

Letter to the Editor

Continuous G-CSF therapy for isolated chronic mucocutaneous candidiasis: Complete clinical remission with restoration of IL-17 secretion

To the Editor:

Patients with genetic defects in T_H17 immunity suffer from recurrent infections with *Candida albicans* (chronic mucocutaneous candidiasis [CMC]).¹ *Candida*-specific T_H17 cells produce mostly IL-17, IL-22, and IFN- γ .² IL-6, IL-23, and IL-1 β contribute to the differentiation of *Candida*-specific T_H17 cells.²

CMC can be an isolated disease transmitted as an autosomal-recessive or autosomal-dominant form¹ or part of a syndrome, such as the hyper-IgE syndrome or the autoimmune polyendocrine syndrome-1. Indeed, patients with hyper-IgE syndrome were found to have low levels of T_H17 cells,³ and patients with autoimmune polyendocrine syndrome have inhibitory autoantibodies against IL-17 and IL-22.⁴ Recently, gain-of-function mutations in STAT1 with increased production of IFN- γ , impairing IL-17 immunity, were found in most cases of autosomal-dominant form of CMC.⁵

We present a female patient, now 59 years old, who has suffered from isolated CMC for more than 33 years.⁶ Molecular analysis done by us recently revealed heterozygous G821A STAT1 coiled-coil domain mutation, which has already been described in several patients with isolated CMC.⁵

The patient has 3 brothers. Two of them are completely healthy, and 1 passed away more than 30 years ago after "a very severe disease." Aside from CMC, the patient suffers from insulin-dependent diabetes mellitus. No other autoimmune disorders or autoantibodies were detected during her follow-up. Following an immunologic work-up done by us in 1995,⁶ which demonstrated depressed monocyte (IL-1 secretion) and neutrophil (chemotaxis) functions, we started intravenous GM-CSF treatment (leucomax, 800 μ g twice a week)⁶ and the patient went rapidly into complete clinical remission with improvement of her monocyte and neutrophil functions.⁶ About 3 years later, because of difficulties in obtaining GM-CSF, treatment was switched to twice a week 400 μ g (5 μ g/kg) subcutaneous G-CSF (filgrastim). G-CSF therapy has been continued successfully for the last 16 years, keeping white blood cells no more than 15,000/mm³ with 80% to 90% granulocytes.

In light of the new insight regarding the pathogenesis of isolated CMC, we looked at the proportions of the patient's T_H17 cells and at IL-17A and F and IFN- γ intracellular and serum levels, while on G-CSF, and found them within normal range (Fig 1, A, B, and D, panel b). Thus, we stopped treatment for a while. After 4 weeks, we observed a flare-up of all clinical findings. Resumption of G-CSF treatment resulted once again in complete clinical remission within 2 weeks.

Thus, and in accordance with the new knowledge of the role of GM-CSF in IL-17 immunity,⁷ we went on studying the T_H17 pathway in the patient. As can be seen (Fig 1, A-C), while on G-CSF treatment (panels b and d), proportions of T_H17 cells and levels of IL-17A, IL-17F, IFN- γ , IL-22, IL-23, and IL-2 were comparable to those in normal controls. However, IL-6 and IL-21 levels were significantly increased compared with normal controls (Fig 1, B and D). When G-CSF treatment was stopped and *Candida*

infections recurred, a marked decrease in proportions of T_H17 cells and in levels of IL-17, IL-6, IL-21, and IL-22 was found. In addition, a significant increase in CD4⁺ cells expressing IFN- γ and in IFN- γ and IL-27 secretion was observed (Fig 1, A-D, panel c). Resumption of G-CSF treatment, which resulted in complete clinical remission within 2 weeks, was followed by restoration of T_H17 cell proportions, together with an increase in IL-17, IL-6, and IL-21 secretion and marked reduction in IFN- γ and IL-27 levels (Fig 1, A and B, panel d).

We also observed treatment-dependent increased expression of STAT3 (but not STAT1) (Fig 2, A) and treatment-independent hyperphosphorylation of STAT1 (Fig 2, B), as seen in other cases of STAT1 gain-of-function mutations.⁵ When the patient was on-treatment, we also noticed increased expression of SOCS1 (but not SOCS3) and tyrosine-phosphorylation of STAT3 (Fig 2, B, panel b). Interestingly enough, when patient was off-treatment, a decrease in both SOCS1 levels and STAT3 phosphorylation was noticed (Fig 2, B, panel c).

On the basis of our findings and the relevant literature,⁸⁻¹⁰ we propose that G-CSF treatment stimulates the production of IL-6,⁸ which may affect IL-17 immunity in 2 putative pathways—activation of STAT3, which promotes T_H17 cell development, or stimulation of IL-17 secretion⁹ and of SOCS1 expression. The latter results in inhibition of STAT1, which results in decreased IFN- γ secretion, and relief of T_H17 suppression.¹⁰

The current report of continuous treatment with G-CSF is the first to show complete clinical remission in the autosomal-dominant form of isolated CMC with STAT1 gain-of-function mutation. Other patients with isolated CMC may be considered for this mode of treatment.

Gizi Wildbaum, PhD^{a,*}
Eduardo Shahar, MD^{a,b,*}
Rina Katz, MSc^b
Nathan Karin, PhD^a
Amos Etzioni, MD^{a,b}
Shimon Pollack, MD^{a,b}

From ^athe Department of Immunology, Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, and ^bthe Institute for Allergy, Immunology and AIDS, Rambam Health Care Campus, Haifa, Israel. E-mail: pollack@rambam.health.gov.il.

Disclosure of potential conflict of interest: N. Karin has consultant arrangements with the BioRap and has grants/grants pending to fund his laboratory. The rest of the authors declare that they have no relevant conflicts of interest.

*These authors contributed equally to this work.

REFERENCES

- Hanna S, Etzioni A. New host defense mechanisms against *Candida* species clarify the basis of clinical phenotypes. *J Allergy Clin Immunol* 2011;127:1433-7.
- Zielinski CE, Mele F, Aschenbrenner D, Jarrossay D, Ronchi F, Gattorno M, et al. Pathogen-induced human TH-17 cells produce IFN- γ or IL-10 and are regulated by IL-1 β . *Nature* 2012;484:514-8.
- Milner J, Brechley JM, Laurence A, Freeman AF, Hill BJ, Elias KM, et al. Impaired TH17 cell differentiation in subjects with autosomal dominant hyper-IgE syndrome. *Nature* 2008;452:773-6.
- Puel A, Doffinger R, Natividad A, Chrabei M, Barcenas-Morales G, Picard C, et al. Autoantibodies against IL-17A, IL-17F and IL-22 in patients with chronic mucocutaneous candidiasis and autoimmune polyendocrine syndrome type 1. *J Exp Med* 2010;207:291-7.
- Liu L, Okada S, Kong XF, Kreins AY, Cypowyj S, Abhyankar A, et al. Gain of function human STAT1 mutations impair IL-17 immunity and underlie chronic mucocutaneous candidiasis. *J Exp Med* 2011;208:1635-48.

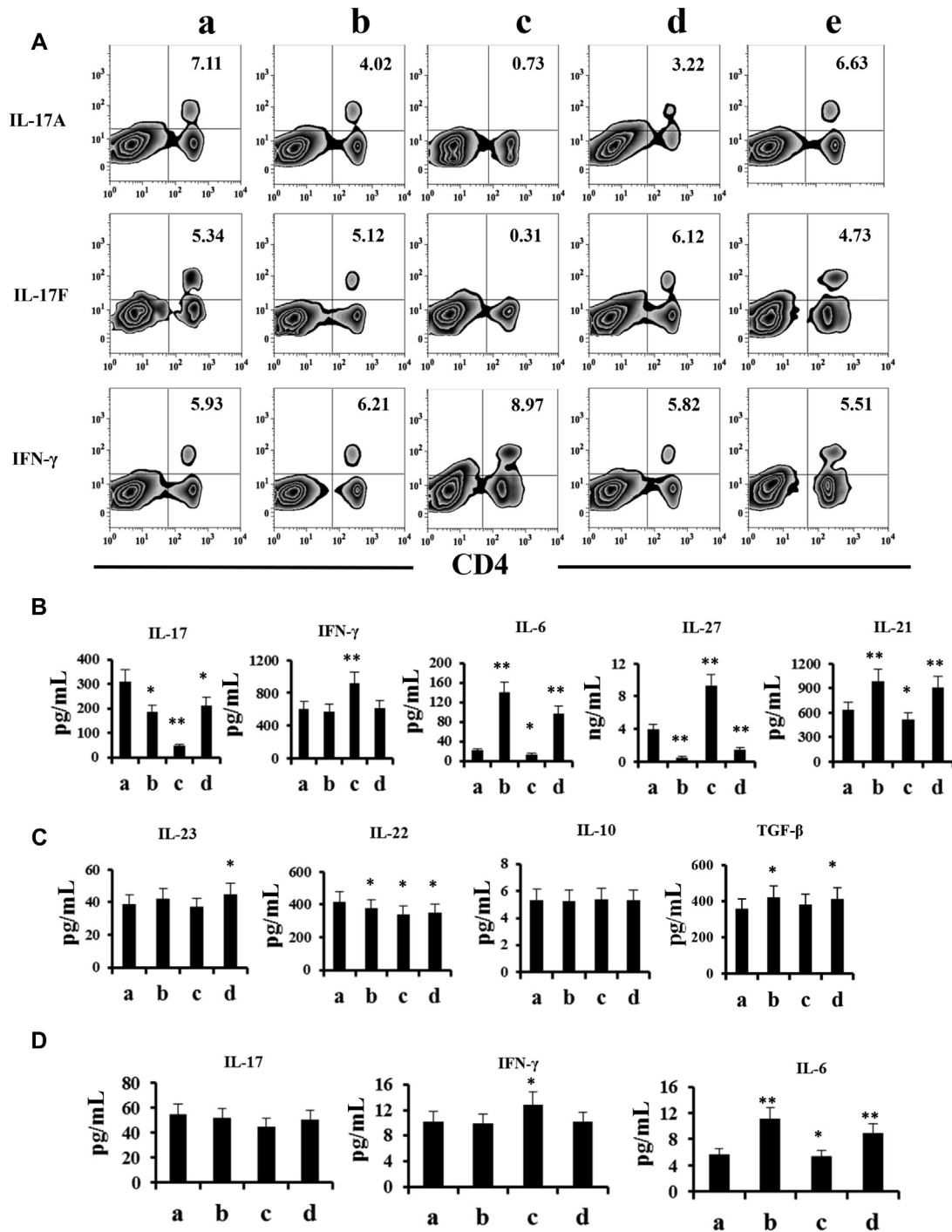


FIG 1. Intracellular and secreted cytokines in patient and controls. Patient's and controls' PBMCs were stimulated with phorbol 12-myristate 13-acetate and ionomycin. Cells were stained with antihuman CD4, and intracellular staining was done with anti-IL-17A, IFN- γ , and IL-17F. Intracellular cytokines were detected by fluorescence-activated cell sorting analysis, and cytokines in cell lysates and serum were measured by ELISA. Proportions of T_H17 ($CD4^+/IL-17A/F^+$) and $CD4^+/IFN-\gamma^+$ cells (**A**) and cytokine concentrations in supernatants of cell lysates (**B** and **C**) and in sera (**D**) are presented. Results are shown as 1 representative experiment out of 3 with similar results (Fig 1, A) or as mean triplicate concentrations ($\pm$ SE) of supernatant or serum cytokines (Fig 1, B-D) (* $P < .05$ and ** $P < .005$ vs control). a, Healthy controls ($n = 3$); b, patient on-treatment; c, patient off-treatment; d, resumption of patient's treatment; e, diabetic control.

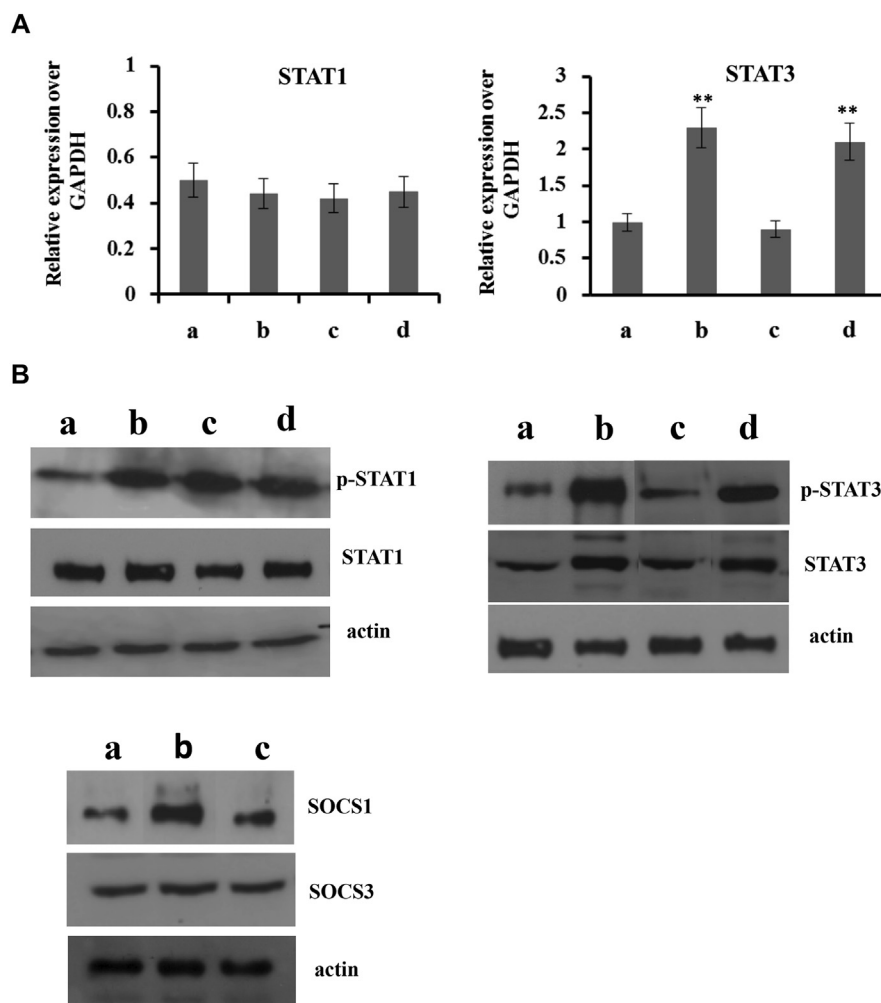


FIG 2. Expression and phosphorylation of transcription factors in PBMCs of patient and controls. Protein expression and tyrosine phosphorylation were detected by using standard Western blotting, whereas relative expression of transcription factors was determined by using real-time PCR normalizing to GAPDH. Results are shown as mean of triplicates $\pm$ SEM (**A**) (** $P < .005$ vs control), or as 1 representative experiment out of 3 with similar results (**B**). *a*, Healthy controls ($n = 3$); *b*, patient on-treatment; *c*, patient off-treatment; *d*, resumption of patient's treatment. *GAPDH*, Glyceraldehyde 3-phosphate dehydrogenase.

6. Shahar E, Kriboy N, Pollack S. White cell enhancement in the treatment of severe candidosis. *Lancet* 1995;346:974-5.
7. McGeachy MJ. GM-CSF: the secret weapon in the TH17 arsenal. *Nat Immunol* 2011;12:521-2.
8. Tian SS, Lamb P, Seidel HM, Stein RB, Rosen J. Rapid activation of the STAT3 transcription factor by granulocyte-colony stimulating factor. *Blood* 1994;84:1760-4.
9. Cypowyj S, Piccard C, Marodi L, Casanova JL, Puel A. Immunity to infection in IL17 deficiency mice and humans. *Eur J Immunol* 2012;42:2246-54.
10. Tamiya T, Kashiwagi I, Takahashi R, Yasukawa H, Yoshimura A. Suppressors of cytokine signaling (SOCS) proteins and JAK/STAT pathways: regulation of T-cell inflammation by SOCS1 and SOCS3. *Arterioscler Thromb Vasc Biol* 2012;981-5.

<http://dx.doi.org/10.1016/j.jaci.2013.04.018>