.F., S.H., and S.B. participated in the clinical care of the patient; C.P. performed genetic and biologic diagnosis of the patient and critically read the manuscript; M.C.-C. and S.H.-B.-A. critically read the manuscript; and A.F. designed the research, critically read the manuscript, and participated in writing the paper.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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To the editor:

Second attempt to discontinue imatinib in CP-CML patients with a second sustained complete molecular response

Recent results from the STop IMatinib (STIM) trial suggest that imatinib may be safely discontinued in some chronic myeloid leukemia (CML) patients with long-lasting complete molecular response (CMR).[1] However, 60% of patients experienced molecular recurrence (MR; detection of *BCR-ABL1* transcripts confirmed by a second analysis) and responded to imatinib reintroduction.

We explored the feasibility of a second discontinuation in (1) CML patients currently treated by imatinib for at least 3 years who had been in sustained CMR for at least 2 years with (2) MR after first attempt of imatinib discontinuation and (3) in second CMR for at least 1 year after imatinib reintroduction. The molecular follow-up was assessed as previously reported.[1]

Sixteen patients were included. Sex ratio (male/female) was 5/11, and the median age was 62 years (range: 45-80 years). At diagnosis, 15 patients were in chronic phase (CP) and 1 patient was in accelerated phase (AP), and Sokal scores were low in 10 patients, intermediate in 3 patients, and high in 2 patients. Ten of the 16 patients received treatment before imatinib initiation. Imatinib was initiated at 400 mg per day in CP-CML patients, and at 600 mg per day in the AP-CML patient with a median time from diagnosis to imatinib initiation of 8 months (range: 1-73 months). The median interval from imatinib initiation to the first CMR was 14 months (range: 5-56 months). Imatinib was then administered during a median duration of 54 months (range: 32-105 months), and the median duration of CMR was 31 months (range: 19-78 months). After the first attempt of imatinib discontinuation, all patients were in MR within a median of 2.5 months (range: 1-8 months) and they obtained a second CMR after imatinib reintroduction within a median of 6 months (range: 1-19 months).

After the second imatinib discontinuation, we observed 2 different molecular patterns. The first group of patients (12/16, 75%) experienced rapid MR after imatinib was discontinuated on the second occasion. They lost their major molecular response (MMR) at a median of 2.1 month (range: 0.7-5.9 months) and were re-treated with a tyrosine kinase inhibitor (TKI; imatinib n = 11; dasatinib n = 1). In this group of patients the median time to the first positive molecular biology test, the median time to TKI reintroduction, and the median of time to the second CMR after TKI reintroduction were all similar in the 2 instances of imatinib discontinuation, but kinetics of molecular recurrence progressed in several ways. Indeed, among the 11/12 patients with available data, the kinetics of the second molecular recurrence were similar to those of the first recurrence for 1 patient (Figure 1A), was slower than the first recurrence in 5 patients (Figure 1B), and faster in 5 patients (Figure 1C), reflecting heterogeneity of recurrence kinetics.

The second group of patients (4/16, 25%) never lost their MMR and remained free of treatment with a median follow-up of 32 months (range: 15-53 months; Figure 1D). However, 2 of these 4 had a MR after a median of 11.6 months after discontinuation (range: 9.1-14.0 months), but remained treatment-free with a follow-up of 15 and 25 months. The other 2 patients had a prolonged CMR after the second imatinib discontinuation with a follow-up of 40 and 53 months. Therefore, according to the STIM criteria, the probability of remaining in CMR after the second imatinib discontinuation was 12.5% (Figure 1D). Interestingly, in the 2 patients in this group who experienced MR this occurred later compared with those of the first group who were re-treated (median: 11.6 months [range: 9.1-14.0 months] vs 2.1 months [range: 0.7-5.9 months]).

In conclusion, our pilot study demonstrated that it seems possible to discontinue TKIs a second time in selected patients.

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