Increased percentages of circulating T follicular helper cells associate with disease subtype and activity in pediatric immune cytopenias

Emily M. Harris,[1] Aleksandra Bourdine,[2] Logan Magin,[2] Megan Elkins,[2] Matthew Nikiciuk,[2,3] Anne Chu,[2,3] Deena Wafadari,[2] Silvia Nastasio,[4] Rebecca Hale,[2] Leetah Senkpeil,[2] Shira Rockowitz,[5-8] Piotr Sliz,[5-10] Craig D. Platt,[2] Brenna LaBere,[2°] Caitlin Montcrieff,[1] Sarah Chamseddine,[2] Toshiro K. Ohsumi,[2] Rachael F. Grace[1#] and Janet Chou[2#]

[1]Dana-Farber/Boston Children's Cancer and Blood Disorders Center, Harvard Medical School; [2]Division of Immunology, Boston Children's Hospital, Harvard Medical School; [3]Northeastern University; [4]Division of Gastroenterology, Hepatology, and Nutrition, Boston Children's Hospital, Harvard Medical School; [5]Children's Rare Disease Collaborative, Boston Children's Hospital; [6]Department of Pediatrics, Harvard Medical School; [7]The Manton Center for Orphan Disease Research, Boston Children's Hospital; [8]Division of Genetics and Genomics, Boston Children's Hospital; [9]Division of Molecular Medicine, Boston Children's Hospital and [10]Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA, USA

[#]RFG and JC contributed equally as senior authors.

[°]Current affiliation: Division of Allergy and Immunology, Phoenix Children's Hospital, Phoenix, AZ, USA.

Correspondence: E.M. Harris
Emily.Harris2@childrens.harvard.edu

Received: April 11, 2025.
Accepted: October 9, 2025.
Early view: October 16, 2025.

https://doi.org/10.3324/haematol.2025.287988

Abstract

Immune thrombocytopenia (ITP), warm autoimmune hemolytic anemia (wAIHA), and Evans syndrome (ES) have unpredictable disease activity and are sometimes associated with monogenetic disorders and/or extra-hematologic autoimmunity. ITP and immune neutropenia remain diagnoses of exclusion, hindering the diagnosis of ES in patients with multiple cytopenias. As there are no predictors of immunological comorbidities in these patients, it is difficult to determine who would benefit from genetic testing and/or evaluations for extra-hematologic autoimmunity. As circulating $CD4^+$ T follicular helper (cTfh) cells promote autoimmunity, we quantified cTfh cells, associated clinical characteristics, and transcriptional signatures in a cohort of 153 pediatric patients with immune cytopenias (85 with ITP, 26 with wAIHA, and 42 with ES). cTfh cell percentages exceeding 9.5% had 76% sensitivity and 86% specificity for distinguishing ES from ITP or wAIHA, irrespective of disease activity, with a positive predictive value of 0.68 and negative predictive value of 0.91. Increased percentages of cTfh cells were associated with active cytopenia and decreased with treatment in patients with improving cytopenias over time, suggesting the utility of cTfh measurement for clinical monitoring. Increased percentages of cTfh cells were also associated with underlying immune disorders and extra-hematologic autoimmunity, thus identifying patients who would benefit from more extensive immunological evaluation. Single-cell RNA-sequencing of cTfh cells and plasma cytokines revealed increased interferon-α/β and interferon-γ signaling in patients with active wAIHA and ES, respectively. These results not only uncover immunological pathways differentiating subtypes of immune cytopenias, but also demonstrate applications of existing clinical tests in diagnosing immune cytopenias and in identifying patients who require more extensive evaluation for immunological comorbidities.

Introduction

Pediatric immune cytopenias are challenging to diagnose and manage because they range from self-resolving, single-lineage cytopenias to life-threatening immune dysfunction affecting multiple lineages and organs. These disorders include immune thrombocytopenia (ITP), warm autoimmune hemolytic anemia (wAIHA), immune neutropenia, and Evans syndrome (ES) affecting multiple lineages. While patients with immune cytopenias can achieve sustained remission,

a relapsing/remitting or chronic course is common, particularly for those with ES. Pediatric mortality due to ES ranges from 7-35%.[3,4] The diagnosis of ES is challenging due to the lack of established diagnostic tests for ITP or immune neutropenia, leading to a high rate of misdiagnosis.[5,6] There are no known markers of disease activity, immunological comorbidities, or treatment response.

T cells, including T regulatory (Treg) and T follicular helper (Tfh) cells, have been implicated in the pathogenesis of ITP in adults but have not been as robustly studied in pediatric immune cytopenias.[7-9] Tfh cells are a subset of CD4$^+$ T cells that reside in lymphoid organs, where they activate B cells.[10] They are essential for germinal center formation and antibody production.[10] Circulating T follicular helper (cTfh) cells are CD4$^+$CXCR5$^+$PD1$^+$ T cells that share T-cell receptor clonotypes and functions with germinal center Tfh cells.[10] Tfh cells are known to drive B-cell expansion, activation, and the production of protective antibodies as well as autoantibodies in patients with autoimmunity.[11-13] Measurement of cTfh cells has been studied for the diagnosis and management of many different diseases, including sickle cell disease,[14] primary antibody deficiency,[15] stem cell transplant,[16] and cancer.[17] cTfh cell expansion has been described in pediatric patients with active untreated ES[18,19] and chronic ITP,[20-23] but the clinical features and transcriptional changes associated with cTfh expansion in patients with immune cytopenias remain incompletely understood. To address these questions, this study evaluated the clinical utility of quantifying cTfh cells in a cohort of 153 pediatric patients with ITP, wAIHA, and ES with different disease activity, treatments, and comorbidities, reflecting the heterogeneity seen in clinical practice.

Methods

Study ohort

This single-center prospective cohort study enrolled 153 pediatric patients with immune cytopenias who had research samples drawn during routine clinical care and 78 healthy controls. This study was approved by the Boston Children's Hospital Institutional Review Board and was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2013. Patients and/or their legal guardians provided informed consent and assent when appropriate. The inclusion criteria were a diagnosis of ITP, wAIHA, or ES as determined by the treating hematologist, including patients with and without immune disorders and extra-hematologic autoimmunity. Patients were included regardless of cytopenia or treatment status at the time of enrollment. Clinical and laboratory data were collected via review of medical records. Primary or secondary immune disorder was defined as an inborn error of immunity (*Online Supplementary Methods*), common variable immunodeficiency,[24] or prior hematopoietic stem cell transplant. Extra-hematologic autoimmunity was defined as autoimmunity affecting another organ system other than blood (erythrocytes, platelets, or leukocytes) (Table 1).

Patients who were receiving immune cytopenia-directed medication at the time of cTfh measurement were considered on treatment. Patients given intravenous immunoglobulin within 4 weeks and rituximab within 6 months before cTfh measurement were considered on treatment. Active cytopenia was defined as the presence of cytopenia in clinical laboratory results at the time of cTfh measurement. Inactive cytopenia was defined as no cytopenia at the time of cTfh measurement.

Improving disease was defined as ability to discontinue treatment and/or cytopenia resolution. Worsening disease was defined as recurrence of a previously resolved cytopenia. No status change was defined as no change in whether cytopenia was present and no change in whether a patient was receiving medication compared to the previous timepoint.

Flow cytometry

cTfh cells were measured by flow cytometry as a percentage of CD4$^+$ T cells expressing CXCR5 and PD-1 (*Online Supplementary Figure S1, Online Supplementary Methods*).

Cytokine measurements

Cytokine levels in plasma were measured with LEGENDplex multiplex bead-based assay panels (*Online Supplementary Methods*).

Single-cell RNA sequencing

Peripheral blood mononuclear cells were freshly isolated by Ficoll-Paque density gradient centrifugation. CD4$^+$ T cells were isolated from the peripheral blood mononuclear cells from controls and patients using magnetic separation (Miltenyi Biotec, 130-096-533). Single-cell RNA sequencing was performed on CD4$^+$ cells from controls and patients with a total of 10,000 cells per library. Sequencing data have been deposited in the National Center for Biotechnology Information Sequence Read Archive (SRA) with accession number PRJNA1233813.

Statistical analysis

Data distribution was tested for normality using D'Agostino & Pearson, Anderson-Darling, Shapiro-Wilk, and Kolmogrov-Smirnov tests. The Mann-Whitney test was used for comparisons between two groups. Kruskal-Wallis and Dunn multiple comparisons tests were used for comparisons of three or more groups. Spearman correlation was used to evaluate associations between two variables. A receiver operating characteristic curve was used to determine sensitivity and specificity. Logistic regression was performed using the mctest package[25] with and without Firth correction. Statistical tests were performed using Graphpad Prism v10.0.0 or R v4.3.1.. Graphs were created with Graphpad Prism v10.0.0.

Results

Clinical characteristics

This study enrolled 78 controls and 153 pediatric patients with immune cytopenias: 85 patients with ITP, 26 with wAIHA, and 42 with ES. Their clinical and demographic characteristics are outlined in Table 1 and *Online Supplementary Table S1*. There were 32 patients with immune disorders, including 29 with genetically defined disorders, one with common variable immunodeficiency lacking a genetic cause, and two with a history of hematopoietic stem cell transplantation (Table 2). All 42 patients with ES had genetic testing performed and 17 (40.5%) had a genetic diagnosis. Of the 26 patients with wAIHA, 22 (84.6%) had genetic testing performed and five (22.7%) of those tested had a positive result. Of the 85 patients with ITP, 36 (42.4%) had genetic testing performed and eight (22.2%) of those tested had a positive result (Figure 1A, B and Table 2).

Table 1. Demographic and clinical features at the time of sampling.

Clinical features	All patients, N=153			Controls, N=78
	ITP N=85	wAIHA N=26	ES N=42	
Age, years, median (range)	10.6 (1-22)	13.3 (0.5-27)	16.9 (0.5-33)	12.0 (1.3-47.1)
Sex, female, N (%)	45 (53)	17 (65)	15 (36)	44 (56.4)
Race, N (%)				
White	46 (54)	19 (73.1)	27 (64.3)	48 (61.5)
Asian	2 (2.4)	0 (0)	1 (2.4)	3 (3.8)
Black	0 (0)	1 (3.8)	1 (2.4)	5 (6.4)
Other	13 (15.3)	3 (11.5)	6 (14.3)	11 (14.1)
Unknown	24 (28.2)	3 (11.5)	7 (16.7)	11 (14.1)
Ethnicity, N (%)				
Hispanic or Latino	11 (12.9)	1 (3.8)	4 (9.5)	12 (15.4)
Unknown	23 (27)	6 (23.1)	10 (23.8)	10 (12.8)
Time from diagnosis, days, median (range)	374 (4-5,827)	372 (2-3,563)	1,420 (38-10,697)	-
Active cytopenia present, N (%)	53 (62)	8 (31)	24 (57)	-
On treatment, N (%)	31 (36)	24 (92)	32 (76)	-
Immunoglobulin replacement	6	5	6	-
Corticosteroid	2	15	8	-
Anti-CD20 therapies	4	7	1	-
Bortezomib	0	1	0	-
Sirolimus	1	3	5	-
Mycophenolate mofetil	1	5	11	-
Azathioprine	0	0	1	-
Thrombopoietin receptor agonists	21	0	6	-
Abatacept	0	0	2	-
Ruxolitinib	0	1	1	-
G-CSF	0	0	1	-
Immune disorders, N (%)	8 (9)	6 (23)	18 (43)	-
Inborn error of immunity	8	4	17	-
CVID	0	0	1	-
Post-HSCT	0	2	0	-
Extrahematologic autoimmunity, N (%)	7 (8)	8 (31)	12 (29)	-
Hepatitis	1	4	4	-
Gastrointestinal tract	3	2	3	-
Thyroiditis	2	1	4	-
Skin	3	1	1	-
Pulmonary	1	0	4	-
Systemic lupus erythematosus	1	2	0	-
Primary sclerosing cholangitis	0	2	0	-
Type 1 diabetes	0	0	1	-
ANA titer ≥1:160, N/N (%)	10/50 (20)	3/15 (20)	7/33 (21)	-

Some patients have more than one type of extra-hematologic autoimmunity. The post-HSCT patients had undergone stem cell transplant for RAG1 deficiency (N=1) and Hurler syndrome (N=1). No patients had undergone splenectomy. ITP: immune thrombocytopenia; wAIHA: warm autoimmune hemolytic anemia; ES: Evans syndrome; G-CSF: granulocyte colony-stimulating factor; CVID: common variable immune deficiency; HSCT: hematopoietic stem cell transplant; ANA: antinuclear antibodies.

Clinical features associated with increased percentages of circulating T follicular helper cells

Percentages of cTfh cells were significantly higher in patients with ES than in those with ITP (P<0.0001), wAIHA (P=0.0007), or controls (P<0.0001) (median 13.05% in those with ES, 4.82% in those with ITP, 6.11% in those with wAIHA, and 5.16% in controls) (Figure 2A). Patients with active or inactive ES had higher percentages of cTfh cells than controls

Table 2. Genetically defined immune disorders in this study cohort.

Immune cytopenia	Genetic disorder	Gene	Variant
Idiopathic thrombocytopenic purpura	ALPS	*FAS*	c.856G >A, p.G286R
	ch19p13.3 duplication*	19p13.3	19p13.3(260911-4342341x3) de novo duplication
	DiGeorge syndrome	22q11.2	22q11.2 deletion
	Kabuki syndrome†	*KMT2D*	c.12415_12416del, p.Val4139Phefs*28
	Kabuki syndrome	*KMT2D*	c.18267G>T, p.V5423F
	Kabuki syndrome	*KMT2D*	c.44221G>T, p.C1474F
	NFKB1 haploinsufficiency	*NFKB1*	c.1153G>A, p.Gly385Ser
	IKZF1 haploinsufficiency	*IKZF1*	c.1537G>C, p.Glu513Gln
Warm autoimmune hemolytic anemia	DOCK11 deficiency	*DOCK11*	c.1162G>A, p.Gly388Arg
	Kabuki syndrome	*KMT2D*	c.13151C>G, p.Ala4384Gly
	NFKB1 haploinsufficiency	*NFKB1*	c.259-1G>C
	RAG1 deficiency, post-HSCT	*RAG1*	c.1210C>T, p.Arg404Trp c.983G>A, p.Cys328Tyr
	STAT1 GOF	*STAT1*	c.604A>G, p.Met202Val
Evans syndrome	ALPS	*FAS*	c.185C>G, p.Pro92Arg
	A20 haploinsufficiency	*TNFAIP3*	c.2281C>T, p.Arg761Cys
	CD25 deficiency	*IL2RA*	c.530G>A, p.Trp177* c.800del, p.Lys267Argfs*95
	CTLA4 haploinsufficiency	*CTLA4*	c.347T>A, p.I116N
	DiGeorge syndrome	22q11.2	22q.11.21 (18661724_21661435)x1 dn, *de novo* deletion of at least 2.9 Mb within cytogenic band 22q11.21
	Kabuki syndrome	*KMT2D*	c.6265_6266delinsCAAT mutation
	Kabuki syndrome	*KMT2D*	c.13696G>T, p.Glu4566
	Kabuki syndrome	*KMT2D*	c.15731_15732delAA
	Kabuki syndrome	-	variant identity unavailable
	NFKB1 haploinsufficiency	*NFKB1*	*NFKB1* c.884G>A, p.Trp295*
	NFKB1 haploinsufficiency	*NFKB1*	*NFKB1* c.1855_1856del, p.Val619PhefsTer10
	NFKB2 haploinsufficiency	*NFKB2*	*NFKB2* c.1354G>A, p.Gly452Ser
	PLAID	*PLCG2*	c.2866C>T, p.Arg956Cys
	SAMD9L deficiency	*SAMDL9*	c.1549T>C, p.Trp517Arg
	SOCS1 haploinsufficiency	*SOCS1*	c.24delA, p.Ala9Profs∗76
	STAT3 GOF	*STAT3*	c.2144 C>T, p.Pro715Leu
	STAT3 GOF	*STAT3*	c.1199A>T, p.Asn400Ile

*This disorder is associated with immune dysregulation and immunodeficiency.[70,71] †Although the numbers of patients with Kabuki syndrome in our cohort were likely influenced by our center's multidisciplinary Roya Kabuki program, others have similarly identified immune cytopenias as a common feature of Kabuki syndrome.[72,73] ALPS: autoimmune lymphoproliferative syndrome; GOF: gain-of-function; HSCT: hematopoietic stem cell transplant; PLAID: PLCG2-associated antibody deficiency and immune dysregulation.

(Figure 2B). In contrast, patients with active or inactive ITP or wAIHA had comparable percentages of cTfh cells to controls, irrespective of disease activity (Figure 2B). Receiver operating characteristic curve analysis revealed that a cTfh percentage exceeding 9.5% had 76% sensitivity and 86% specificity for distinguishing ES from ITP or wAIHA (Figure 2C). cTfh measurements greater than 9.5% had a positive predictive value of 0.68 (95% confidence interval [95% CI]: 0.54-0.80) and a negative predictive value of 0.91 (95% CI: 0.84-0.95) for ES as compared to ITP or wAIHA alone. cTfh percentages were highest in patients with active ES off treatment (Figure 2D). Among patients with ES, only those with normal blood cell counts without treatment had cTfh percentages comparable to those of controls (Figure 2D).

We next studied cTfh percentages in patients with longitudinal measurements over time. Of the 51 patients with more than one measurement of cTfh percentages, 27 had ITP, six had wAIHA, and 18 had ES. Most patients with ES with improving disease had normalization of cTfh percentages over time (Figure 2E, *Online Supplementary Figure S2*). Among those with persistently active ES, most had cTfh percentages that remained above 11.3%, which we previously published as the 97.5th percentile of cTfh percentages in 210 controls.[26] In contrast, most patients with ITP (88.5%, N=23) and wAIHA (87.5%, N=7) had cTfh values that remained <11.3% (Figure 2E, *Online Supplementary Figure S2*). There was no significant correlation between disease duration and cTfh percentages in patients with ITP, wAIHA, or ES (*Online Supplementary Figure S3*).

To identify specific immune features associated with cTfh cell expansion, we substratified cTfh percentages by clinical features: (i) primary or secondary immune disorder, (ii) extra-hematologic autoimmunity, or (iii) elevated anti-nuclear antibody (ANA) titer. Patients with a primary or secondary immune disorder or extra-hematologic autoimmunity accompanying their immune cytopenia had significantly higher cTfh percentages than those lacking these features (Figure 2F). We next investigated these associations within each subtype of immune cytopenia. Among patients with ITP and ES, those with an associated primary or secondary immune disorder had increased percentages of cTfh cells compared to those with a history of immune cytopenias alone (*Online Supplementary Figure S4*). Extra-hematologic autoimmunity was also associated with increased cTfh cell percentages in patients with ITP but not ES or wAIHA, suggesting that extra-hematologic autoimmunity denotes a clinically distinct subgroup of patients with ITP (*Online Supplementary Figure S4*).

As immune cytopenias can be a presenting feature of systemic lupus erythematosus, ANA is often measured as part of the diagnostic evaluation for these disorders. Using an ANA threshold associated with systemic lupus erythematosus,[27] we found no difference in cTfh between patients who had an ANA titer ≥1:160 or <1:160 in the total cohort (Figure 2F) or in patients with ITP or ES. Among patients with wAIHA, cTfh percentages were increased in patients with ANA ≥1:160 (*Online Supplementary Figure S4*). We next assessed percentages of Treg cells, which have been implicated in the pathogenesis of immune cytopenias.[28] $CD4^{+}CD25^{hi}CD127^{low}$ Treg cells were measured by a CLIA-approved clinical flow cytometry test in 42 study participants (15 with ITP, 8 with wAIHA, and 19 with ES). Percentages of Treg cells were not different among these patients and did not correlate with cTfh percentages (*Online Supplementary Figure S5*).

Having identified several clinical features associated with elevated cTfh percentage, we used logistic regression to model these relationships. A diagnosis of ES (odds ratio [OR]=13.25, 95% CI: 4.43-44.92, q=0.0001), immune disorder, defined as a primary or secondary immune disorder and/or extra-hematologic autoimmunity (OR=9.88, 95% CI: 3.42-32.22, q=0.0001), and active cytopenia (OR=4.90, 95% CI: 1.73-15.79, q=0.0015) were associated with increased percentages of cTfh cells

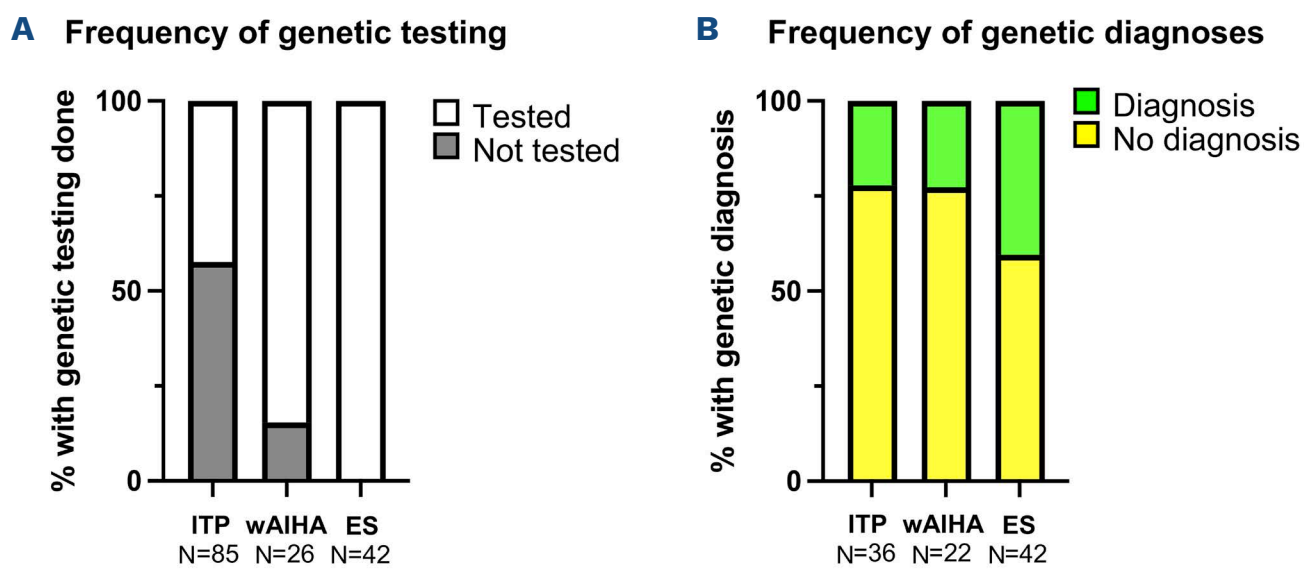

Figure 1. Genetic testing results. (A) Frequency of genetic testing in patients with immune thrombocytopenia (ITP), warm autoimmune hemolytic anemia (wAIHA), and Evans syndrome (ES). (B) Frequency of genetic diagnosis in patients with ITP, wAIHA, and ES who had genetic testing performed.

(Figure 2G, *Online Supplementary Table S2*). Treatment with cytopenia-directed medications at the time of sampling was inversely associated with cTfh percentages (OR=0.30, 95% CI: 0.09-0.90, q=0.0165) (Figure 2G).

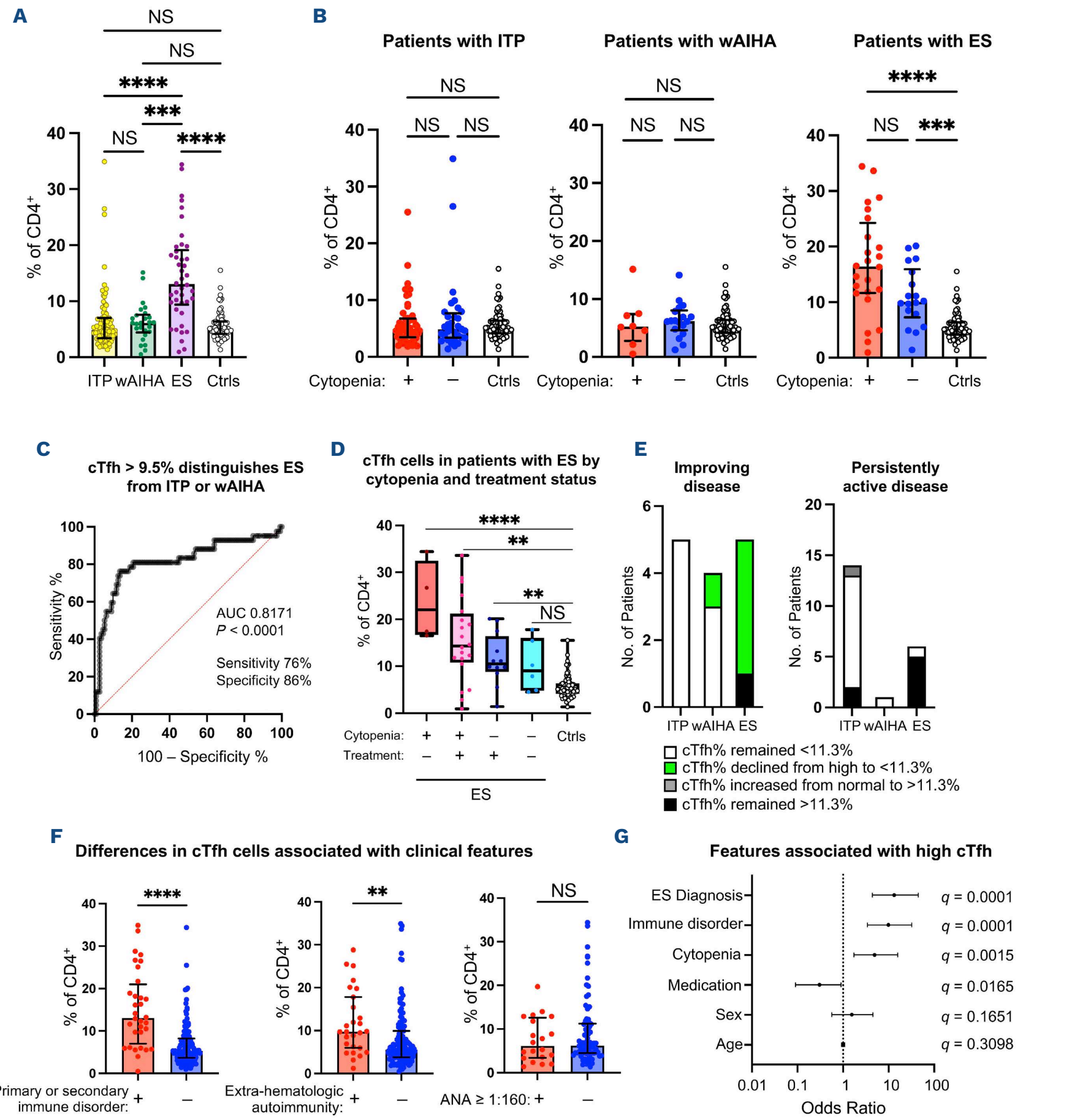

Figure 2. Quantification of circulating T follicular helper cells and associated clinical characteristics in patients with immune cytopenias. (A) Circulating T follicular helper cells (cTfh), measured as percentage of CD4⁺ cells, in peripheral blood samples from patients with immune thrombocytopenia (ITP), warm autoimmune hemolytic anemia (wAIHA), Evans syndrome (ES), and controls; NS=P>0.05, ***P<0.001, ****P<0.0001 by the Kruskal-Wallis test. (B) cTfh, measured as percentage of CD4⁺ cells, by presence (+) or absence (-) of cytopenia at the time of sample collection in patients with ITP, wAIHA, and ES as compared to controls. (C) Receiver operating characteristic curve comparing cTfh values in patients with ES as compared to non-ES immune cytopenias (isolated ITP or wAIHA). (D) cTfh, measured as percentage of CD4⁺ cells, by presence (+) or absence (-) of active cytopenia at the time of sample collection and whether patient was receiving (+) or not receiving (-) medication treatment at the time of sample collection, in patients with ES and controls; NS=P>0.05, **P<0.01, ****P<0.0001 by the Kruskal-Wallis test. (E) Longitudinal evaluation of cTfh in patients with improving disease and persistently active disease showing number of patients with ITP (N=26),

Continued on following page.

wAIHA (N=8), and ES (N=18) who had cTfh values that remained <11.3% (white), declined from high to <11.3% (green), increased from normal to >11.3% (gray), and remained >11.3% (black) over time. (F) cTfh, measured as percentage of $CD4^+$ cells, in patients with immune cytopenias with (+) and without (-) extra-hematologic autoimmunity, a primary or secondary immune disorder, and antinuclear antibody titer ≥1:160. Primary immune disorder refers to a genetically defined inborn error of immunity (N=29). Secondary immune disorder refers to patients who developed immune cytopenia after a stem cell transplant or met clinical criteria for common variable immune deficiency without a genetic diagnosis (N=2). Antinuclear antibodies were measured in a subset of participants: 58.8% (N=50) of patients with ITP, 57.7% (N=15) of patients with wAIHA, and 76.2% (N=32) of patients with ES. NS=*P*>0.05, ***P*<0.01, *****P*<0.0001 by the two-tailed Mann-Whitney test. (G) Logistic regression analysis of clinical factors associated with high percentages of cTfh cells (>11.3%). Immune disorders include primary immune disorders (inborn errors of immunity), secondary immune disorders, and extra-hematologic autoimmunity. Each patient was included only once for each variable. False discovery rate-adjusted *q* values are shown. NS: not statistically significant; Ctrls: controls; AUC: area under the curve; ANA: antinuclear antibodies.

Transcriptional profiles of circulating T follicular helper cells in patients with immune cytopenias

We hypothesized that the transcriptional profiles of cTfh cells from patients with immune cytopenias differed from controls, even in those with normal percentages. We used single-cell RNA-sequencing to analyze cTfh cells from patients with ES (subdivided into those with active or inactive ES and those on or off treatment), active or inactive wAIHA on treatment, and active ITP off treatment (Figure 3A, B). Pathway analysis was performed on differentially expressed genes (fold-change of at least ±1.5, adjusted *P*<0.05) in each group compared to controls. cTfh cells from patients with active ES off treatment exhibited transcriptional profiles indicative of increased interferon (IFN) signaling, which encompassed targets downstream of IFN-α/β (*OASL, IFITM3*) and IFN-γ (*IFNG, CXCR3, IL21*) signaling, activation of the T-cell receptor and effector kinases (PI3K/AKT) that support the metabolic demands of cell activation, and apoptosis (Figure 3C, D). Additionally, these cells also had decreased interleukin (IL)-7 receptor signaling, a pathway important for T-cell survival.[29] In contrast, patients with active or inactive ES on treatment had reduced signaling downstream of T-cell activation, the Th1 pathway, and IFN-γ (Figure 3C, D), reflecting treatment effects rather than disease activity. In patients with active ES on treatment, cTfh cells exhibited upregulation of the vitamin D receptor/retinoic acid receptor axis known to suppress genes important for the differentiation of Tfh and cTfh cells.[30] Patients with inactive ES on treatment had upregulated signaling in the PD-1 pathway, which inhibits T-cell activation and mediates T-cell exhaustion. Compared to controls, cTfh cells from patients with inactive ES off treatment had no pathways enriched for differentially expressed genes relevant to T-cell development and/or function, consistent with their clinical status. Furthermore, all patients except for those with inactive ES off treatment had transcriptional evidence of increased apoptosis, indicating persistent differences in cTfh transcriptional profiles even in the absence of active cytopenias.

Among patients with wAIHA, there was increased expression of genes indicative of IFN-α/β signaling in those with and without active cytopenias, even though all patients in these cohorts were receiving immunomodulatory treatments. Patients with active wAIHA had increased inflammatory signaling compared to controls, evidenced by increased expression of targets downstream of IFN-γ, the pathway that fuels T-cell activation, and IL-8. In contrast, those with inactive wAIHA had reduced antigen presentation and less Th1 compared to controls. Lastly, the transcriptional profiles of cTfh cells from patients with active ITP off treatment exhibited a lack of inflammatory signaling. Compared to controls, patients with active ITP off treatment had decreased expression of genes important for T-cell activation (Notch1, STAT3, and CD28 co-stimulation), IL-3, a cytokine produced by activated T cells, and transforming growth factor (TGF)- β, a regulator of Tfh cell development (Figure 3C). Despite the normal percentages of cTfh cells in patients with wAIHA and ITP, single-cell RNA-sequencing analysis showed that increased IFN signaling distinguished the cTfh cells in those with wAIHA.

We next used unsupervised hierarchical clustering of differentially expressed genes in the IFN-α/β and IFN-γ pathways to compare the disease groups (Figure 4). Patients with active ES off treatment and active wAIHA on treatment clustered together with the most robust IFN signatures. Those with active ES off treatment had the highest expression of genes indicative of IFN-γ signaling (*IFNG, CXCR3, IL21*), while patients with active wAIHA on treatment had the highest expression of genes downstream of IFN-α/β signaling (*IFIT1, MX1, OAS1/2/3, ISG15/18*, among others). Patients with inactive wAIHA on treatment and active ES on treatment formed the next cluster (Figure 4). cTfh cells from patients with inactive wAIHA on treatment exhibited increased expression of IFN-stimulated genes compared to those with active ES on treatment, underscoring the robust IFN signature in patients with wAIHA regardless of the presence of cytopenia. Patients with inactive ES on or off treatment, those with active ITP, and controls formed the last cluster; among these, patients with ITP clustered most closely with controls. Collectively, these findings identify type I and type II IFN signaling as immunological mechanisms distinguishing subgroups of patients with immune cytopenias.

Cytokine profiles

As the circulating cytokine milieu is known to affect the

transcriptional profiles of cTfh cells, we measured 23 cytokines in the plasma of patients with ES, wAIHA, and ITP and in controls. These cytokines included those induced by IFN-α/β, IFN-γ, NF-κB, and Th1/Th2/Th17 effector T cells (Figure 5, *Online Supplementary Figure S6*). We found that CXCL9, a cytokine induced by IFN-γ, was significantly higher in patients with ES (P=0.0008) than in controls (Figure 5A), consistent with the increased IFN-γ signaling seen in cTfh cells of patients with active ES. Plasma CXCL9 levels were higher in patients with wAIHA than in controls (P=0.0357) (Figure 5A), also concordant with the pattern of increased IFN-γ signaling observed in cTfh cells from patients with active wAIHA on treatment. In our total cohort, there was a modest but statistically significant correlation between CXCL9 levels and cTfh percentages (P=0.0003, r=0.3267) (Figure 5B). Additionally, levels of the chemokine ligand CCL2 were significantly higher in patients with wAIHA than in controls (P=0.0170) (Figure 5C). IFN-α/β upregulates the expression of CCL2, which activates and recruits T cells and macrophages to sites of inflammation.[31-33] The increased

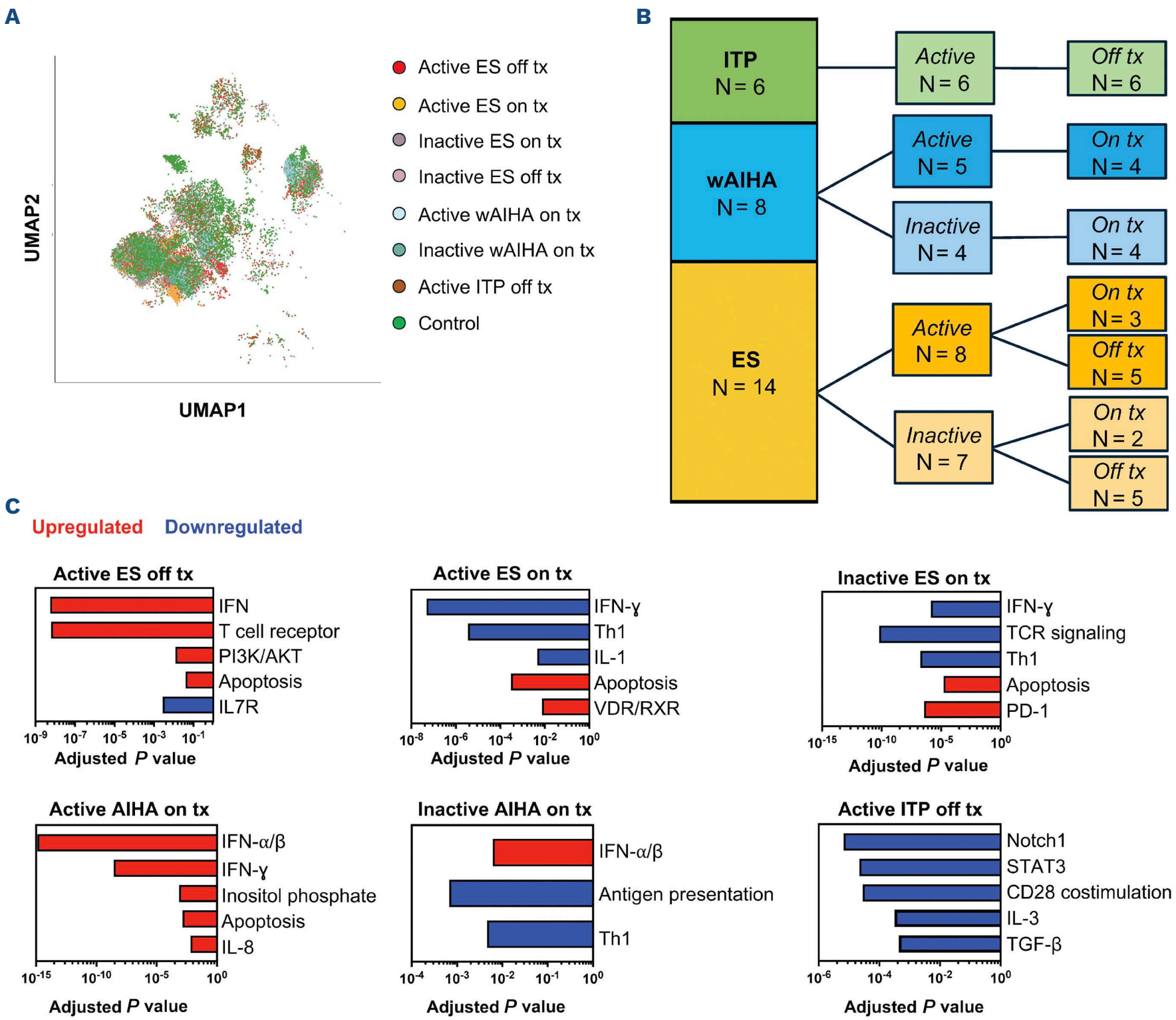

Figure 3. Analysis of transcriptional profiles of circulating T follicular helper cells obtained by single-cell RNA-sequencing. (A) Uniform manifold approximation and projection of circulating T follicular helper (cTfh) cells in patients with the indicated immune cytopenias. (B) Clinical characteristics of the patients studied in this analysis. (C) Pathway analysis of differentially expressed genes (-1.5<fold change<1.5, adjusted P value <0.05) in cTfh cells from the indicated groups of patients compared to controls. UMAP: uniform manifold approximation and projection; ES: Evans syndrome; tx: treatment; wAIHA: warm autoimmune hemolytic anemia; ITP: immune thrombocytopenia; IFN: interferon; PI3K: phosphoinositide 3-kinase; IL7R: interleukin-7 receptor; Th1: T helper 1; IL-1: interleukin-1; VDR/RXR: vitamin D receptor/retinoid X receptor; TCR: T-cell receptor; PD-1: programmed cell death-1; IL-8: interleukin-8; STAT3: signal transducer and activator of transcription 3; CD28: cluster of differentiation 28; IL-3: interleukin-3; TGF-β: transforming growth factor-beta.

circulating CCL2 levels were concordant with the transcriptional signature of IFN-α/β signaling in cTfh cells from patients with wAIHA. We identified no correlation between CCL2 levels and cTfh percentages (Figure 5D), suggesting that cTfh expansion is associated with type II, rather than type I, IFN signaling. There were no significant elevations in the levels of any other cytokines evaluated in patients with ITP, wAIHA, or ES compared to controls (*Online Supplementary Figure S6*).

Discussion

Quantification of cTfh cells is a clinically available test that can aid in diagnosing ES, identifying patients with immune disorders and extra-hematologic autoimmunity, and monitoring immune cytopenia activity. Patients with ES had higher cTfh percentages than those with ITP or wAIHA, and those with active ES had the highest percentages. We found that cTfh cell expansion was also associated with the presence of extra-hematologic autoimmunity and underlying immune disorders, identifying patients with immune cytopenias who would benefit from immunological evaluations. cTfh values decreased with treatment and in patients with improving cytopenias over time, suggesting that cTfh measurement may be useful for clinical monitoring. Transcriptional studies of cTfh cells and circulating cytokine profiles revealed IFN-γ and IFN-α/β as key pathways associated with active ES and wAIHA, respectively, which could be potential therapeutic targets.

Quantification of cTfh percentages can aid in the diagnosis of ES, as a cTfh percentage exceeding 9.5% had 76% sensitivity and 86% specificity for distinguishing ES from ITP or wAIHA. Kumar *et al*. have previously shown that 24 pediatric patients with active ES had increased cTfh percentages compared to 22 patients with chronic ITP and 24 healthy individuals, none of whom was receiving treatment.[18] Our study shows that cTfh percentages distinguish ES from ITP or wAIHA in patients with a spectrum of disease activity and cytopenia-directed treatments. This is clinically relevant because the lack of diagnostic tests for ITP or immune neutropenia, which remain diagnoses of exclusion, often makes it difficult to determine whether a patient with multiple cytopenias has ES or cytopenias of other etiologies.[6,34,35] Given the finding of cTfh cell expansion in patients with ES, cTfh measurement may facilitate the diagnosis of ES in a patient with cytopenias of unclear etiology. Future prospective validation studies using sam-

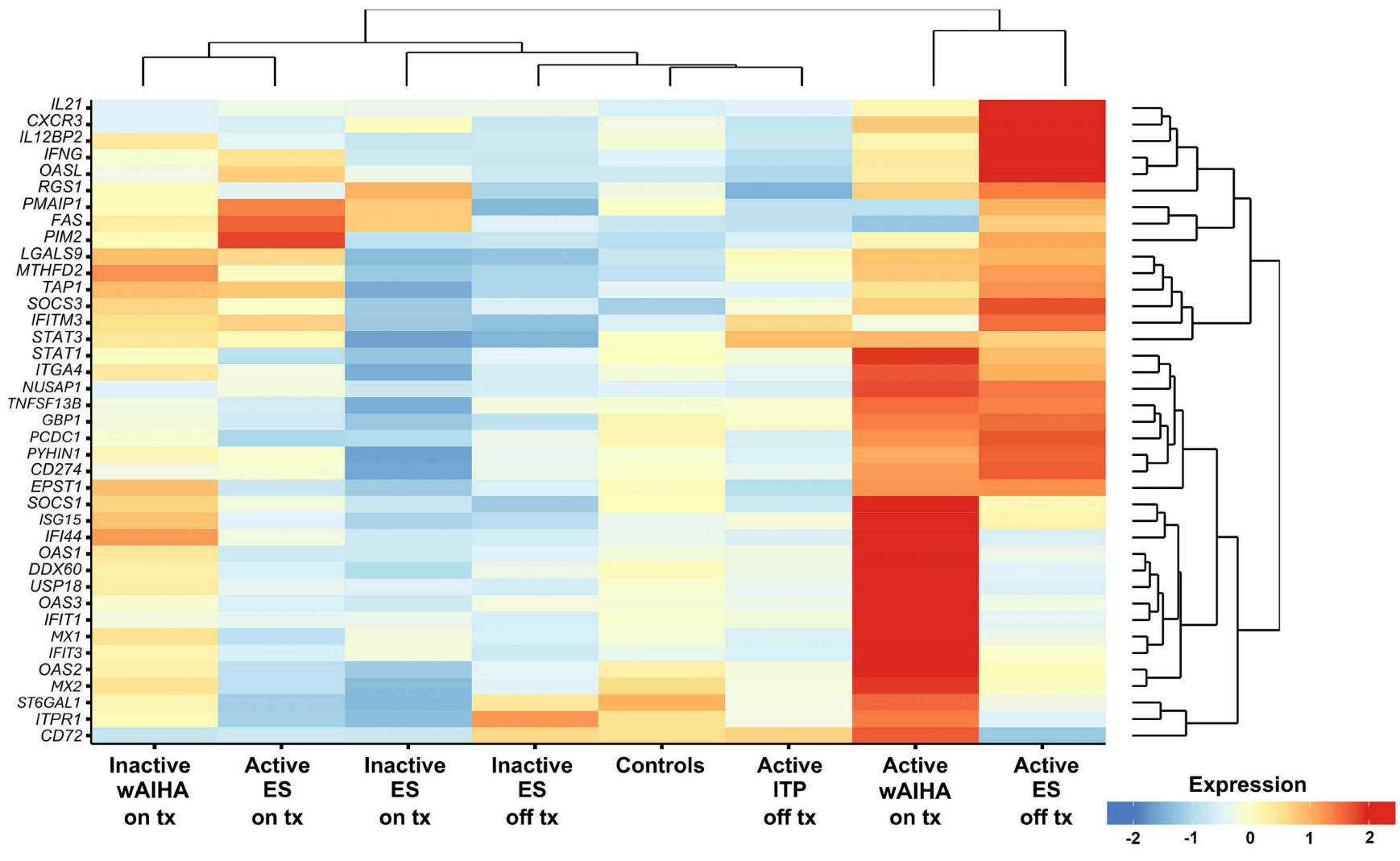

Figure 4. Unsupervised hierarchical clustering of differentially expressed genes in the interferon-α/β and interferon-γ pathways. Patients were categorized based on cytopenia type (Evans syndrome, immune thrombocytopenia, warm autoimmune hemolytic anemia), activity, and treatment. Active indicates patients with active cytopenia present at the time of sample collection. Inactive indicates patients without active cytopenias present at the time of sample collection. wAIHA: warm autoimmune hemolytic anemia; tx: treatment; ES: Evans syndrome; ITP: immune thrombocytopenia.

ples collected at the time of diagnosis will be essential to establish the diagnostic utility of cTfh.

Presence of high cTfh could be integrated into clinical practice as a marker of immune dysregulation. Among patients with ES, cTfh percentages were comparable to those in controls only in patients without active cytopenias off treatment. Longitudinal cTfh measurements normalized in patients with ES with improving disease, suggesting that cTfh normalization may reflect treatment efficacy. Larger cohorts studied longitudinally are needed to establish the clinical utility of cTfh as a biomarker for patient monitoring. cTfh cell percentages can be measured using equipment and techniques readily available in most clinical laboratories with a low reagent cost.[26]

Logistic regression analysis showed that elevated cTfh percentages were also associated with immune disorders, including inborn errors of immunity, secondary immune disorders, and extra-hematologic autoimmunity. It is often not apparent at diagnosis which patients with immune cytopenias have immune disorders, as predictors of underlying immune disorders in pediatric immune cytopenias are lacking.[36] It is consequently difficult to determine which patients require functional immunological testing, genetic testing, and multidisciplinary evaluation for extra-hematologic autoimmunity, which is not currently standard of care for all patients with immune cytopenias. We found that increased percentages of cTfh cells were associated with underlying immune disorders in patients with ITP as well as ES (*Online Supplementary Figure S2*). Therefore, cTfh cell measurements may also identify patients with immune cytopenias who require functional immunological and genetic testing as well as multidisciplinary evaluation for extra-hematologic autoimmunity. Measurement of cTfh cells helps support additional diagnostic testing, including genetic testing, for underlying immune disorders.

There are numerous studies evaluating T cells in patients with ITP that are complementary to the current study (*Online Supplementary Table S3*). Some of these studies identified increased percentages of Tfh and decreased Treg cells in patients with ITP, while others showed no increase in cTfh

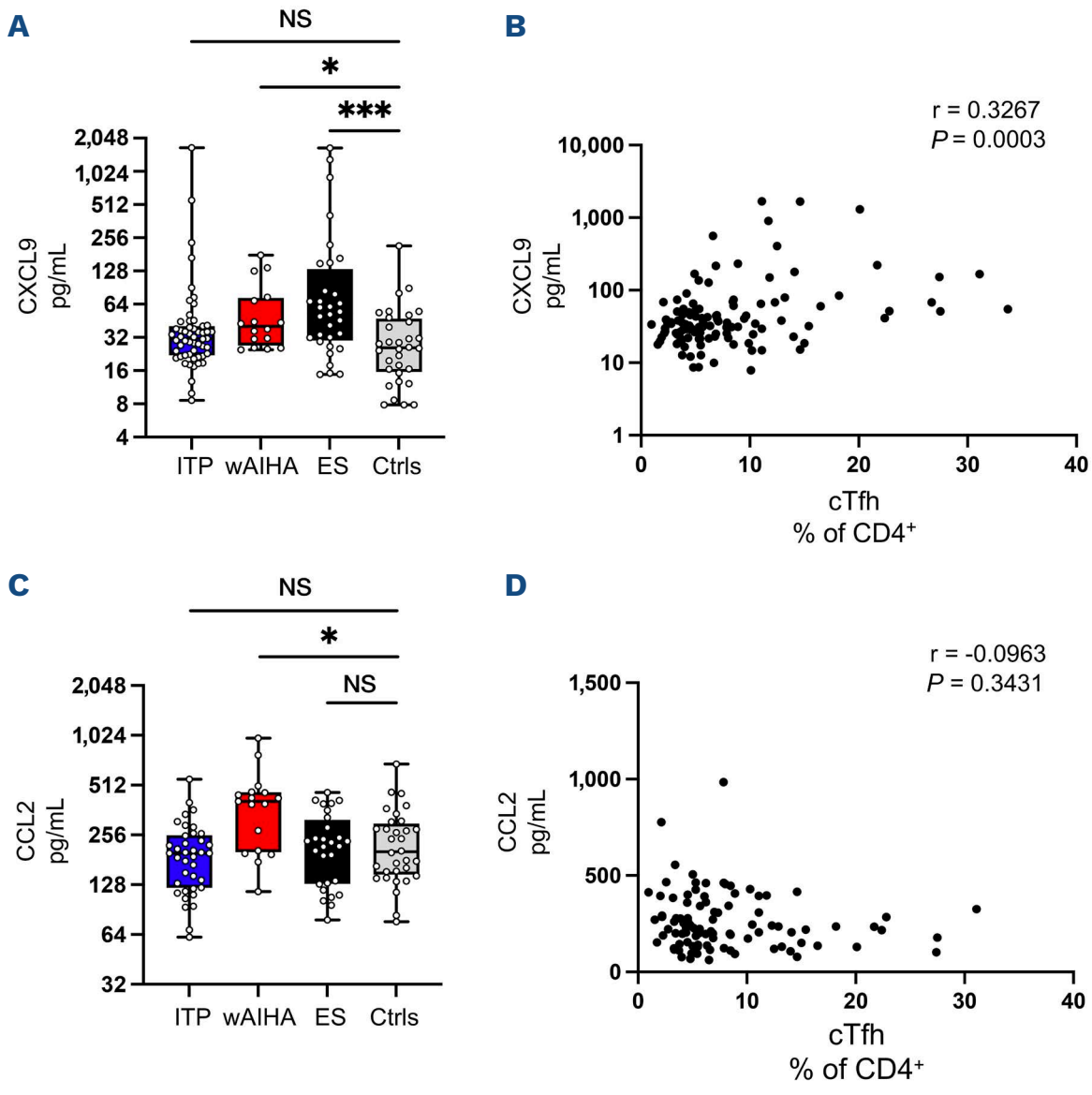

Figure 5. Cytokine levels. (A) C-X-C motif chemokine ligand 9 (CXCL9) levels in plasma samples from patients with immune thrombocytopenia (ITP), warm autoimmune hemolytic anemia (wAIHA), Evans syndrome (ES), and controls (Ctrls); NS=*P*>0.05, *P<0.05, ***P<0.001 by the Kruskal-Wallis test to correct for multiple comparisons. (B) Spearman correlation of CXCL9 levels with circulating T follicular helper cell (cTfh) percentages in all patients and controls. (C) C-C motif chemokine ligand 2 (CCL2) levels in plasma samples from patients with ITP, wAIHA, ES, and controls; *P<0.05 by the Kruskal-Wallis test to correct for multiple comparisons. (D) Spearman correlation of CCL2 levels with cTfh percentages in all patients and controls. NS: not statistically significant; Ctrls: controls.

cells in pediatric patients with ITP.[10,18,21,22,37-40] It is important to note that there are key methodological differences across studies in how cTfh and Treg cells are measured and in the ages of the patient populations studied.[41] The variability in markers used to define cTfh and Treg cells complicates comparisons across studies.[41–44] Furthermore, most prior studies of cTfh cells in immune cytopenias focused on adults with ITP, who have distinct clinical phenotypes from children with ITP[45] and differences in baseline immunity.[46,47] Even within a given age group, there are additional clinical features, such as the presence of antiplatelet antibodies, which may be associated with increased cTfh cells in patients with ITP.[22] In our cohort, patients with ITP who had elevated cTfh cells were those patients who had underlying genetic causes of autoimmunity and/or multisystem autoimmunity.

In addition to differences in cTfh percentages among patients with ES, wAIHA, and ITP, we showed that these disorders can be distinguished by the transcriptional changes in cTfh cells. cTfh cells from patients with active ES off treatment exhibited a gene signature indicative of IFN-γ and IFN-α/β, consistent with increased circulating levels of CXCL9, a chemokine ligand induced by IFN-γ. These findings are concordant with those of Kumar *et al*. who, using targeted transcriptomics of flow-sorted $CD4^+CD127^+CD25^{low}CXCR5^+$ T cells from patients with active untreated ES, showed increased expression of *IFNG* and *CXCL9*, with the latter declining after sirolimus treatment.[18,19] We also found that multiple types of immunomodulatory treatment reduced expression of IFN-γ gene targets, which further declined with improving disease. We note that the correlation between CXCL9 and cTfh percentages was modest, suggesting that IFN-γ may affect other aspects of the cTfh population, including function. However, patients on treatment without active cytopenias still had increased expression of genes indicative of apoptosis and PD-1 signaling, demonstrating that normalization of cTfh percentages does not necessarily indicate normalization of gene expression. Only patients off treatment and without cytopenias had cTfh cell transcriptional profiles comparable to those of controls.

The transcriptional signature of cTfh cells in wAIHA was previously unknown. We identified a robust type I IFN signature in cTfh cells from patients with wAIHA in tandem with increased expression of CCL2, a target of IFN-α. The causal link between type I IFN and AIHA has been previously established by reversible AIHA caused by pegylated IFN-α and by the development of AIHA in patients with type I interferonopathy.[48–51] These findings highlight type I and type II IFN pathways as potential therapeutic targets for the treatment of AIHA and ES, respectively. Future studies are needed to functionally validate the IFN pathway findings and to elucidate the mechanistic relationship between CXCL9 levels and cTfh percentages. Future studies are also needed to determine whether blocking type I or II IFN with JAK inhibitors may be an effective treatment option for patients with wAIHA, regardless of a genetically defined defect impairing regulation of JAK/STAT signaling. In contrast, cTfh cells from patients with ITP had no transcriptional evidence of activation, so additional investigations are needed to identify markers of active ITP beyond thrombocytopenia. We acknowledge that Th1 skewing has been identified in many adult cohorts with ITP.[52-55] However, studies have shown that Th1 skewing in children with ITP is much more variable, with some studies showing that only a minority of children with ITP have Th1 skewing.[56,57]

Monogenic immune disorders, known as inborn errors of immunity, are present in at least 65% of pediatric patients with ES,[58,59] but data regarding the frequency of inborn errors of immunity in children with ITP and wAIHA remain sparse.[60,61] In this cohort, 40.5% of patients with ES, 22.7% of patients with wAIHA and 22.2% of patients with ITP who underwent genetic testing had a positive result. However, these estimates are limited by the lack of uniform genetic testing across all patient groups. Given the high prevalence of inborn errors of immunity in patients with ES,[58,59] standard of care includes genetic and functional immunological testing.[62] In contrast, genetic testing is not routinely offered to patients with wAIHA and ITP, even though both are increasingly recognized in immune disorders.[60,61,63] The relatively high rate of genetic findings in patients with ITP and wAIHA in this cohort suggests that genetic evaluation should also be considered in children with single-lineage immune cytopenias, especially those with increased cTfh cells. Identification of a genetic disorder informs treatment with targeted or curative therapies, screening for extra-hematologic autoimmunity, and risk of malignancy.[64,65]

This study has several limitations. Due to the study design, samples were not all collected at the time of diagnosis. Prospective validation studies at the time of diagnosis are needed to determine whether increased percentages of cTfh cells in a patient with a single-lineage immune cytopenia predict an increased risk of future development of ES and/or extra-hematologic autoimmunity. Additionally, the number of longitudinal samples was limited, so the effect of specific treatments on cTfh over time could not be evaluated. As ES is a rare disease, future multicenter studies are needed for validation of these findings. As we have shown an expansion of cTfh cell percentages and increased IFN-γ signaling in patients with ES, future studies are needed to elucidate the mechanism by which these immunological features contribute to disease activity. Many studies have shown that cTfh cells induce B-cell activation and differentiation into antibody-secreting cells in disorders characterized by a loss of tolerance, including systemic lupus erythematosus, chronic graft-*versus*-host disease, and primary immune regulatory disorders.[66-69] We thus hypothesize that cTfh cells have a similar role in promoting ES disease activity, although future studies are needed to identify the epitopes recognized by autoreactive T and B cells in ES and wAIHA.

In conclusion, this study shows how quantification of cTfh cells can advance clinical characterization of immune cytopenias by identifying disease subtypes as well as patients with genetic and immunological co-morbidities and by indicating changes in disease activity. Although cTfh cells are known to drive B-cell proliferation and lead to autoantibody production, further study is needed to understand why cTfh cell expansion occurs. Our results highlight CCL2 and CXCL9 as two chemokine ligands that can be used as indicators of type I and type II IFN signaling, respectively. We have identified type I and type II IFN signaling as inflammatory pathways upregulated in wAIHA and active ES, respectively, thereby setting the foundation for future studies investigating these pathways as therapeutic targets. As measurements of cTfh, CXCL9, and CCL2 are relatively inexpensive clinically available tests, these results address the urgent need for better diagnostic tools for patients with immune cytopenias.

Disclosures

RFG has received research funding from Novartis, Sobi, and Agios and has provided consultancy for Sobi, Sanofi, and Agios. CM is an employee of Takeda Pharmaceuticals U.S.A. Inc. and holder of Takeda stock/stock options. The remaining authors have no conflicts of interest.

Contributions

EMH, RFG, and JC conceptualized the study. EMH, AB, LM, AC, DW, RH, BL, and CM obtained informed consent. EMH, AB, LM, ME, MN, AC, BL, SN, and SC processed the patients' samples, performed experiments, and contributed to data collection and analysis. EMH, DW, CDP, SC, CM, RFG, and JC contributed to clinical data collection. EMH, ME, SR, PS, TKO, and JC contributed to single-cell RNA sequencing data collection and analysis. EMH, TKO, RFG, and JC verified and analyzed the data and generated figures. EMH, RFG, and JC wrote the initial version of the manuscript. AB, LM, ME, DW, SN, RH, LS, RH, BL, SC, TKO, RFG, and JC provided feedback and revised the manuscript. All authors read and approved the final version.

Acknowledgments

We are grateful to the patients and their families for their participation in this study.

Funding

This work was supported by grants from the National Institutes of Health (T32HL007574 to EMH; T32AI007512 to SC, BL, and JC; K12TR004381 to RH; T32DK007477 to SN; R01DK130465 to JC). Funding for this study was also obtained from the Thrasher Research Fund (EMH), Immune Deficiency Foundation (EMH), Boston Children's Hospital Cell Discovery Network (JC), Perkins Fund (JC), and Lin Family Fund (JC). Whole exome sequencing was done through the Boston Children's Hospital Children's Rare Disease Collaborative.[1,2] The sponsors of this study are public or nonprofit organizations that support science. They were not involved in gathering, analyzing, or interpreting the data. The content of this publication is solely the responsibility of the authors and does not necessarily reflect the official views or policies of the National Institutes of Health or the United States Department of Health and Human Services.

Data-sharing statement

Single-cell RNA-sequencing data have been deposited in the National Center for Biotechnology Information Sequence Read Archive (SRA) with accession number PRJNA1233813. Additional de-identified data will be shared upon reasonable request by email to the corresponding author.

References

1. French CE, Andrews NC, Beggs AH, et al. Hospital-wide access to genomic data advanced pediatric rare disease research and clinical outcomes. NPJ Genom Med. 2024;9(1):60.
2. Rockowitz S, LeCompte N, Carmack M, et al. Children's rare disease cohorts: an integrative research and clinical genomics initiative. NPJ Genom Med. 2020;5:29.
3. Pincez T, Fernandes H, Leblanc T, et al. Long term follow-up of pediatric-onset Evans syndrome: broad immunopathological manifestations and high treatment burden. Haematologica. 2022;107(2):457-466.
4. Barcellini W, Fattizzo B. How I treat warm autoimmune hemolytic anemia. Blood. 2021;137(10):1283-1294.
5. Teachey DT, Lambert MP. Diagnosis and management of autoimmune cytopenias in childhood. Pediatr Clin North Am. 2013;60(6):1489-1511.
6. Arnold DM, Nazy I, Clare R, et al. Misdiagnosis of primary immune thrombocytopenia and frequency of bleeding: lessons from the McMaster ITP Registry. Blood Adv. 2017;1(25):2414-2420.
7. Bu S, Liu M, Yang L, et al. The function of T cells in immune thrombocytopenia. Front Immunol. 2025;16:1499014.
8. Li Q, Marcoux G, Hu Y, et al. Autoimmune effector mechanisms associated with a defective immunosuppressive axis in immune thrombocytopenia (ITP). Autoimmun Rev. 2024;23(12):103677.
9. Audia S, Mahévas M, Nivet M, Ouandji S, Ciudad M, Bonnotte B. Immune thrombocytopenia: recent advances in pathogenesis and treatments. Hemasphere. 2021;5(6):e574.
10. Crotty S. T follicular helper cell biology: a decade of discovery and diseases. Immunity. 2019;50(5):1132-1148.
11. Caldirola MS, Martínez MP, Bezrodnik L, Zwirner NW, Gaillard MI. Immune monitoring of patients with primary immune regulation disorders unravels higher frequencies of follicular T cells with different profiles that associate with alterations in B cell subsets. Front Immunol. 2020;11:576724.
12. Wei X, Niu X. T follicular helper cells in autoimmune diseases. J Autoimmun. 2023;134:102976.
13. Harris EM, Chamseddine S, Chu A, et al. T cell and autoantibody profiling for primary immune regulatory disorders. medRxiv. 2025 Jan 27. doi: 10.1101/2024.02.25.24303331 [preprint, not

peer-reviewed].
14. Godefroy E, Zhong H, Pham P, Friedman D, Yazdanbakhsh K. TIGIT-positive circulating follicular helper T cells display robust B-cell help functions: potential role in sickle cell alloimmunization. Haematologica. 2015;100(11):1415-1425.
15. Bogaert DJA, De Bruyne M, Debacker V, et al. The immunophenotypic fingerprint of patients with primary antibody deficiencies is partially present in their asymptomatic first-degree relatives. Haematologica. 2017;102(1):192-202.
16. Ma N, Wu WB, Zhao XY, et al. Targeting TFH cells is a novel approach for donor-specific antibody desensitization of allograft candidates: an in vitro and in vivo study. Haematologica. 2024;109(4):1233-1246.
17. Dobson R, Du PY, Rásó-Barnett L, et al. Early detection of T-cell lymphoma with T follicular helper phenotype by RHOA mutation analysis. Haematologica. 2021;107(2):489-499.
18. Kumar D, Prince C, Bennett CM, et al. T-follicular helper cell expansion and chronic T-cell activation are characteristic immune anomalies in Evans syndrome. Blood. 2022;139(3):369-383.
19. Kumar D, Nguyen TH, Bennett CM, et al. mTOR inhibition attenuates cTfh cell dysregulation and chronic T-cell activation in multilineage immune cytopenias. Blood. 2023;141(3):238-243.
20. Chen Z, Zheng Q, Wang Y, et al. CXCL13/CXCR5 axis facilitates TFH expansion and correlates with disease severity in adults with immune thrombocytopenia. Thromb Res. 2024;244:109196.
21. Yao X, Li C, Yang J, Wang G, Li C, Xia Y. Differences in frequency and regulation of T follicular helper cells between newly diagnosed and chronic pediatric immune thrombocytopenia. Blood Cells Mol Dis. 2016;61:26-36.
22. Xie J, Cui D, Liu Y, et al. Changes in follicular helper T cells in idiopathic thrombocytopenic purpura patients. Int J Biol Sci. 2015;11(2):220-229.
23. Audia S, Rossato M, Santegoets K, et al. Splenic TFH expansion participates in B-cell differentiation and antiplatelet-antibody production during immune thrombocytopenia. Blood. 2014;124(18):2858-2866.
24. Bonilla FA, Barlan I, Chapel H, et al. International Consensus Document (ICON): common variable immunodeficiency disorders. J Allergy Clin Immunol Pract. 2016;4(1):38-59.
25. Imdadullah M, Aslam M, Altaf S. mctest: an R package for detection of collinearity among regressors. The R Journal. 2016;8(2):499-509.
26. LaBere B, Nguyen AA, Habiballah SB, et al. Clinical utility of measuring CD4+ T follicular cells in patients with immune dysregulation. J Autoimmun. 2023;140:103088.
27. Granel J, Fernandes H, Bader-Meunier B, et al. Antinuclear antibody-associated autoimmune cytopenia in childhood is a risk factor for systemic lupus erythematosus. Blood. 2024;143(16):1576-1585.
28. Kargar M, Torabizadeh M, Purrahman D, Zayeri ZD, Saki N. Regulatory factors involved in Th17/Treg cell balance of immune thrombocytopenia. Curr Res Transl Med. 2023;71(2):103389.
29. Fry TJ, Mackall CL. The many faces of IL-7: from lymphopoiesis to peripheral T cell maintenance. J Immunol. 2005;174(11):6571-6576.
30. Kim J, Lee H, Lee JE, et al. Liver X receptor controls follicular helper T cell differentiation via repression of TCF-1. Proc Natl Acad Sci U S A. 2023;120(9):e2213793120.
31. Gschwandtner M, Derler R, Midwood KS. More than just attractive: how CCL2 influences myeloid cell behavior beyond chemotaxis. Front Immunol. 2019;10:2759.
32. Deshmane SL, Kremlev S, Amini S, Sawaya BE. Monocyte chemoattractant protein-1 (MCP-1): an overview. J Interferon Cytokine Res. 2009;29(6):313-326.
33. Lehmann MH, Torres-Domínguez LE, Price PJR, Brandmüller C, Kirschning CJ, Sutter G. CCL2 expression is mediated by type I IFN receptor and recruits NK and T cells to the lung during MVA infection. J Leukoc Biol. 2016;99(6):1057-1064.
34. Newburger PE. Autoimmune and other acquired neutropenias. Hematology Am Soc Hematol Educ Program. 2016;2016(1):38-42.
35. Vrbensky JR, Moore JE, Arnold DM, Smith JW, Kelton JG, Nazy I. The sensitivity and specificity of platelet autoantibody testing in immune thrombocytopenia: a systematic review and meta-analysis of a diagnostic test. J Thromb Haemost. 2019;17(5):787-794.
36. Grace RF, Lambert MP. An update on pediatric ITP: differentiating primary ITP, IPD, and PID. Blood. 2022;140(6):542-555.
37. Sun M, Wang X, Zhang N, et al. Imbalance of follicular regulatory T (Tfr) cells/follicular helper T (Tfh) cells in adult patients with primary immune thrombocytopenia. Exp Biol Med (Maywood). 2023;248(11):959-965.
38. Sun M, Wang X, Wang L, et al. The role of follicular regulatory T cells/follicular helper T cells in primary immune thrombocytopenia. Acta Haematol. 2023;146(4):267-276.
39. Audia S, Rossato M, Trad M, et al. B cell depleting therapy regulates splenic and circulating T follicular helper cells in immune thrombocytopenia. J Autoimmun. 2017;77:89-95.
40. Dai L, He L, Wang Z, et al. Altered circulating T follicular helper cells in patients with chronic immune thrombocytopenia. Exp Ther Med. 2018;16(3):2471-2477.
41. Chamseddine S, Harris EM, Chou J. T follicular helper cells in primary immune regulatory disorders. J Allergy Clin Immunol. 2025;156(6):1465-1476.
42. Nishimoto T, Kuwana M. CD4+CD25+Foxp3+ regulatory T cells in the pathophysiology of immune thrombocytopenia. Semin Hematol. 2013;50(suppl 1):S43-49.
43. Elsayh KI, Saad K, Osman NS, et al. Regulatory T-lymphocyte subsets in children with chronic immune thrombocytopenia after high-dose of dexamethasone. Pediatr Res. 2022;92(5):1432-1436.
44. Bao W, Bussel JB, Heck S, et al. Improved regulatory T-cell activity in patients with chronic immune thrombocytopenia treated with thrombopoietic agents. Blood. 2010;116(22):4639-4645.
45. Despotovic JM, Grimes AB. Pediatric ITP: is it different from adult ITP? Hematology. 2018;2018(1):405-411.
46. Forconi CS, Nixon C, Wu HW, et al. T-follicular helper cell profiles differ by malaria antigen and for children compared to adults. Elife. 2025;13:RP98462.
47. Chou J, Thomas PG, Randolph AG. Immunology of SARS-CoV-2 infection in children. Nat Immunol. 2022;23(2):177-185.
48. Cauli C, Serra G, Chessa L, et al. Severe autoimmune hemolytic anemia in a patient with chronic hepatitis C during treatment with peginterferon alfa-2a and ribavirin. Haematologica. 2006;91(6 Suppl):ECR26.
49. Wang S, Qin E, Zhi Y, Hua R. Severe autoimmune hemolytic anemia during pegylated interferon plus ribavirin treatment for chronic hepatitis C: a case report. Clin Case Rep. 2017;5(9):1490-1492.
50. Andriani A, Bibas M, Callea V, et al. Autoimmune hemolytic anemia during alpha interferon treatment in nine patients with hematological diseases. Haematologica. 1996;81(3):258-260.
51. Frémond ML, Hully M, Fournier B, et al. JAK inhibition in Aicardi-Goutières syndrome: a monocentric multidisciplinary real-world approach study. J Clin Immunol. 2023;43(6):1436-1447.
52. Georgi JA, Middeke JM, Bornhäuser M, Matzdorff A, Trautmann-Grill K. Deciphering the genetic basis of immune thrombocytopenia: current evidence for genetic predisposition

in adult ITP. Blood Adv. 2023;7(14):3710-3724.
53. Ma D, Zhu X, Zhao P, et al. Profile of Th17 cytokines (IL-17, TGF-β, IL-6) and Th1 cytokine (IFN-γ) in patients with immune thrombocytopenic purpura. Ann Hematol. 2008;87(11):899-904.
54. Rocha AMC, Souza C, Rocha GA, et al. The levels of IL-17A and of the cytokines involved in Th17 cell commitment are increased in patients with chronic immune thrombocytopenia. Haematologica. 2011;96(10):1560-1564.
55. Zhu X, Ma D, Zhang J, et al. Elevated interleukin-21 correlated to Th17 and Th1 cells in patients with immune thrombocytopenia. J Clin Immunol. 2010;30(2):253-259.
56. Mouzaki A, Theodoropoulou M, Gianakopoulos I, Vlaha V, Kyrtsonis MC, Maniatis A. Expression patterns of Th1 and Th2 cytokine genes in childhood idiopathic thrombocytopenic purpura (ITP) at presentation and their modulation by intravenous immunoglobulin G (IVIg) treatment: their role in prognosis. Blood. 2002;100(5):1774-1779.
57. Semple J, Milev Y, Cosgrave D, et al. Differences in serum cytokine levels in acute and chronic autoimmune thrombocytopenic purpura: relationship to platelet phenotype and antiplatelet T-cell reactivity. Blood. 1996;87(10):4245-4254.
58. Hadjadj J, Aladjidi N, Fernandes H, et al. Pediatric Evans syndrome is associated with a high frequency of potentially damaging variants in immune genes. Blood. 2019;134(1):9-21.
59. Amaya-Uribe L, Rojas M, Azizi G, Anaya JM, Gershwin ME. Primary immunodeficiency and autoimmunity: a comprehensive review. J Autoimmun. 2019;99:52-72.
60. Joshi N, Lango Allen H, Downes K, et al. The role of genetic sequencing in the diagnostic work-up for chronic immune thrombocytopenia. Blood Adv. 2025;9(7):1497-1507.
61. Grace RF, Steele M, Kalashnikova T, et al. Pediatric autoimmune hemolytic anemia is associated with a high incidence of an underlying immune disorder and high mortality rate. Blood. 2024;144(Supplement 1):795.
62. Kim TO, Despotovic JM. Primary and secondary immune cytopenias: evaluation and treatment approach in children. Hematol Oncol Clin North Am. 2019;33(3):489-506.
63. Kim TO, Geris JM, Flanagan JM, et al. Genetic variants in canonical Wnt signaling pathway associated with pediatric immune thrombocytopenia. Blood Adv. 2024;8(21):5529-5538.
64. von Hardenberg S, Klefenz I, Steinemann D, et al. Current genetic diagnostics in inborn errors of immunity. Front Pediatr. 2024;12:1279112.
65. Chan AY, Torgerson TR. Primary immune regulatory disorders: a growing universe of immune dysregulation. Curr Opin Allergy Clin Immunol. 2020;20(6):582-590.
66. Forcade E, Kim HT, Cutler C, et al. Circulating T follicular helper cells with increased function during chronic graft-versus-host disease. Blood. 2016;127(20):2489-2497.
67. Gao X, Luo K, Wang D, et al. T follicular helper 17 (Tfh17) cells are superior for immunological memory maintenance. Elife. 2023;12:e82217.
68. Tchen J, Simon Q, Chapart L, et al. PD-L1- and IL-4-expressing basophils promote pathogenic accumulation of T follicular helper cells in lupus. Nat Commun. 2024;15(1):3389.
69. Tangye SG, Ma CS. Molecular regulation and dysregulation of T follicular helper cells - learning from inborn errors of immunity. Curr Opin Immunol. 2021;72:249-261.
70. Dolan M, Mendelsohn NJ, Pierpont ME, Schimmenti LA, Berry SA, Hirsch B. A novel microdeletion/microduplication syndrome of 19p13.13. Genet Med. 2010;12(8):503-511.
71. Seidel MG, Duerr C, Woutsas S, et al. A novel immunodeficiency syndrome associated with partial trisomy 19p13. J Med Genet. 2014;51(4):254-263.
72. Bianchi C, Margot H, Fernandes H, et al. Autoimmune cytopenia and Kabuki syndrome in paediatrics: insights in 11 patients. Br J Haematol. 2024;204(5):1899-1907.
73. Rossini L, Ricci S, Montin D, et al. Immunological aspects of Kabuki syndrome: a retrospective multicenter study of the Italian Primary Immunodeficiency Network (IPINet). J Clin Immunol. 2024;44(5):105.