Trisomy 21 dysregulates T cell lineages toward an autoimmunity-prone state associated with interferon hyperactivity

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T21 show elevated levels of basal IFN signaling and hypersensitivity to IFN-inducible phosphoepitopes demonstrates that T cell subsets with DS are depleted of naïve subsets and enriched for differentiated subsets, express higher levels of markers of activation and senescence (e.g., IFN-γ, Granzyme B, PD-1, KLRG1), and overproduce cytokines tied to autoimmunity (e.g., TNF-α). Conventional CD4+ T cells display increased differentiation, polarization toward the Th1 and Th17 states, and overproduction of the autoimmunity-related cytokines IL-17A and IL-22. Plasma cytokine analysis confirms the Th1 and Th1/17 states, and overproduction of the cytokines correlated positively with IFN hyperactivity. Finally, mass cytometry analysis of 8 different cell types from people with and without DS, including T cells, demonstrated that T21 causes a consistent activation of the IFN transcriptional response (9). Additionally, plasma proteomics analyses demonstrated that people with DS have signs of chronic autoinflammation, including elevated levels of proinflammatory cytokines tied to autoimmunity (e.g., TNF-α, IL-17A, and IL-22). Plasma cytokine analysis confirms the presence of multiple autoimmunity-related cytokines (e.g., TNF-α, IL-17A–D, IL-22) in people with DS, independent of diagnosis of autoimmunity. Although Tregs are more abundant in DS, functional assays show that CD8+ and CD4+ effector T cells from T21 are resistant to Treg-mediated suppression, regardless of Treg karyotype. Transcriptome analysis of white blood cells and T cells reveals strong signatures of T cell differentiation and activation that correlate positively with IFN hyperactivity. Finally, mass cytometry analysis of 8 IFN-inducible phosphoepitopes demonstrates that T cell subsets with T21 show elevated levels of basal IFN signaling and hypersensitivity to IFN-α stimulation. Therefore, these results point to T cell dysregulation associated with IFN hyperactivity as a contributor to autoimmunity in DS.

Significance

Triplication of human chromosome 21, or trisomy 21 (T21), causes the condition known as Down syndrome (DS). People with DS show a markedly different disease spectrum relative to typical people, being highly predisposed to conditions such as Alzheimer’s disease, while being protected from other conditions, such as most solid malignancies. Interestingly, people with DS are affected by high rates of autoimmune disorders, whereby the immune system mistakenly attacks healthy tissues. This manuscript reports an exhaustive characterization of the T cells of people with DS, demonstrating many alterations in this key immune cell type that could explain their high risk of autoimmunity. These results reveal opportunities for therapeutic intervention to modulate T cell function and improve health outcomes in DS.

D own syndrome (DS) is caused by trisomy 21 (T21), the most common chromosomal abnormality in the human population, affecting ∼1 in 700 newborns (1). Besides developmental delays and highly variable cognitive deficits, T21 causes an altered disease spectrum, wherein people with DS are protected from certain diseases, such as most solid malignancies (2), but highly predisposed to others, such as Alzheimer’s disease (AD) (3). People with T21 are highly predisposed to develop a specific spectrum of autoimmune conditions, including autoimmune thyroid disease, celiac disease, type 1 diabetes, and a range of autoimmunity and autoimmune-inflammatory skin conditions (4–6). With the exception of AD, the high prevalence of which is due to the presence of the amyloid precursor protein gene (APP) on chromosome 21 (chr21), the molecular and cellular bases of this different disease spectrum are unclear.

Several genes involved in immune control are encoded on chr21, including 4 of the 6 interferon (IFN) receptor subunits (IFNRs): the 2 type I IFNRs (IFNAR1, IFNAR2), one type II IFN (IFNGR2), and IL10RB, which encodes a subunit of the receptors for type III IFN ligands, IL-10, IL-22, and IL-26 (7). The role of IFN signaling in development of autoimmunity is well documented, with many genome-wide association studies revealing strong genetic associations between polymorphisms in components of the IFN pathway and autoimmune disorders (reviewed in ref. 8). Recently, transcriptome analysis of 4 different cell types from people with and without DS, including T cells, demonstrated that T21 causes a consistent activation of the IFN transcriptional response (9). Additionally, plasma proteomics analyses demonstrated that people with DS have signs of chronic autoinflammation, including elevated levels of proinflammatory cytokines tied to autoimmunity (e.g., TNF-α, IL-17A, and IL-22). Plasma cytokine analysis confirms the presence of multiple autoimmunity-related cytokines (e.g., TNF-α, IL-17A–D, IL-22) in people with DS, independent of diagnosis of autoimmunity. Although Tregs are more abundant in DS, functional assays show that CD8+ and CD4+ effector T cells from T21 are resistant to Treg-mediated suppression, regardless of Treg karyotype. Transcriptome analysis of white blood cells and T cells reveals strong signatures of T cell differentiation and activation that correlate positively with IFN hyperactivity. Finally, mass cytometry analysis of 8 IFN-inducible phosphoepitopes demonstrates that T cell subsets with T21 show elevated levels of basal IFN signaling and hypersensitivity to IFN-α stimulation. Therefore, these results point to T cell dysregulation associated with IFN hyperactivity as a contributor to autoimmunity in DS.

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PNAS Latest Articles | 1 of 11
cytokines, such as IL-6, TNF-α, MCP-1, and IL-22 (10). Thus, it is clear that people with DS display signs of chronic inflammation, even in the absence of diagnosed autoimmune disorders.

Less is known about alterations in cellular immunity that could contribute to the high prevalence of autoimmune disorders and the chronic inflammatory state in DS. Histopathologic studies of thymic tissue from people with DS showed hypoplasia and compromised architecture, even in newborns (11, 12), which could explain the lower thymic output of mature αβ+ thymocytes observed in DS (13). This decreased thymic output is consistent with decreased naïve T cell percentages; however, so is the reported increase in central memory and terminally differentiated T lymphocytes (13–17). Although it has been shown that individuals with T21 exhibit increased CD8/CD4 ratios and higher frequencies of regulatory CD4+ T cells (Tregs) in peripheral blood (17–20), characterization of CD8+ and CD4+ T cell subsets has been limited to the expression of a few phenotypic markers in the pediatric population (14, 17, 19, 21).

In this study, we used multiparametric, high-dimensional flow cytometry to comprehensively characterize the peripheral T cell compartment of individuals with DS by measuring the expression of a large panel of phenotypic markers. We also performed functional assays, cytokine measurements, and transcriptome analyses. We found that CD8+ T cells from individuals with T21 express markers of activated and senescent states and respond to stimulation more potently than their euploid counterparts, overproducing autoimmune-related cytokines. Conventional CD4+ T cells (Tconvs) show a polarized state toward increased production of IL-17A, and plasma levels of IL-17A, IL-17B, IL-17C, and IL-17D are elevated in DS. Interestingly, we found that Tregs from individuals with T21 show neither significant phenotypic differences nor impaired functional capacity relative to control Tregs. Indeed, our results from allogeneic “criss-cross” experiments indicate that the functional deficiency previously reported for Tregs with T21 is not cell-autonomous (18). Instead, we find that T21 CD8+ T and CD4+ Tconvs cells are resistant to Treg-mediated suppression, regardless of Treg karyotype. Transcriptome analysis of bulk white blood cells (WBCs) and T cells revealed gene expression signatures indicative of T cell differentiation and activation which correlated positively with IFN activity. Finally, mass cytometry experiments revealed that T cells of people with DS display both elevated basal IFN signaling and hyperactivation upon IFN-α stimulation. Altogether, these results reveal that multiple alterations observed in the T cell compartment of adults with T21 mimic those observed in autoimmune conditions, even in the absence of clinical manifestation of autoimmunity, concurrent with IFN hyperactivity.

Results

Trisomy 21 Reshapes the Peripheral T Cell Compartment away from the Naive State. In order to investigate the impact of T21 on the peripheral T cell compartment, we used flow cytometry to define alterations in the numbers and/or frequencies of different functional subsets of T cells present in blood from adult individuals, including total T cells (CD3+), CD8+, CD4+ Tconvs, and Tregs, as well as a plethora of phenotypic markers (Dataset S1) (22). First, we analyzed the numbers and frequencies of major T cell subsets in greater detail by reducing the flow cytometry of antigen-induced activation. To obtain an overview of the T cell subsets in people with DS, we reduced the flow cytometry data to 2 dimensions by applying the t-distributed stochastic neighbor embedding (t-SNE) algorithm, where we considered the differential expression of 12 parameters, including surface markers, transcription factors, and signaling and activation molecules (SI Appendix, Fig. S2 A and B). These analyses were performed in conjunction with Flow Self-Organizing Maps (FlowSOM) clustering (25), which resolved 9 T cell subpopulations: potentially naïve (PNa, CD45RA+ CCR7+ cells, which include true naïve and stem cell memory-like T cells or TSCMs), central memory (TCM), effector memory (TEM), and terminally differentiated effector (TEMRA) CD8+ T cells; PNa and memory CD4+ Tconvs; Tregs; double-positive CD4+/CD8+ T cells; and non-CD4+/CD8+ T cells (Fig. 1D and SI Appendix, Fig. S2B). The most prominent change among CD3+ T cells in people with T21 was a reduction in the proportion of PNa cells among CD8+ and CD4+ T cells, concurrent with an increase in the frequency of TEMRA CD8+ T cells (Fig. 1E). We next analyzed the CD8+ and CD4+ T cell subsets in greater detail by manual gating using surface markers to discriminate the following 5 subpopulations (SI Appendix, Fig. S2C): truly naïve, TSCM, TCM, TEM, and TEMRA (26). This exercise confirmed that, among total CD8+ T cells, people with DS have lower levels of naïve CD8+ T cells and higher levels of TEM and TEMRA populations (Fig. 1F). When analyzed in terms of frequency among all CD8+ T cells, TSCMs were not found to be different; however, when calculated as a fraction of all CD45RA+ cells, TSCMs were found to be more common identified as TSCMs in the literature (27), there is an increase in TSCMs in people with DS (Fig. 1F). This discrepancy can be explained by the fact that the increase in TSCMs occurs in the background of decreased CD45RA+ CCR7+ cells (SI Appendix, Fig. S2D). Among CD4+ T cells, there was a similar depletion of naïve cells and an increase in the TCM population along with an increase in TSCMs among PNa CD4+ T cells (Fig. 1G). Next, we investigated alterations in Tregs by measuring expression of FOXP3, a key transcription factor in their development and function (28, 29). Since FOXP3+ T cells may not be functionally homogeneous, due to the transient expression of FOXP3 during activation of Tconvs, we used the expression of CD45RA and FOXP3 to distinguish 3 phenotypically distinct subpopulations (30): FOXP3+ non-Tregs (CD45RA-/FOXP3low), PNa Tregs (CD45RA+/FOXP3low), and effector Tregs (CD45RA-/FOXP3high) (SI Appendix, Fig. S2E). Although there is no re-distribution among the PNa and effector Tregs, effector Tregs express more FOXP3 in people with DS (SI Appendix, Fig. S2F). As expected, effector Tregs express the most FOXP3 relative to the other populations (SI Appendix, Fig. S2F), showing the classical CD25high CD127low phenotype and producing the lowest levels of IFN-γ (SI Appendix, Fig. S2G).

Altogether, these results indicate that T21 disrupts peripheral T cell homeostasis, affecting the frequencies and total numbers of key T cell subtypes, thus justifying a deeper characterization of these populations.


**Trisomy 21 Drives CD8+ T Cell Differentiation toward a Hyperactivated State.** Having observed an increase in both frequency and number of total CD8+ T cells in people with DS, along with a redistribution away from the naïve state, we investigated the expression of markers of activation, proliferation, and senescence in this T cell subset (31). First, we evaluated the levels of granzyme B (GZMB), IFN-γ, and TNF-α after stimulating CD8+ T cells with phorbol 12-myristate 13-acetate (PMA)/Ionomycin and found that all 3 effector markers were increased fraction in TSCMs, even TEMRAs showed increases in the CD45RO and CCR7 marker expression (PNa, TCM, TEM, and TEMRA) (Fig. 2A). Moreover, the T-bet/EOMES ratio is higher in DS, consistent with an enhanced effector phenotype (Fig. 2G).

Next, we examined cytokines produced by CD8+ T cells in vitro upon stimulation with anti-CD3/CD28. Remarkably, 28 of the 29 cytokines detected were more abundant in the supernatant of T21 CD8+ T cells, 10 of them significantly (Fig. 2D, SI Appendix, Fig. S3D, and Dataset S3). We observed elevated levels of cytokines known to be produced by CD8+ T cells, including TNF-α, IFN-γ, IL-2, and MIP-1α (32–35). Notably, overproduction of GM-CSF by CD8+ T cells has been observed in autoimmune conditions (36), and overproduction of antiinflammatory IL-10 is indicative of negative feedback within this subset (37). Others and we have observed elevation of many of the cytokines overproduced by CD8+ T cells in the plasma of people with DS, including TNF-α, IL-2, MIP-1α, IL-10, Eotaxin, and IL-8 (SI Appendix, Fig. S3E) (10, 38). However, extended analysis of cytokine profiles of people with DS revealed no differences between those with diagnoses of autoimmune conditions and those without (Dataset S2 and SI Appendix, Fig. S3F).

Lastly, we measured the expression of the transcription factors T-bet (T-box 21, TBX21) and Eomesodermin (EOMES), which play well-recognized roles in CD8+ T cell differentiation, senescence, and exhaustion (39). In CD8+ T cells, T-bet is up-regulated upon activation and is associated with induction of effector functions, including cytotoxicity, whereas EOMES expression is higher in long-term, stable, self-renewing memory CD8+ T cells, and has also been associated with a dysfunctional phenotype (39). We observed that the frequency of T-bet+/EOMES+ CD8+ T cells is increased in people with DS (Fig. 2E). We also examined how the expression of T-bet and EOMES associated with effector markers of CD8+ T cell function and found an increase in IFN-γ+ TNF-α+ T-bet+/EOMES+ CD8+ T cells in people with DS (Fig. 2F). Moreover, the T-bet/EOMES ratio is higher in DS, consistent with an enhanced effector phenotype (Fig. 2G).

**CD8+ T Cells from People with DS Show Signs of Increased Senescence.** The fact that people with DS showed an increase in differentiated CD8+ T cell subsets prompted us to determine whether these cells had acquired a senescent phenotype, as is observed in chronic inflammatory settings (40). To test this, we measured expression of the inhibitory receptors PD-1, TIM-3, and BTLA, and the senescence markers CD57 and KLRG1. Samples with T21 showed an increased frequency of CD8+ T cells expressing PD-1 and TIM-3 (Fig. S4A), which is expressed by most naïve CD8+ T cells (41). Next, we investigated coexpression of these receptors and found a greater proportion of CD8+ T cells coexpressing all 3 inhibitory receptors in T21 samples (Fig. 3B). Individuals with DS also have a higher percentage of CD8+ T cells positive for CD57 and KLRG1 (Fig. 3C), as well as higher numbers of cells expressing both markers (Fig. 3D). Of note is that individuals with T21 also exhibit a higher frequency of KLRG1+ CD57– CD8+ T cells, again indicative of an activated state (42) (Fig. 3D). Importantly, many of these increases in senescence markers were also observed when comparing phenotypically matched subsets of CD8+ T cells (Fig. 3E and SI Appendix, Fig. S4 B and C).
In the CD8+ T cell response, the effector state precedes the memory state and may divert to a senescent phenotype upon chronic stimulation. Accordingly, cells can exist in an intermediate state where they express both activation and inhibitory/senescent markers. When we measured coexpression of these markers, samples with T21 showed an elevated frequency of cells that coexpress IFN-γ or TNF-α with PD-1, KLRG1, and CD57 (Fig. 3F and SI Appendix, Fig. S4D). Finally, we measured the expression of T-bet and EOMES in CD57+ and KLRG1+ CD8+ T cells, which revealed that adults with DS exhibit increased T-bet (but not EOMES) in KLRG1+ CD57+ and KLRG1+ CD57− CD8+ T cells (SI Appendix, Fig. S4E). This intermediate phenotype has been associated with senescence of CD8+ T cells in aged individuals (43). Overall, these findings indicate that CD8+ T cells from people with DS show signs of differentiation toward activated and senescent states. However, upon ex vivo stimulation, they remain...
cytokine IL-21 was not detected in these assays (Dataset S3). We found no differences in IL-17 isoforms or IL-22 between people with DS or without a confirmed diagnosis of an autoimmune condition (SI Appendix, Fig. S5C). Thus, dysregulation of the CD4+ compartment could also contribute to the autoimmune-related cytokine profile of DS.

**Conventional T Cells from People with Trisomy 21 Are Polarized toward the Th1 and Th1/17 States.** Given our findings that individuals with T21 show anomalies in the distribution of naïve/memory CD4+ T cells (Fig. 1G), we next investigated their polarization into biologically distinct subsets by evaluating the expression of chemokine receptors (SI Appendix, Fig. S5A). Th1 and Th1/17 subsets were enriched among CD4+ Tconv from people with T21, with no differences in Th17, Th2, and Th17 subsets (Fig. 4A). Evaluation of cytokines produced by CD4+ Tconv stimulated in vitro with anti-CD3/CD28 revealed elevated levels of IL-10, IL-17A, IL-22, and MIP-3α in T21 samples (Fig. 4B, SI Appendix, Fig. SSB, and Dataset S3). Other IL-17 isoforms were below detection limits in these assays (Dataset S3). The increased levels of IL-10 are consistent with self-regulation within this subset (44), and the elevated levels of IL-17A and IL-22 are consistent with the observed increase in the Th1/17 subset (45, 46). The increase in MIP-3α (CCL20, the ligand for CCR6) is indicative of increased CCR6-dependent signaling, a hallmark of the Th1/17 and Th17 subsets (45, 47). Differences in Th1 cytokines, such as TNF-α and IFN-γ, did not reach statistical significance (Fig. 4B). Of note is that the Th1 cytokine IL-21 was not detected in these assays (Dataset S3). We next investigated plasma levels of several IL-17 isoforms, IL-22, MIP-3α, and IL-21, which revealed increases in IL-17A, IL-17B, IL-17C, IL-17D, IL-22, and MIP-3α, but not in IL-17E, IL-17F, and IL-21 in people with DS (Fig. 4C and Dataset S3). We found no differences in IL-17 isoforms or IL-22 between people with DS or without a confirmed diagnosis of an autoimmune condition (SI Appendix, Fig. S5C). Thus, dysregulation of the CD4+ compartment could also contribute to the autoimmune-related cytokine profile of DS.

**Effector T Cells with Trisomy 21 Are Resistant to Treg-Mediated Suppression.** The enhanced activation phenotype observed in CD8+ and CD4+ T cells of people with DS, which is accompanied by increased numbers of FOXP3+ Tregs (Fig. 1C), prompted us to test for differences in Treg functionality. We analyzed 14 phenotypic markers to study the proliferation, activation, inhibitory potential, functional specialization, and effector properties of Tregs, which revealed minor effects of karyotype (SI Appendix, Fig. S5 D and E). PNA Tregs expressed more of both Ki-67 and the activation marker Helios, but less PD-1 (SI Appendix, Fig. S5 D and E). In contrast, effector Tregs, which express more FOXP3 in people with DS (SI Appendix, Fig. S2F), also express more of the costimulatory molecule GITR (SI Appendix, Fig. S5 D and E).

To further test Treg functionality, we performed allogeneic “criss-cross” suppression assays (24, 48–50), in which Tregs from people with and without DS were isolated and titrated into responder cells with or without T21. In this way, we could independently define the impact of karyotype on responder cells (either CD8+ or CD4+ Tconv cells) versus impacts on Treg suppressive capacity. We observed that Tregs of either karyotype
showed a similar ability to suppress responder CD8+ T cells and CD4+ Tconv cells from D21 individuals (Fig. 5 A and C). In contrast, both CD8+ T and CD4+ Tconv cells with T21 were more resistant to Treg-mediated suppression, regardless of the Treg karyotype (Fig. 5 B and C). Further analysis of effectors cells upon stimulation with anti-CD3/CD28 in the absence of Tregs revealed that T21 CD8+ T cells are less sensitive to suppression by Tregs, which could be responsible for the gene expression changes observed in a given experimental condition (52). However, ~90% of DEGs are encoded elsewhere in the genome, indicating the existence of signaling cascades dysregulated by the trisomy. In order to identify these signaling pathways, we employed the Upstream Regulator analysis tool of the Ingenuity Pathway Analysis (IPA) software (52), a bioinformatics tool that uses thousands of available gene expression datasets and myriad other experimental evidence to predict the “upstream regulators” (i.e., driving factors) responsible for the gene expression changes observed in a given experimental condition (52). IPA revealed that the top 2 upstream regulators predicted to be activated in WBCs with T21 are CD3 and CD28, the coreceptors mediating T cell activation (Fig. 6A). Therefore, although the samples were processed immediately after blood draw without any stimulation, samples with T21 resemble samples known to have activated T cells. Importantly, these predictions are identical when we employed only DEGs not encoded on chr21, indicating that these predictions are driven by the genome-wide signature (non-chr21, Fig. 6A).

To further investigate this phenomenon, we employed the same analysis on RNA sequencing (RNAseq) data previously generated from bulk T cells (CD45+CD3+CD14−CD19−CD56−) (9), which revealed that 5 of the top 10 upstream regulators predicted to be activated in T21 samples belong to the IFN pathway (IFN alpha, IRF7, IRF1, IFNA2, IFNG) (Fig. 6C). Interestingly, the top upstream regulator is CSF2/GM-CSF (Fig. 6 C and D), a cytokine overproduced by CD8+ T cells with T21 (Fig. 2D and SI Appendix, Fig. S3D). Once again, these signatures are driven mostly by DEGs not encoded on chr21, and enriched for canonical IFN-stimulated genes, leading to highly elevated IFN scores in T cells of people with DS (Fig. 6D).

Next, we performed Gene Set Enrichment Analysis (GSEA) to further explore to what degree the gene expression changes observed are consistent with differences in cell composition and/or specific signaling events. We completed this analysis for both WBCs and T cells, which produced very similar results (Dataset S6). When we analyzed the T cell transcriptome data with the GSEA Hallmark gene sets, the most prominently enriched gene sets were IFN gamma, mTORC1, and IFN alpha (Fig. 6E, SI Appendix, Fig. S7D, and Dataset S6). Of note is that mTOR has been shown to be chronically activated in DS (53) (SI Appendix, Dataset S6). Of note is that mTOR has been shown to be chronically activated in DS (53).
For example, among the top signatures associated with T21 were genes elevated in PD-1 high CD8+ T cells over naïve CD8+ T cells (e.g., T-bet/TBX21, EOMES) and genes upregulated during exposure of CD4+ T cells to the superantigen toxic shock syndrome toxin (e.g., Ki-67/MKI67 and other cell cycle genes) (Fig. 6F, SI Appendix, Fig. S7 E and F, and Dataset S6). Of note is that these signatures are mostly nonoverlapping (SI Appendix, Fig. S7E), indicating that different fractions of the T21-associated signature can be attributed to shifts in the CD8+ and CD4+ subsets toward activated effector states. Lastly, the T cell RNAseq not only confirmed transcriptional induction of T-bet/TBX21 and EOMES in T cells of people with DS but also revealed that expression of both factors correlated positively with the IFN alpha scores (Fig. 6F and G and SI Appendix, Fig. S7 F and G).
Altogether, these results indicate that the clear signs of T cell activation and differentiation in people with DS correlate positively with increased IFN signaling.

**T Cell Subsets with Trisomy 21 Are Hypersensitive to IFN Stimulation.**

We next hypothesized that the IFN signature observed in T cells from people with DS could be driven by the IFNRs encoded on chr21, which we previously demonstrated to be overexpressed in bulk T cells at the mRNA level (9). We also showed previously that fibroblasts with T21 respond to lower doses of types I and II IFN similarly to T cells from people with DS (9). We next hypothesized that the IFN signature observed in T cells from people with DS could be driven by the IFNRs encoded on chr21, which we previously demonstrated to be overexpressed in bulk T cells at the mRNA level (9). We also showed previously that fibroblasts with T21 respond to lower doses of types I and II IFN similarly to T cells from people with DS (9).

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Altogether, these results reveal that T cells from individuals with T21 show higher levels of basal IFN signaling and are also hyperresponsive to type I IFN stimulation.

Discussion

Despite substantial research efforts, the mechanisms by which T21 causes the immune anomalies reported in people with DS remain unclear. The situation is even more complex when investigating the adult immune system, as it becomes harder to dissect the direct effects of the extra chromosome versus environmental and lifestyle variables working across the lifespan of people with DS. Most of what is known regarding the peripheral immune system in people with DS comes from studies in children. These reports agree on an immune profile that resembles one of older typical people: a skewed distribution of the naïve/effector memory subsets, an increased CD8/CD4 ratio of unstimulated values for each sample. Vertical dashed line represents the no-change midline. Horizontal dashed line represents P value of 0.05 as calculated by Student t test. (C) Dot plots displaying geometric mean metal intensity (gMMI) for the indicated epitopes and cell types, with lines connecting the basal and IFN-α-stimulated values for each sample. (D) Scatter plots showing the gMMI of pSTAT1, pSTAT4, pSTAT6, and p4E-BP1 among the indicated T cell subsets in people with and without T21 before and after stimulation with IFN-α. Data in C are shown as mean ± SEM with significance determined by 2-way ANOVA with Sidak’s posttest. *P < 0.05; **P < 0.01.
reveal why this population is protected from diverse solid malignancies (2).

Previous efforts to characterize the polarization of CD4+ Tconv in adults with T21 have led to contradictory conclusions (19, 21). We show here that CD4+ Tconv from people with DS are polarized toward the Th1 and Th1/17 states, overproducing IL-17A and IL-22, concurrent with higher levels of circulating IL-17A-D and IL-22 in plasma compared to controls. The role of IL-17–expressing cells in the pathogenesis of inflammatory and autoimmune disease is well known, particularly in autoimmune diseases characterized by high levels of type I IFN signaling, such as in systemic lupus erythematosus (SLE) and dermatomyositis (62). Furthermore, it has been hypothesized that type I IFN and IL-17 act in concert to sustain and amplify autoimmune and inflammatory responses (62), making them a dangerous combination involved in the pathogenesis of autoimmune diseases, which could explain the increased incidence of autoimmune conditions observed in DS. Interestingly, the increase in IL-10 could be indicative of dampening mechanisms preventing the widespread development of autoimmunity in DS (44).

Our findings demonstrate that Tregs show increased numbers and higher FOXP3 expression in people with T21, yet our analysis did not detect major phenotypic differences. Furthermore, when allogenic cross-suppression assays were performed, T21 Tregs were perfectly capable of suppressing D21 CD8+ T cells and CD4+ Tconv cells. However, both effector CD8+ T and CD4+ Tconv cells from people with DS were resistant to Treg-mediated suppression, independent of Treg karyotype. Resistance to suppression has previously been reported in different autoimmune diseases such as type I diabetes (63) and SLE (50). Although the mechanisms leading to effector T cell resistance in DS are unknown at this point, continued exposure to cytokines that are elevated in people with DS, such as TNF-α (10), have been shown to induce T cell resistance to suppression (64). Moreover, both CD8+ and CD4+ Tconv cells from people with DS show increased proliferation rates upon stimulation compared to controls while producing higher levels of effector molecules, which could potentially explain their resistance to Treg-mediated suppression.

Repeatedly, our work points to hyperactive IFN signaling as a potential driver of the observed dysregulation of T cell homeostasis in DS. RNAseq analysis revealed a positive correlation between IFN transcriptional scores, CD3/CD28 activation scores, and expression of T-bet and EOMES (39, 43). Furthermore, GSEA analysis revealed that T cells from adults with DS have transcriptome signatures indicative of both increased differentiation and hyperactivation. The previously observed overexpression of IFN-γ and IFN-α in T cells and other cell types with T21 (9) and the clear hyperreactivity that diverse T cell subsets show upon IFN-α stimulation could have profound impacts in shaping the T cell compartment.

Finally, our results point to inhibition of IFN signaling as a therapeutic strategy in DS, particularly for the T cell-driven autoimmune conditions that are more prevalent in this population. IFN signaling can be blocked by a number of strategies, including inhibitors of JAK kinases which are approved for the treatment of autoimmune conditions such as rheumatoid arthritis (65). In fact, we recently reported 2 cases of JAK inhibition in individuals with DS, with remarkable therapeutic benefit for alopecia areata, a T cell-driven autoimmune disorder more prevalent in DS (66). We believe our results justify a thorough investigation of the potential therapeutic benefits of anti-IFN strategies in people with DS.

Methods

Study Approval. All donors were enrolled under a study protocol approved by the Colorado Multiple Institutional Review Board, known as the Crnic Institute’s Human Trisome Project (COMIRB #15-2170, www.trisome.org). Written informed consent was obtained from parents or guardians of each participant, and assent was obtained from participants over the age of 7 y who were cognitively able to assent. Cohort information can be found in Dataset S2.

Flow Cytometry. Blood samples were processed and stained according to standard procedures. For more details on antibodies, flow cytometry, and software, see SI Appendix, Materials and Methods.

In Vitro Assays. In vitro suppression assays were performed according to a protocol modified from ref. 67. For more details, see SI Appendix, Materials and Methods.

RNAseq. For RNAseq of WBCs, blood samples were immediately processed using a previously described protocol (10). For more details, see SI Appendix, Materials and Methods.

Mass Cytometry Antibody Staining and Data Acquisition. The procedure for mass cytometry analyses and the antibody–metal conjugates used are detailed in SI Appendix, Materials and Methods.

Meso Scale Discovery Assays (MSD). Cytokines were measured from plasma samples of 128 individuals (Datasets S2E and S3C) and from supernatant of in vitro stimulated CD8+ and CD4+ Tconv cells of 20 individuals with anti-CD3/CD28 (Datasets S2B and S3 A and B) using V-PLEX immunoassays and a Meso QuickPlex SQ120 from Meso Scale Diagnostics per manufacturer’s instructions.

Statistical Analysis. All statistical analyses are detailed in SI Appendix, Materials and Methods.

Data Availability. RNAseq datasets have been deposited in the Gene Expression Omnibus (GEO) under accession numbers GSE128622 for WBCs (68) and GSE84531 for T cells (51). All other datasets and code have been deposited in the Open Science Framework database and can be accessed at https://osf.io/zzpyu/ (22) and https://osf.io/p5kmf/ (56).

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