Signal transducer and activator of transcription 1 (*STAT1*) gain-of-function mutations and disseminated coccidioidomycosis and histoplasmosis

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Background: Impaired signaling in the IFN-γ/IL-12 pathway causes susceptibility to severe disseminated infections with mycobacteria and dimorphic yeasts. Dominant gain-of-function mutations in signal transducer and activator of transcription 1 (*STAT1*) have been associated with chronic mucocutaneous candidiasis.

Objective: We sought to identify the molecular defect in patients with disseminated dimorphic yeast infections.

Methods: PBMCs, EBV-transformed B cells, and transfected U3A cell lines were studied for IFN-γ/IL-12 pathway function. STAT1 was sequenced in probands and available relatives. Interferon-induced STAT1 phosphorylation, transcriptional responses, protein-protein interactions, target gene activation, and function were investigated.

Results: We identified 5 patients with disseminated *Coccidioides immitis* or *Histoplasma capsulatum* with heterozygous missense mutations in the *STAT1* coiled-coil or DNA-binding domains. These are dominant gain-of-function mutations causing enhanced STAT1 phosphorylation, delayed dephosphorylation, enhanced DNA binding and transactivation, and enhanced interaction with protein inhibitor of activated STAT1. The mutations caused enhanced IFN-γ–induced gene expression, but we found impaired responses to IFN-γ restimulation.

Conclusion: Gain-of-function mutations in *STAT1* predispose to invasive, severe, disseminated dimorphic yeast infections, likely through aberrant regulation of IFN-γ–mediated inflammation. (J Allergy Clin Immunol 2013;131:1624-34.)

Key words: *Signal transducer and activator of transcription 1, IFN-γ, progressive multifocal leukoencephalopathy,* Histoplasma capsulatum*,* Coccidioides immitis*, thrush*

The IFN-γ/IL-12 signaling pathway controls extrapulmonary infections with bacteria, such as nontuberculous mycobacteria, BCG, *Mycobacterium tuberculosis*, and *Salmonella* species,[1,2] as well as the dimorphic fungi *Histoplasma capsulatum*,[3] *Paracoccidioides brasiliensis*,[4] and *Coccidioides immitis*.[5,6] Stimulation of IFN-γ and IFN-α receptors leads to phosphorylation of signal transducer and activator of transcription 1 (STAT1), which homodimerizes and heterodimerizes before translocating to the nucleus, where interferon-induced genes are activated.[7] Complete recessive mutations in *STAT1* cause susceptibility to viral, mycobacterial, and bacterial infections, whereas heterozygous inhibitory *STAT1* mutations cause mild disseminated BCG or nontuberculous mycobacterial infections.[8-10] Recently, dominant gain-of-function mutations in *STAT1* were described as causing chronic mucocutaneous candidiasis (CMC), impaired STAT1 dephosphorylation, and diminished numbers of IL-17–producing T cells.[11,12]

The regulation of STAT1 activity includes the suppressor of cytokine signaling and protein inhibitor of activated STAT (PIAS)

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Abbreviations used
CMC: Chronic mucocutaneous candidiasis
CSF: Cerebrospinal fluid
CT: Computed tomography
DMA: Mono/dimethylarginine antibody
GAS: Gamma-activated sequence
GFP: Green fluorescent protein
ISRE: Type I interferon response element
L-AmB: Liposomal amphotericin B
MRI: Magnetic resonance imaging
NIH: National Institutes of Health
PIAS: Protein inhibitor of activated STAT
PML: Progressive multifocal leukoencephalopathy
pSTAT1: Phosphorylated STAT1
SAMe: S-adenosylmethionine
siRNA: Small interfering RNA
STAT1: Signal transducer and activator of transcription 1
WB: Western blotting
WT: Wild-type

families of proteins.[13,14] Posttranslational modifications of STATs (acetylation, methylation, SUMOylation, and ISG15ylation among others) also regulate their function and response. PIAS1 is thought to interfere with STAT1 DNA binding and to recruit other transcriptional coregulators. PIAS proteins have also been shown to have E3 ligase activity and to promote protein SUMOylation.[15]

We identified 5 patients with disseminated dimorphic fungal infections who had mutations in *STAT1*: 2 patients had disseminated refractory coccidioidomycosis beginning in childhood or adolescence without CMC, and 3 patients had disseminated histoplasmosis and CMC, including 1 patient who also had progressive multifocal leukoencephalopathy (PML). These are gain-of-function mutations that ultimately lead to delayed dephosphorylation of STAT1, lower STAT1 methylation, enhanced STAT1/PIAS1 association, and an impaired response to IFN-γ restimulation.

METHODS

Patients and blood samples

All samples were collected under approved National Institutes of Health (NIH) protocols; all patients or their parents provided written informed consent. Healthy volunteer blood samples were obtained under approved protocols through the Department of Transfusion Medicine, NIH.

Cell lines

EBV-transformed B-cell lines derived from patients and healthy donors were maintained in RPMI 1640 with 20% FCS, 2 mmol/L L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37°C in a humidified 5% CO_2 incubator. STAT1-deficient U3A cells (generously provided by G. Stark, Cleveland Clinic, Cleveland, Ohio) were maintained in complete Dulbecco modified Eagle medium (see the Methods section in this article's Online Repository at www.jacionline.org).

STAT1 sequencing

Genomic DNA (PureGene Gentra DNA isolation kit; Qiagen, Hilden, Germany) and total RNA (STAT-60 RNA isolation kit; Tel-Test, Friendswood, Tex) were extracted from EBV-transformed B-cell lines or polymorphonuclear leukocytes. Primers spanning exons and flanking splice sites of human genomic *STAT1* and full-length cDNA were designed with Primer Select (Lasergene; DNASTAR, Madison, Wis). Genomic amplification was performed with Platinum PCR Supermix High Fidelity (Invitrogen, Carlsbad, Calif). Samples were treated with ExoSAP (Affymetrix, Santa Clara, Calif), and 1 μL of the resulting product was used in sequencing reactions with Big Dye Terminators v3.1 (Applied Biosystems, Foster City, Calif), purified with Performa DTR short-well plate kit (Edge BioSystems, Gaithersburg, Md), and run on an Applied Biosystems 3730XL sequencer. Alignment was to the consensus sequence NM_007315.3 using Sequencer software (Gene Codes, Ann Arbor, Mich).

Constructs

Mutated STAT1 sequences or green fluorescent protein (GFP)–tagged constructs were created with a STAT1 expression vector (BioInnovatise, Rockville, Md). Wild-type (WT) and mutant STAT1 plasmids were isolated with the QIAprep Miniprep Kit (Qiagen), according to the manufacturer's recommendations, and all mutations were verified by means of sequencing. Transient transfection of U3A cells was done with the Nucleofactor Amaxa device (Lonza, Walkersville, Md), according to the manufacturer's recommendations.

Reporter gene assay

U3A cells were cotransfected with WT and/or mutant STAT1 expression constructs along with a plasmid containing tandem interferon-response elements (gamma-activated sequence [GAS] and type I interferon response element [ISRE]) driving a luciferase reporter gene (1 μg; Panomics, Fremont, Calif). A Renilla expression vector was cotransfected to measure transfection efficiency. Cells were stimulated with human IFN-γ or IFN-α2b at 1000 IU/mL for 6 hours. Luciferase activity was evaluated with a dual luciferase assay (Promega, Madison, Wis; see the Methods section in this article's Online Repository). Data are expressed as the fold increase in response to interferon over the WT unstimulated samples.

Evaluation of STAT1 activation

Phosphorylated STAT1 (pSTAT1) was assayed in U3A and EBV-B cells stimulated with IFN-γ (400 IU/mL) or IFN-α (1,000 IU/mL). For evaluation of dephosphorylation, pSTAT1 kinetics were assayed in cells stimulated with IFN-γ from 30 to 120 minutes. Cell lysates were recovered and analyzed by means of Western blotting (WB) and flow cytometry. For immunoprecipitation, cell lysates were incubated with anti-STAT1 antibody and protein G–Sepharose (Amersham Biosciences, Piscataway, NJ) overnight at 4°C, and immunoreactive proteins were resolved by means of WB.

Downregulation of PIAS1

High-purity small interfering RNA (siRNA) oligonucleotides that target PIAS1 and a control siRNA were obtained from Darmacon (Thermo Scientific, Lafayette, Colo). U3A cells were transiently transfected with the siRNA (ON-TARGET*plus* SMARTpool siRNA, 50 nmol/L) through eletroporation (Nucleofactor Amaxa), cotransfected with WT or mutant STAT1 constructs, and stimulated with IFN-γ (400 IU/mL).

Real-time PCR

Total RNA was extracted from cultured cells (PBMCs isolated from venous blood by means of density centrifugation and transfected U3A cells) with the RNeasy Mini Kit (Qiagen). For real-time PCR, 1 μg of total RNA was reverse transcribed (Invitrogen), and the resulting cDNA was amplified by means of PCR with the ABI 7500 Sequencer and TaqMan expression assays (Applied Biosystems). Glyceraldehyde-3-phosphate dehydrogenase was used as a normalization control. The data were analyzed with the $2^{-\Delta\Delta CT}$ method, and results were expressed as mean fold induction.

Statistical analysis

Results are reported as means ± SDs, unless otherwise stated. Differences between groups were assessed by using the Student *t* test (GraphPad Prism;