filaggrin staining in several cases. This finding could be explained by *FLG* mutations for some patients, but there were others for whom even full *FLG* sequencing did not show any concomitant filaggrin mutation(s). This observation is supported by a recent case report (Katayama et al., 2017), and it is known that XLI per se may be associated with a mild reduction of the stratum granulosum (Metze and Traupe, 2016). Hence, there might be other genetic or regulatory modifiers of XLI than primary *FLG* mutations.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

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STAT1 Gain-of-Function and Dominant Negative STAT3 Mutations Impair IL-17 and IL-22 Immunity Associated with CMC

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TO THE EDITOR

Chronic mucocutaneous candidiasis (CMC) is characterized by recurrent *Candida albicans* infections associated with an impaired IL-17 and IL-22 response (Puel et al., 2012). Several monogenetic defects have been identified to cause CMC (Ling et al., 2015; Puel et al., 2012), but the most frequent CMC form is a result of heterozygous autosomal dominant gain-of-function mutations in signal transducer and activator of transcription (STAT) 1 (Liu et al., 2011; Toubiana et al., 2016). There are also syndromic CMC entities, such as autosomal dominant hyper-IgE syndrome (HIES) (Hagl et al., 2016; Schimke et al., 2010). Autosomal dominant HIES is caused by impaired STAT3 signaling due to heterozygous *STAT3* mutations and presents next to CMC with high serum

Abbreviations: CMC, chronic mucocutaneous candidiasis; HIES, hyper-IgE syndrome; PBMC, peripheral blood mononuclear nuclear cell; STAT, signal transducer and activator of transcription; Th, T helper

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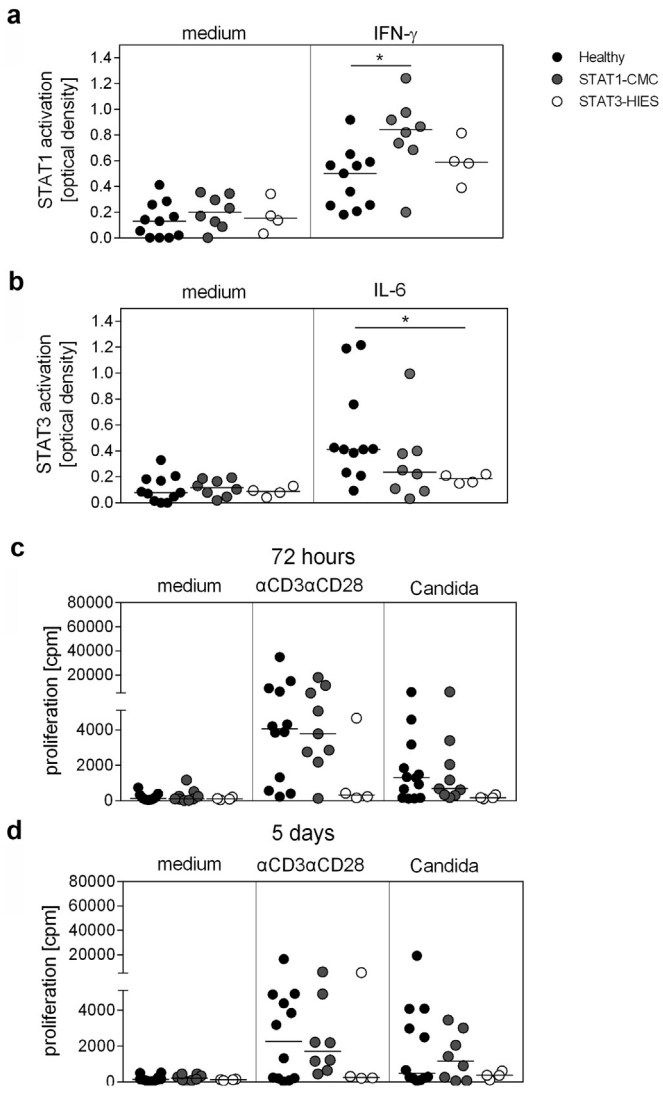

Figure 1. Altered STAT1/STAT3 DNA-binding activity and proliferation in STAT1-CMC and STAT3-HIES patients. PBMCs of healthy control subjects (black dots), STAT1-CMC patients (grey dots), and STAT3-HIES patients (white dots) were assessed for (**a**) STAT1 or (**b**) STAT3 DNA-binding activity and (**c**) proliferation. Cells were treated with (**a**) 100 U/ml IFN-γ or (**b**) 20 ng/ml IL-6 for 1 hour. Nuclear lysates were analyzed for DNA-binding capacity by using TransAM assay. Proliferation was assessed upon treatment with anti-CD3/CD28 or *Candida albicans* for (**c**) 72 hours or (**d**) 5 days by incorporation of radiolabeled [^{3}H]thymidine into DNA for 6 hours. Median is indicated. *Significant differences ($P < 0.05$), calculated by Mann-Whitney test and confirmed by bootstrap analysis. CMC, chronic mucocutaneous candidiasis; cpm, counts per minute; HIES, hyper-IgE syndrome; PBMC, peripheral blood mononuclear nuclear cell.

IgE levels, recurrent bacterial infections, healing defects after pneumonia, and skeletal symptoms. STAT3 is essential in T helper (Th) 17 cell differentiation and inflammation control (Milner et al., 2008; Renner et al., 2008), whereas STAT1 counteracts STAT3 and thus Th17 immunity (Hu and Ivashkiv, 2009; Liu et al., 2011; Toubiana et al., 2016). Here, we provide a thorough comparison of Th17 and Th22 immunity in *C. albicans* host defense in STAT1-CMC and STAT3-HIES patients. All patients gave written informed consent, and this study was approved by the review boards of the Technical University and the Ludwig Maximilian University Munich.

To directly compare the reciprocal effects of STAT1 and STAT3 in clinically and genetically defined STAT1-CMC and STAT3-HIES patients (see Supplementary Table S1 online), we stimulated control and patients' peripheral blood mononuclear cells (PBMCs) with IL-6 or IFN-γ and extracted nuclear proteins. STAT DNA binding assessed by TransAM Transcription Factor ELISA (Active Motif, La Hulpe, Belgium) showed significantly increased STAT1 DNA-binding activity in response to IFN-γ in STAT1-CMC patients' cells compared with control cells (Figure 1a), likely explained by impaired nuclear dephosphorylation or DNA dissociation of STAT1 due to STAT1 gain-of-function mutations (Liu et al., 2011; Mizoguchi et al., 2014). Our STAT1-CMC patients showed a slightly decreased STAT3 DNA-binding after IL-6 stimulation (Figure 1b), likely caused by the overactive STAT1 protein. A negative effect of overactive STAT1 on STAT3 function is proven by the fact that inhibition of STAT1 phosphorylation rescues STAT3 activity (Zheng et al., 2015). Underlying mechanisms may be that STAT1 protein competes for binding sites at the IL-6 receptor complex or sequesters STAT3 in STAT1/STAT3 heterodimers, leading to reduced nuclear translocation or DNA-binding (Hu and Ivashkiv, 2009). In the nucleus, overactive STAT1 may compete for DNA-binding sites with STAT3. Although the dominant negative STAT3 mutations have no effect on STAT1 activity, STAT3 nuclear activity was significantly reduced in the STAT3-HIES patients (Figure 1a and b).

Analogous to STAT3's pivotal role in cell proliferation, PBMCs of STAT3-HIES patients showed an impaired proliferation after stimulation with T-cell activating CD3/CD28-antibodies or *C. albicans,* whereas an overactive STAT1 protein in STAT1-CMC did not affect cell growth (Figure 1 c and d). Consequently, the impaired Th17 immunity of STAT1-CMC patients is not due to a generally defective lymphocyte proliferation.

To investigate how the altered STAT1/STAT3 function affects differentiation of IL-17- and IL-22-producing cells, we analyzed Th cell subtypes by flow cytometry on single cell level and cytokine secretion by ELISA upon activation by anti-CD3/CD28 or *C. albicans* at two different time points (Figure 2). After 72 hours of stimulation with anti-CD3/CD28, STAT1-CMC and STAT3-HIES patients had significantly lower frequencies of IL-17-only-producing, IL-17/IL-22-co-producing, and previously described IL-17/IFN-γ-co-producing *Candida*-specific Th17 cells (Zielinski et al., 2012) compared with healthy controls, highlighting impaired Th17 immunity in both patient

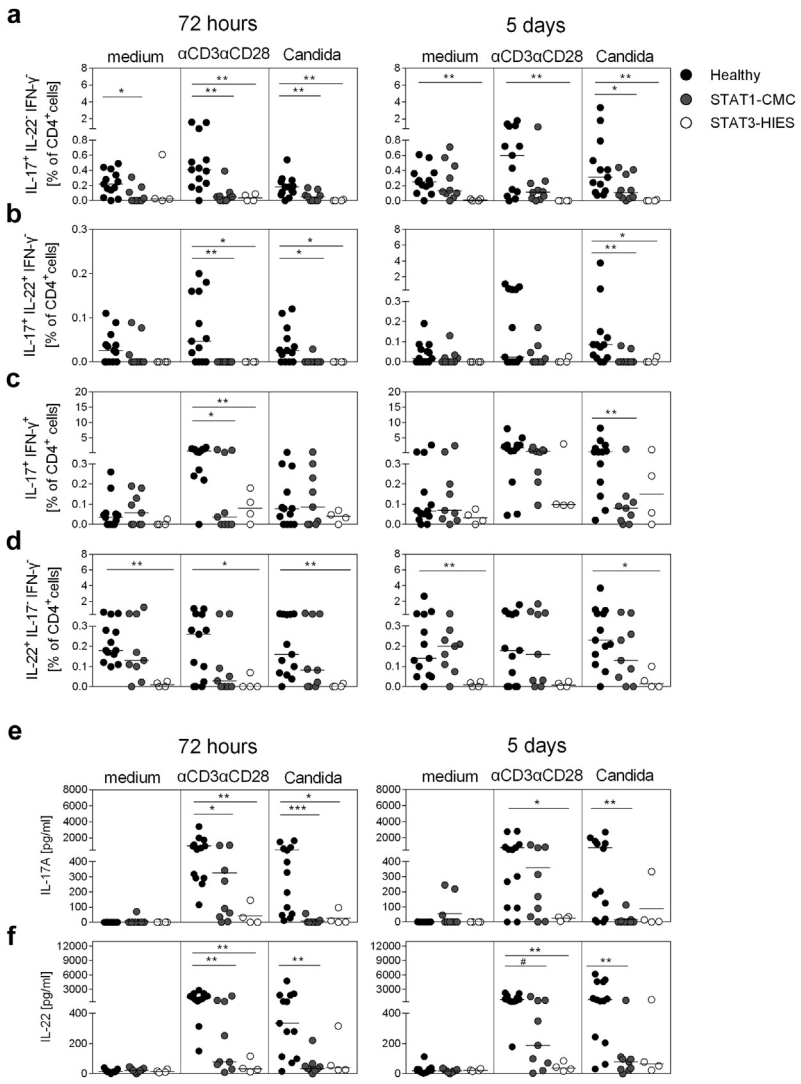

Figure 2. Frequencies of Th17 cell subsets and Th22 cells and cytokine secretion in STAT1-CMC and STAT3-HIES. PBMCs of healthy control subjects (black dots), STAT1-CMC patients (grey dots), and STAT3-HIES patients (white dots) were stimulated with anti-CD3/CD28 or *Candida albicans* or were left untreated for 72 hours and 5 days. (**a**) Percentages of IL-17-only-producing Th17 cells (IL-17$^+$IL-22$^-$IFN-γ$^-$CD4$^+$), (**b**) IL-17/IL-22-co-producing cells (IL-17$^+$IL-22$^+$IFN-γ$^-$CD4$^+$), (**c**) *Candida*-specific Th17 cells (IL-17$^+$IFN-γ$^+$CD4$^+$), and (**d**) Th22 cells (IL-22$^+$IL-17$^-$IFN-γ$^-$CD4$^+$) are shown. (**e**) Reduced IL-17 and (**f**) IL-22 secretion in STAT1-CMC and STAT3-HIES. Cell free supernatants were analyzed for IL-17A or IL-22 by ELISA. Median is indicated. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ calculated by Mann-Whitney test and confirmed by bootstrap analysis. $^{\#}$Significant difference ($P < 0.05$) calculated by Mann-Whitney test, which was not confirmed by bootstrap analysis. CMC, chronic mucocutaneous candidiasis; HIES, hyper-IgE syndrome; PBMC, peripheral blood mononuclear cell.

groups (Figure 2a–c). Th22 cells, which have also been associated with host defense against *C. albicans* (Eyerich et al., 2009, 2011), were reduced in both patient groups at 72 hours after T-cell stimulation (Figure 2d). The herein observed reduction of Th22 cells in STAT3-HIES patients has not yet been reported and hints to a central role of STAT3 in Th22 cell differentiation in humans, which has been suggested for mice (Backert et al., 2014). The percentage of Th22 cells and IL-17/IFN-γ-co-producing, *Candida*-specific Th17 cells normalized within 5 days in STAT1-CMC but not in STAT3-HIES cells, suggesting that the dampening effect on STAT3 activity by an overactive STAT1 resolves over time in vitro (Figure 2c and d). To analyze anti-fungal Th17 and Th22 responses in more detail, we challenged PBMCs directly with *C. albicans*. Compared with healthy controls, PBMCs of STAT1-CMC and STAT3-HIES patients had significantly lower IL-17-only-producing and IL-17/IL-22-co-producing Th17 cells at both time points (Figure 2a and b). At 72 hours after *Candida* challenge, neither patients' cells nor control cells showed a significant differentiation to IL-17/IFN-γ-co-producing, *Candida*-specific Th17 cells (Figure 2c). After 5 days, IL-17/IFN-γ-co-producing, *Candida*-specific Th17 cells were decreased in both patient groups compared with control cells. In contrast to the anti-CD3/CD28 stimulation, after *Candida*-challenge the impairment of the Th17 and Th22 response persisted not only in STAT3-HIES patients but also in STAT1-CMC patients even after 5 days (Figure 2a–d).

The impairment of Th17 and Th22 cells after anti-CD3/CD28 as well as *C. albicans* stimulation was proven by a reduced secretion of IL-17A and IL-22 in both patient groups (Figure 2e and f). Similar to the Th22 cell differentiation, the reduction of IL-22 secretion was more persistent after stimulation with *C. albicans* compared with anti-CD3/CD28 in STAT1-CMC patients. Although IL-17/IFN-γ-co-producing, *Candida*-specific Th17 cells normalized within 5 days in STAT1-CMC patients with anti-CD3/CD28, secreted IL-17 increased only marginally. IL-17 and IL-22 accumulated over time in the supernatants and may superimpose subtle changes which are assessed by sensitive flow cytometric single-cell analyses.

Because STAT1-CMC and STAT3-HIES are rare diseases and, as a consequence, sample numbers were small, we confirmed all significant *P*-values by calculating 95% nonparametric bootstrap confidence intervals, a statistical method addressed to small sample sizes.

Overall, our data highlight the importance of a STAT1/STAT3 balance in immunity against *C. albicans* infections. Despite differences in the clinical presentation of STAT1-CMC and STAT3-HIES, both diseases show comparable reduced Th17 and Th22 immunity. However, the two patient groups show one important difference: although induction of Th17 and Th22 cell differentiation was possible in STAT1-CMC patients to a variable degree in vitro, as shown by an increase in cell frequency, it was impossible to overcome Th17 and Th22 defects in STAT3-HIES patients. These findings imply a less persistent impairment in

Th17/Th22 cell differentiation in STAT1-CMC patients compared with STAT3-HIES patients. Because IL-17 and IL-22 directly influence epidermal immunity and host defense against *C. albicans* and IL-22 activates STAT3 in keratinocytes, a comparison of the effects of Th17 cytokines on epithelial cells from both patient groups in the context of our results may help to explain why STAT3-HIES patients suffer not only from CMC but also from other skin infections (Conti and Gaffen, 2015; Eyerich et al., 2011; Moriwaki et al., 2015; Nograles et al., 2008). Our results suggest a strong T-cell receptor engagement as a therapeutic strategy to enhance STAT3 activity, improving antifungal responses in STAT1-CMC. Thus, we provide evidence that stratification of patients with chronic mucocutaneous *Candida* infections is relevant.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

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