Impaired natural killer cell functions in patients with signal transducer and activator of transcription 1 *(STAT1)* gain-of-function mutations

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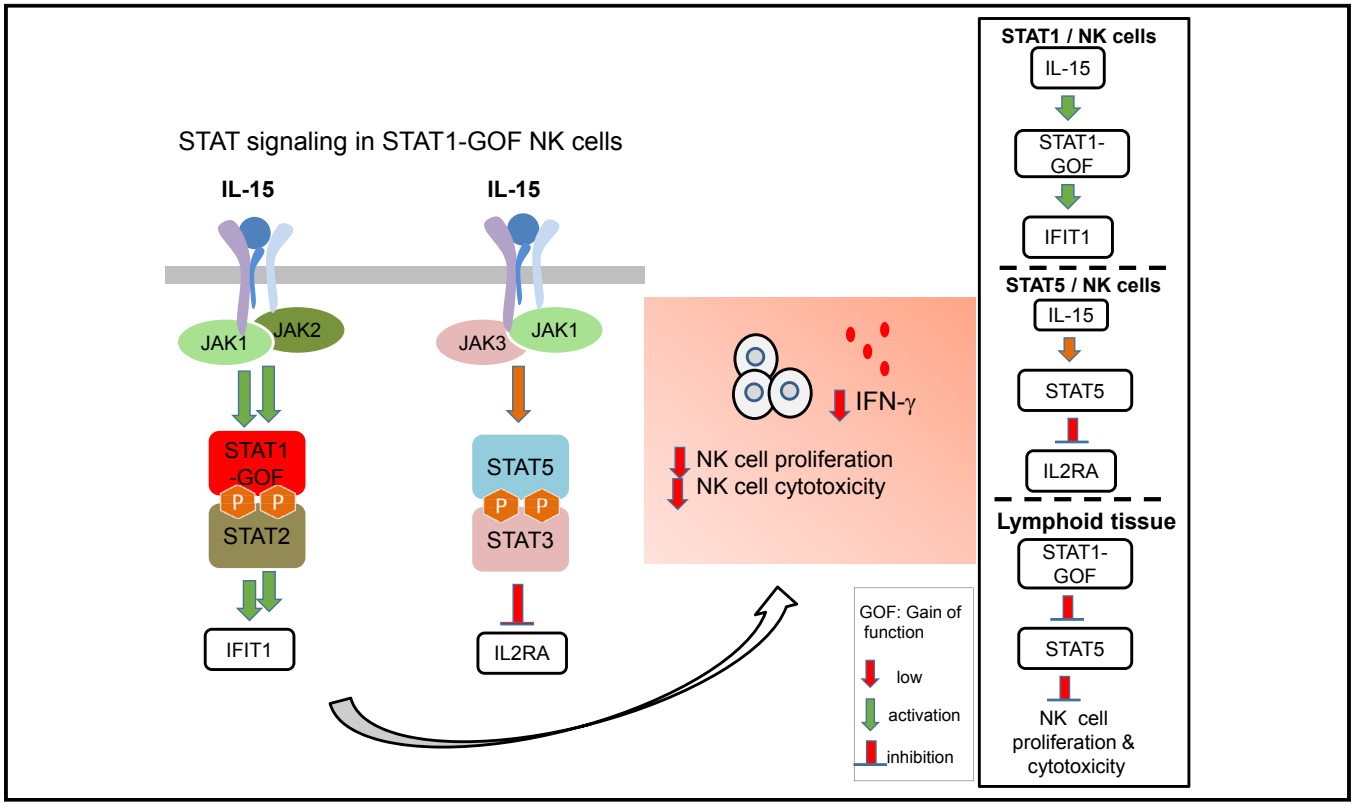
GRAPHICAL ABSTRACT



Background: Gain-of-function (GOF) mutations affecting the coiled-coil domain or the DNA-binding domain of signal transducer and activator of transcription 1 *(STAT1)* cause chronic mucocutaneous candidiasis disease. This condition is characterized by fungal and bacterial infections caused by impaired generation of T_H17 cells; meanwhile, some patients with chronic mucocutaneous candidiasis disease might also have viral or intracellular pathogen infections.

Objective: We sought to investigate the effect of *STAT1* GOF mutations on the functioning of natural killer (NK) cells.

Methods: Because STAT1 is involved in the signaling response to several cytokines, we studied NK cell functional activities and STAT1 signaling in 8 patients with *STAT1* GOF mutations.

Results: Functional analysis of NK cells shows a significant impairment of cytolytic and degranulation activities in patients with *STAT1* GOF mutations. Moreover, NK cells from these

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patients display lower production of IFN-γ in response to IL-15 and reduced proliferation after stimulation with IL-2 or IL-15, suggesting that STAT5 signaling is affected. In addition, signaling studies demonstrate that the increased phosphorylation of STAT1 in response to IFN-α is associated with detectable activation of STAT1 and increased STAT1 binding to the interferon-induced protein with tetratricopeptide repeats 1 *(IFIT1)* promoter in response to IL-15, whereas STAT5 phosphorylation and DNA binding to IL-2 receptor α *(IL2RA)* are reduced or not affected in response to the same cytokine.

Conclusion: These observations suggest that persistent activation of STAT1 might affect NK cell proliferation and functional activities. (J Allergy Clin Immunol 2017;140:553-64.)

Key words: *Natural killer cells, cytotoxic activity, IL-15, signal transducer and activator of transcription 1, signal transducer and activator of transcription 5, candidiasis*

Abbreviations used

ChIP:	Chromatin immunoprecipitation
CMCD:	Chronic mucocutaneous candidiasis disease
E/T:	Effector/target
FITC:	Fluorescein isothiocyanate
GOF:	Gain of function
IFIT1:	Interferon-induced protein with tetratricopeptide repeats 1
IL2RA:	IL-2 receptor α
MFI:	Mean fluorescence intensity
NK:	Natural killer
PE:	Phycoerythrin
PRL:	Prolactin
SOCS:	Suppressor of cytokine signaling
STAT:	Signal transducer and activator of transcription

Chronic mucocutaneous candidiasis is a condition characterized by impairment of mucosal immunity against *Candida albicans*. It can be observed in patients with various inborn defects of the immune response, including disorders caused by mutations in autoimmune regulator *(AIRE)*, signal transducer and activator of transcription 3 *(STAT3)*, *IL12B*, *IL12RB1*, *IL17F*, *IL17RA*, or RAR-related orphan receptor C *(RORC)*.[1-3] In 2011, 2 different groups reported that monoallelic mutations of *STAT1* account for a large proportion of patients with genetic predisposition to *C albicans* infections of the skin, nails, and mucous membranes.[4,5] These mutations, causing chronic mucocutaneous candidiasis disease (CMCD), were shown to be gain-of-function (GOF) mutations and to affect the coiled-coil or DNA-binding domain of *STAT1*. The discovery of these genetic defects and their pathogenesis have contributed to reveal the role of T_H17 cells in the immune response against *Candida* species infections in patients with primary immunodeficiencies.[4-10]

STAT1 is a transcription factor that regulates many of the biological effects mediated by type I interferons and other cytokines in many cell types, including natural killer (NK) cells.[11-13]

NK lymphocytes constitute an important cell subset in the immune response against intracellular pathogens and virally infected cells.[14] Functioning of NK cells is controlled by an array of different activating and inhibitory receptors[15] that, on engagement by specific cell ligands, can either induce or suppress the process of killing. During early viral infection, STAT1 expression is dramatically induced and NK cell activity can be influenced by STAT1 phosphorylation. After type I interferon secretion, NK cells acquire potent effector functions and secrete immunomodulatory cytokines (ie, IL-15) that regulate NK cell expansion.[13]

Fungal infections constitute the most common manifestation of CMCD, but viral infections, including recurrent mucocutaneous infections caused by reactivation of varicella-zoster virus and herpes simplex viruses, debilitating *orf* infection, or severe invasive infection caused by chicken pox, cytomegalovirus, or EBV have also been reported.[16-21] The broad spectrum of infections observed in patients with CMCD suggests that susceptibility to pathogens is not entirely due to the lack of T_H17 cells, but other cell types, particularly NK cells, might play a role in the pathogenesis of CMCD. To define the role of NK cells in the susceptibility of patients with CMCD to intracellular pathogens and viruses, we have investigated NK functional activities and STAT1 and STAT5 signaling in response to cytokines in these patients.

Here we report that NK cells from patients with *STAT1* GOF mutations display increased STAT1 phosphorylation in response to IL-2 and IL-15 but reduced STAT5 activation. This is associated with impaired NK cell proliferation in response to IL-2 or IL-15 and reduced IFN-γ secretion in response to IL-15.

METHODS

Patients

We investigated 8 patients affected by CMCD from 7 families. Some of the patients had been previously screened for other mutations (ie, *AIRE* or *CARD9*) to rule out other causes of CMCD. In all patients described herein, we found heterozygous mutations in the coiled-coil or DNA-binding domains of *STAT1*: L283M in P1 and his mother P2, L351F in P3, L400V in P6, T837A in P8,[21,22] T385M in P4 and P5,[23] and A267V in P7.[4] Clinical data from patients were collected from medical records and are briefly summarized in Table I. Fungal and bacterial infections were observed in all patients with CMCD, whereas viral infections were observed in patients P3 and P8.[21,22]

More detailed information on the Methods used are reported in this article's Online Repository at www.jacionline.org.

RESULTS

STAT1 phosphorylation in NK cells of patients with *STAT1* GOF mutations

To investigate the mechanism of NK cells activation in response to cytokines, we explored STAT1 phosphorylation in response to IFN-α (40,000 U/mL), IL-2 (100 ng/mL), and IL-15 (50 ng/mL) by using flow cytometry in NK cells from both healthy control subjects and patients with *STAT1* GOF mutations. After stimulation, STAT1 phosphorylation was examined by staining with specific antibodies directed against phosphorylated tyrosine residues. Analysis of STAT1 signaling in freshly isolated NK cells (see Fig E1, *A*, in this article's Online Repository at www.jacionline.org) showed higher STAT1 phosphorylation in response to IFN-α in cells from patients compared with those from control subjects, suggesting that *STAT1* GOF mutations result in abnormal functioning of STAT1 signaling in NK cells (Fig 1, *A*). This observation is in line with previous reports showing that *STAT1* GOF mutations are related to increased STAT1 phosphorylation.[5,18,24,25] Then we investigated STAT1