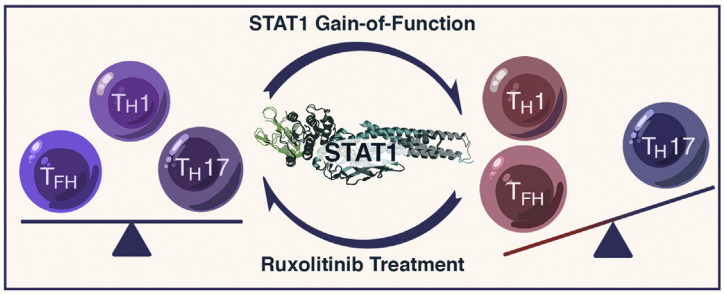
Ruxolitinib reverses dysregulated T helper cell responses and controls autoimmunity caused by a novel signal transducer and activator of transcription 1 *(STAT1)* gain-of-function mutation

Katja G. Weinacht, MD, PhD,[a,b]*‡ Louis-Marie Charbonnier, PhD,[c]* Fayhan Alroqi, MD,[c] Ashley Plant, MD,[a,b] Qi Qiao, PhD,[d] Hao Wu, PhD,[c,d] Clement Ma, PhD,[a,b] Troy R. Torgerson, MD, PhD,[e,f] Sergio D. Rosenzweig, MD, PhD,[g,h] Thomas A. Fleisher, MD,[h] Luigi D. Notarangelo, MD,[c,i] Imelda C. Hanson, MD,[j] Lisa R. Forbes, MD,[j] and Talal A. Chatila, MD, MSc[c]

Boston and Cambridge, Mass, Seattle, Wash, Bethesda, Md, and Houston, Tex

GRAPHICAL ABSTRACT



Background: Gain-of-function (GOF) mutations in the human signal transducer and activator of transcription 1 *(STAT1)* manifest in immunodeficiency and autoimmunity with impaired T_H17 cell differentiation and exaggerated responsiveness to type I and II interferons. Allogeneic bone marrow transplantation has been attempted in severely affected patients, but outcomes have been poor.

Objective: We sought to define the effect of increased STAT1 activity on T helper cell polarization and to investigate the therapeutic potential of ruxolitinib in treating autoimmunity secondary to *STAT1* GOF mutations.

Methods: We used *in vitro* polarization assays, as well as phenotypic and functional analysis of *STAT1*-mutated patient cells.

Results: We report a child with a novel mutation in the linker domain of *STAT1* who had life-threatening autoimmune cytopenias and chronic mucocutaneous candidiasis. Naive lymphocytes from the affected patient displayed increased T_H1

From [a]the Division of Hematology/Oncology, [c]the Division of Immunology, and [d]the Program in Molecular and Cellular Medicine, Boston Children's Hospital, Department of Pediatrics, Harvard Medical School, Boston; [b]the Department of Pediatric Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston; [e]the Department of Immunology, Seattle Children's Hospital; [f]the Department of Pediatrics, Immunology Division, University of Washington, Seattle; [g]the Primary Immunodeficiency Clinic, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda; [h]the Department of Laboratory Medicine, Clinical Center, National Institutes of Health, Bethesda; [i]Harvard Stem Cell Institute, Cambridge; and [j]Baylor College of Medicine and Texas Children's Hospital, Department of Pediatrics, Section of Immunology, Allergy and Rheumatology and Center for Human Immunobiology, Houston.

*These authors contributed equally to this work.

‡Katja G. Weinacht, MD, PhD, is currently affiliated with the Division of Stem Cell Transplantation and Regenerative Medicine, Lucile Packard Children's Hospital, Stanford School of Medicine, Stanford, Calif.

Supported by National Institutes of Health grants 2R01AI085090 and 1R56AI115699 (to T.A.C.).

Disclosure of potential conflict of interest: K. G. Weinacht's institution has received grants from the National Institutes of Health (NIH) and National Institute of Allergy and Infectious Diseases (NIAID). T. R. Torgerson has received consultancy fees from Baxalta Biosciences, CSL Behring, and ADMA Biosciences and payment for developments of educational presentations from Baxalta Biosciences, CSL Behring, and RWJF, and his institution has received grants from Baxalta Biosciences, CSL Behring, and NIH. T. A. Fleisher is employed by the NIH; has received board membership from the American Academy of Allergy, Asthma & Immunology (AAAAI); received payment for lectures from the Louisiana Allergy, Asthma and Immunology Society and Alaska Allergy, Asthma and Immunology Society; and received royalties from Elsevier. L. D. Notarangelo has received board membership from Novimmune and consultancy fees from Sigma-Tau; is employed by Boston Children's Hospital; and has received royalties from UpToDate, and his institution has received grants from the NIH and March of Dimes. L. R. Forbes's institution has received grants from a Chao Physician–Scientist grant from Baylor College of Medicine. T. A. Chatila's institution has received grants 2R01AI085090 and 1R56AI115699 from the NIH. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication March 5, 2016; revised October 18, 2016; accepted for publication November 2, 2016.

Available online January 27, 2017.

Corresponding author: Katja G. Weinacht, MD, PhD, Division of Stem Cell Transplantation and Regenerative Medicine, Stanford School of Medicine, 300 Pasteur Dr, H320, Stanford, CA 94305. E-mail: kgw1@stanford.edu.

The CrossMark symbol notifies online readers when updates have been made to the article such as errata or minor corrections

0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2016.11.022

and follicular T helper cell and suppressed T_H17 cell responses. The mutation augmented cytokine-induced STAT1 phosphorylation without affecting dephosphorylation kinetics. Treatment with the Janus kinase 1/2 inhibitor ruxolitinib reduced hyperresponsiveness to type I and II interferons, normalized T_H1 and follicular T helper cell responses, improved T_H17 differentiation, cured mucocutaneous candidiasis, and maintained remission of immune-mediated cytopenias. Conclusions: Autoimmunity and infection caused by *STAT1* GOF mutations are the result of dysregulated T helper cell responses. Janus kinase inhibitor therapy could represent an effective targeted treatment for long-term disease control in severely affected patients for whom hematopoietic stem cell transplantation is not available. (J Allergy Clin Immunol 2017;139:1629-40.)

Key words: STAT1 *gain of function, IFN-γ, ruxolitinib, autoimmunity, T_H1 cell, T_H17 cell, follicular T helper cell, T helper cell polarization*

Signal transducer and activator of transcription 1 (STAT1) is a member of the STAT family of transcription factors which play a key role in the cellular response to interferons and is a central component in many other signaling pathways, including interleukins, growth factors, and hormones. In response to extracellular receptor stimulation, Janus kinase (JAK) activation leads to phosphorylation of cytoplasmic STAT1, followed by homodimerization or heterodimerization with other phosphorylated STAT family members. The dimers translocate into the nucleus and bind designated promoter elements to activate transcription of their respective target genes.[1-3]

STAT1 is the target of both loss-of-function and gain-of-function (GOF) mutations. Whereas the former are associated with susceptibility to mycobacterial and/or viral infections, the latter give rise to a mixed phenotype of autoimmunity, mucocutaneous candidiasis, and invasive fungal infections related to augmented T_H1 and diminished T_H17 cell responses.[4-9] *STAT1* GOF mutations prompt a signal-induced increase in levels of phosphorylated STAT1 (phospho-STAT) and amplified transcription of interferon-responsive genes, which lead to autoimmunity.[5,6,10] Delayed dephosphorylation with ensuing accumulation of phospho-STAT1 in the nucleus has been proposed as a mechanistic basis in most reported cases.[1,6,11-15] How increased STAT1 activity compromises T_H17 immunity to result in chronic mucocutaneous candidiasis and other invasive fungal and viral infections is less well understood.[12,16,17] Excessive production of interferons, IL-27, and programmed death (PD) 1 ligand can directly impair T_H17 cell differentiation.[18,19] Alternatively, predominance of STAT1 signaling over STAT3 signaling might deviate the response to IL-6, IL-21, and IL-23 away from STAT3, which normally mediates T_H17 cell development.[1,18]

The clinical management of patients with *STAT1* GOF mutations remains challenging.[20-22] In particular, controlling autoimmunity is difficult because conventional immunosuppression adds to the already increased risk of infections. Therapy-refractory or life-threatening disease is considered a noncanonical indication for allogeneic hematopoietic stem cell transplantation; however, the immune phenotype of *STAT1* GOF mutations amplifies the transplant-related risk for uncontrolled infections and graft-versus-host disease, contributing further to the poor prognosis.[21,23]

Abbreviations used
ATG: Anti-thymocyte globulin
BSA: Body surface area
GOF: Gain of function
IC_{50}: Half-maximal inhibitory concentration
ICOS: Inducible costimulator
JAK: Janus kinase
PD: Programmed death
STAT: Signal transducer and activator of transcription
T_C1: Cytotoxic T type 1
T_{FH}: Follicular T helper

Liu et al[24] were the first to provide proof of principle that JAK inhibitors can successfully treat STAT1-mediated hyperresponsiveness to interferons in patients with vascular and pulmonary syndrome caused by mutations in *TMEM173*, which encodes the stimulator of interferon genes. Higgins et al[10] reported hair regrowth in a patient with alopecia areata secondary to a *STAT1* GOF mutation after treatment with ruxolitinib. Most recently, Mössner et al[25] observed improvement of chronic mucocutaneous candidiasis with ruxolitinib and a reactive increase in IL-17A/F levels.

Here we describe the immunophenotypic analysis of a patient with life-threatening autoimmune cytopenias and a novel GOF mutation in the linker domain of *STAT1*. Importantly, in addition to increasing T_H1 and suppressing T_H17 cell differentiation, the augmented STAT1 activity dysregulated follicular T helper (T_{FH}) cell responses. This finding was corroborated in a different patient with known $STAT1^{T385M}$ GOF mutation in the DNA-binding domain who presented solely with chronic mucocutaneous candidiasis and opportunistic infections but without clinical evidence of autoimmunity.[13,26,27] Long-term treatment with the JAK inhibitor ruxolitinib decreased the increased STAT1 phosphorylation, reversed the dysregulated T_H1 and T_{FH} cell development, improved the previously impaired T_H17 response, and enabled effective control of the autoimmune cytopenias. This is the first report demonstrating mechanistic evidence that pharmacologic manipulation of the JAK-STAT pathway in patients with *STAT1* GOF mutations leads to reversal of the immune dysregulation phenotype and provides proof of principle that JAK inhibitors are not only effective in treating active autoimmune disease and immunodeficiency secondary to hyperresponsiveness of STAT1 but also in reversing the aberrant priming of naive cells, thereby maintaining long-term disease control and sustained remission.

METHODS

Patients and healthy subjects

All study participants were recruited after obtaining written informed consent approved by the Boston Children's Hospital Institutional Review Board.

Pharmacotherapy

The IL-1 receptor antagonist anakinra (Kineret; Sobi, Stockholm, Sweden) was administered intravenously twice daily at a dose of 100 mg.

Four infusions with equine anti-thymocyte globulin (ATG; Atgam; Pfizer, New York, NY) were administered intravenously at a dose of 40 mg/kg body