Monogenic mutations differentially affect the quantity and quality of T follicular helper cells in patients with human primary immunodeficiencies

Cindy S. Ma, PhD,[a,b] Natalie Wong, BSc Hons,[a] Geetha Rao, MSc,[a] Danielle T. Avery, BApplSci,[a] James Torpy, MPhil,[a] Thomas Hambridge, BSc Hons,[a] Jacinta Bustamante, MD, PhD,[c,d] Satoshi Okada, MD, PhD,[e] Jennifer L. Stoddard, BS,[f] Elissa K. Deenick, PhD,[a,b] Simon J. Pelham, MSc,[a,b] Kathryn Payne, BSc Hons,[a] Stéphanie Boisson-Dupuis, PhD,[c,g] Anne Puel, PhD,[c] Masao Kobayashi, MD, PhD,[e] Peter D. Arkwright, FRCPCH, DPhil,[h] Sara Sebnem Kilic, MD,[i] Jamila El Baghdadi, PhD,[j] Shigeaki Nonoyama, MD, PhD,[k] Yoshiyuki Minegishi, MD, PhD,[l] Seyed Alireza Mahdaviani, MD,[m] Davood Mansouri, MD,[m] Aziz Bousfiha, MD,[n] Annaliesse K. Blincoe, BHB, MBChB,[o] Martyn A. French, MB ChB, MD, FRACP, FRCPath, FRCP,[p,q] Peter Hsu, FRACP, PhD,[r] Dianne E. Campbell, FRACP, PhD,[r] Michael O. Stormon, MBBS, FRACP,[r] Melanie Wong, MBBS, PhD, FRACP, FRCPA,[r] Stephen Adelstein, MBBCh, PhD, FRACP, FRCPA,[s] Joanne M. Smart, MBBS, FRACP,[t] David A. Fulcher, MBBS, PhD, FRACP, FRCPA,[u] Matthew C. Cook, MBBS, PhD, FRACP, FRCPA,[v,w,x] Tri Giang Phan, MBBS, PhD, FRACP, FRCPA,[a,b] Polina Stepensky, MD,[y] Kaan Boztug, MD,[z,aa] Aydan Kansu, MD,[bb] Aydan İkincioğullari, MD,[cc] Ulrich Baumann, MD,[dd] Rita Beier, MD,[ee] Tony Roscioli, FRACP, PhD,[b,ff,gg] John B. Ziegler, MD, FRACP,[hh] Paul Gray, FRACP,[hh] Capucine Picard, MD, PhD,[c,d] Bodo Grimbacher, MD,[ii] Klaus Warnatz, MD, PhD,[ii] Steven M. Holland, MD,[jj] Jean-Laurent Casanova, MD, PhD,[c,g,kk,ll] Gulbu Uzel, MD,[jj] and Stuart G. Tangye, PhD[a,b]

Darlinghurst, Melbourne, Perth, Westmead, Acton, Canberra, and Randwick, Australia, Paris, France, Hiroshima, Saitama, and Tokushima, Japan, Bethesda, Md, New York, NY, Manchester, United Kingdom, Bursa and Ankara, Turkey, Rabat and Casablanca, Morocco, Tehran, Iran, Auckland, New Zealand, Jerusalem, Israel, Vienna, Austria, and Hannover, Essen, and Freiburg, Germany

From [a]the Immunology Research Program, Garvan Institute of Medical Research, Darlinghurst; [b]St Vincent's Clinical School, UNSW Australia, Melbourne; [c]the Laboratory of Human Genetics of Infectious Diseases, Necker Branch, INSERM U1163, Institut IMAGINE, Necker Medical School, University Paris Descartes, Paris; [d]the Study Center for Primary Immunodeficiencies, Assistance Publique-Hôpitaux de Paris (AP-HP), Necker Hospital for Sick Children, Paris; [e]the Department of Pediatrics, Hiroshima University Graduate School of Biomedical & Health Sciences, Hiroshima; [f]the Clinical Center, National Institutes of Health, Bethesda; [g]St Giles Laboratory of Human Genetics of Infectious Diseases, Rockefeller Branch, Rockefeller University, New York; [h]the University of Manchester, Royal Manchester Children's Hospital, Manchester; [i]the Department of Pediatric Immunology, Uludag University Medical Faculty, Görükle, Bursa; [j]the Genetics Unit, Military Hospital Mohamed V, Hay Riad, Rabat; [k]the Department of Pediatrics, National Defense Medical College, Tokorozawa, Saitama; [l]the Division of Molecular Medicine, Institute for Genome Research, University of Tokushima; [m]Pediatric Respiratory Diseases Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran; [n]the Clinical Immunology Unit, Pediatric Infectious Diseases Department, Averroes University Hospital, King Hasan II University, Casablanca; [o]Starship Children's Hospital, Auckland; [p]the Department of Clinical Immunology, Royal Perth Hospital; [q]the School of Pathology and Laboratory Medicine, University of Western Australia, Perth; [r]Children's Hospital at Westmead; [s]Clinical Immunology, Royal Prince Alfred Hospital, Sydney; [t]the Department of Allergy and Immunology, Royal Children's Hospital Melbourne; [u]the Department of Immunology, Westmead Hospital, University of Sydney; [v]Australian National University Medical School and [w]the John Curtin School of Medical Research, Australian National University, Acton; [x]the Department of Immunology, Canberra Hospital, Acton; [y]Pediatric Hematology-Oncology and Bone Marrow Transplantation Hadassah, Hebrew University Medical Center, Jerusalem; [z]CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna; [aa]the Department of Paediatrics and Adolescent Medicine, Medical University of Vienna; [bb]the Department of Pediatric Gastroenterology and [cc]the Department of Pediatric Immunology and Allergy, Ankara University Medical School; [dd]Paediatric Pulmonology, Allergy and Neonatology, Hanover Medical School; [ee]Pediatric Haematology and Oncology, University Hospital Essen; [ff]the Kinghorn Centre for Clinical Genomics, Victoria St Darlinghurst; [gg]the Department of Medical Genetics, Sydney Children's Hospital, Randwick; [hh]Sydney Children's Hospital, Randwick, and School of Women's and Children's Health, University of New South Wales, Sydney; [ii]the Center for Chronic Immunodeficiency, University Medical Center Freiburg, University of Freiburg; [jj]the Laboratory of Clinical Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda; [kk]the Pediatric Hematology and Immunology Unit, Necker Hospital for Sick Children, AP-HP, Paris; and [ll]the Howard Hughes Medical Institute, New York.

Supported by project and program grants from the National Health and Medical Research Council (NHMRC) of Australia (to E.K.D., S.G.T., C.S.M., D.A.F., and M.C.C.; 596813, 1016953, 1066694, and 1027400), the German Federal Ministry of Education and Research (BMBF 01EO1303 to B.G. and K.W.), and Rockefeller University Center for 541 Clinical and Translational science (5UL1RR024143, to J.L.C.). C.S.M. is a recipient of a Career Development Fellowship (1008820), and S.G.T. a Principal Research Fellowship (1042925) from the NHMRC of Australia.

Disclosure of potential conflict of interest: E. K. Deenick has received research support from the National Health and Medical Research Council, Australia. S. J. Pelham has received research support from University of New South Wales (Australian Postgraduate Award [PhD Scholarship]). D. E. Campbell is employed by NSW Health, has received research support from NHMRC, the Ilhan Food Allergy Foundation, and the University of Sydney; has received payment for development of educational presentations from the Australian Medical Council; and has received travel support from ALLSA, NSW Health, and ASCIA. M. C. Cook has received research support from the National Health and Medical Research Council, Australia. K. Boztug has received research support from the Austrian Science Fund (FWF; grant no. P24999). B. Grimbacher has received research support from BMBF, the European Union, Helmholtz, DFG, and DLR; is employed by UCL and UKL-FR; and has received lecture fees from CSL, Baxter, and Biotest. K. Warnatz has received consultancy fees from Biotest, CSL Behring, and Baxter; has received research support from Baxter; and has received lecture fees from Baxter, CSL Behring, Pfizer, Biotest, Novartis Pharma, Roche, Octapharma, Glaxo Smith Kline, Stallergenes AG, UCB Pharma, and the American Academy of Allergy, Asthma & Immunology. J.-L. Casanova has received research support from the National Institutes of Health (5UL1RR024143); has received consultancy fees from Biogen Idec, Merck, Sanofi-Aventis, Regeneron, and Bioaster; and has received research support from Merck, Biogen Idec, and Pfizer. S. G. Tangye has received research support from the National Health and Medical Research Council of Australia and has received travel support from ESID. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication February 20, 2015; revised May 4, 2015; accepted for publication May 13, 2015.

Corresponding author: Stuart G. Tangye, PhD, or Cindy S. Ma, PhD, Immunology Division, Garvan Institute of Medical Research, 384 Victoria St, Darlinghurst, NSW 2010, Australia. E-mail: s.tangye@garvan.org.au. Or: c.ma@garvan.org.au.

0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2015.05.036

Background: Follicular helper T (T_{FH}) cells underpin T cell–dependent humoral immunity and the success of most vaccines. T_{FH} cells also contribute to human immune disorders, such as autoimmunity, immunodeficiency, and malignancy. Understanding the molecular requirements for the generation and function of T_{FH} cells will provide strategies for targeting these cells to modulate their behavior in the setting of these immunologic abnormalities.
Objective: We sought to determine the signaling pathways and cellular interactions required for the development and function of T_{FH} cells in human subjects.
Methods: Human primary immunodeficiencies (PIDs) resulting from monogenic mutations provide a unique opportunity to assess the requirement for particular molecules in regulating human lymphocyte function. Circulating follicular helper T (cT_{FH}) cell subsets, memory B cells, and serum immunoglobulin levels were quantified and functionally assessed in healthy control subjects, as well as in patients with PIDs resulting from mutations in *STAT3*, *STAT1*, *TYK2*, *IL21*, *IL21R*, *IL10R*, *IFNGR1/2*, *IL12RB1*, *CD40LG*, *NEMO*, *ICOS*, or *BTK*.
Results: Loss-of-function (LOF) mutations in *STAT3*, *IL10R*, *CD40LG*, *NEMO*, *ICOS*, or *BTK* reduced cT_{FH} cell frequencies. *STAT3* and *IL21/R* LOF and *STAT1* gain-of-function mutations skewed cT_{FH} cell differentiation toward a phenotype characterized by overexpression of IFN-γ and programmed death 1. IFN-γ inhibited cT_{FH} cell function *in vitro* and *in vivo*, as corroborated by hypergammaglobulinemia in patients with *IFNGR1/2*, *STAT1*, and *IL12RB1* LOF mutations.
Conclusion: Specific mutations affect the quantity and quality of cT_{FH} cells, highlighting the need to assess T_{FH} cells in patients by using multiple criteria, including phenotype and function. Furthermore, IFN-γ functions *in vivo* to restrain T_{FH} cell–induced B-cell differentiation. These findings shed new light on T_{FH} cell biology and the integrated signaling pathways required for their generation, maintenance, and effector function and explain the compromised humoral immunity seen in patients with some PIDs. (J Allergy Clin Immunol 2015;■■■:■■■-■■■.)

Key words: *Follicular helper T cells, humoral immunity, primary immunodeficiencies, cytokine signaling*

Abbreviations used
Bcl-6: B-cell lymphoma 6
cT_{FH}: Circulating follicular helper T
DN: Double-negative
DP: Double-positive
GC: Germinal center
GOF: Gain of function
IL-10R: IL-10 receptor
IL-12R: IL-12 receptor
IL-21R: IL-21 receptor
LOF: Loss of function
PD-1: Programmed death 1
PID: Primary immunodeficiency
STAT: Signal transducer and activator of transcription
TD: T-dependent
T_{FH}: Follicular helper T
T_{FR}: Follicular regulatory T
Treg: Regulatory T

Naive $CD4^+$ T cells differentiate into distinct populations of effector cells with specialized functions. Such fine tuning ensures the generation of appropriate immune responses that efficiently clear pathogens and generate long-term protective immunity after infection or vaccination.[1] The $CD4^+$ T cells responsible for mediating the differentiation of naive B cells into memory cells and plasma cells, thereby providing effective humoral immunity against T-dependent (TD) antigen, are follicular helper T (T_{FH}) cells.[2-5] T_{FH} cells express increased levels of CXCR5, programmed death 1 (PD-1), B-cell lymphoma 6 (Bcl-6), and several molecules involved in T-cell/B-cell interactions and localize to follicles of secondary lymphoid tissues.[2-4] Differentiation of naive $CD4^+$ T cells into T_{FH} cells is a complex process requiring integration of signals delivered by dendritic cells, B cells, cytokines, specific signaling pathways, and transcription factors.[2-5] The critical role of T_{FH} cells in eliciting long-lived humoral immunity is evidenced by impaired generation of germinal centers (GCs), memory B cells, and antibodies to TD antigen in mice and human subjects who lack genes that promote T_{FH} cell formation.[2-6] Antibody-mediated autoimmune conditions can also be caused by dysregulated T_{FH} cell function.[7-9] Thus delineating molecular requirements underlying T_{FH} cell generation and function are important in understanding how these cells operate and in identifying pathways that could be targeted in the settings of vaccination, immunodeficiency, or autoimmunity.

Although studies of mice and some human immune disorders have taught us much about T_{FH} cells, our understanding of human T_{FH} cell biology remains incomplete, largely because of limited access to lymphoid tissues, where T_{FH} cells are located. However, progress has been made by studying circulating $CD4^+CXCR5^+$ T cells as correlates of tissue T_{FH} cells. Subsets of circulating follicular helper T (cT_{FH}) cells have been reported, with $CCR6^+$, $CCR6^+PD\text{-}1^+$, or $CCR7^{lo}PD\text{-}1^{hi}$ subsets being superior to other subsets in providing B-cell help.[10,11] These subsets correlated with antibody responses to influenza virus after vaccination in young adults, but not older adults,[12] and are increased in patients with autoimmune diseases[8,10,13-16] and decreased in patients with HIV infection.[11] Similarly, $CD4^+CXCR5^+PD\text{-}1^+CXCR3^-$ T cells were identified as the circulating counterpart of lymphoid T_{FH} cells, and their frequencies positively correlated with neutralizing antibodies in patients with HIV infection.[17] Although these studies generally confirmed that $PD\text{-}1^+CXCR3^-/CCR6^+$ cT_{FH} cells are a reliable correlate of T_{FH} cells in human lymphoid tissue, the identity of the circulating B-helper human $CD4^+$ T cells remains contentious because other studies demonstrated that $CXCR5^+CXCR3^+$ or even $CXCR5^-CD4^+$ T cells exhibit detectable B-helper function[11,15,18,19] and correlate with influenza vaccine responsiveness.[18,20]

To assess the molecular requirements for the generation and function of human cT_{FH} cells, we investigated more than 110 subjects with 14 different monogenic mutations that underlie primary immunodeficiencies (PIDs). Our findings identify mutations that have distinct quantitative effects, qualitative effects, or both on human cT_{FH} cells, providing an explanation for humoral immune defects in some PIDs, as well as insights into mechanisms regulating human T_{FH} cell differentiation and function.