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Th1/interferon-γ bias in 22q11.2 deletion syndrome is driven by memory T cells and exacerbated by IL-7

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ABSTRACT

The aim of this study was to investigate the impact of thymic dysplasia on the phenotypic and functional characteristics of T cells in patients with 22q11.2 deletion syndrome, including T-cell phenotype, transcriptional profile, cytokine production, as well as the possibility of utilizing IL-7 to recover their numbers and function.

We found a strong bias towards Th1 response in pediatric and young adult 22q11.2DS patients, expansion of $CXCR5^+$ follicular helper cells and $CXCR3^+CCR6^-$ Th1 cells, increased production of cytokines IFN-γ, IL-10, IL-2, IL-21 and TNF-α. This Th1 skew was primarily driven by expanded terminally differentiated T cells. IL-7 further reduced naive T cells, increased cytokine production and caused an upregulation of exhaustion markers.

Thus, Th1 bias in T cell populations persists from infancy into adolescence and is accompanied by accelerated maturation of T cells into memory stages. This phenotype is exacerbated by IL-7 which causes further decrease in naïve T cells in vitro.

1. Introduction

22q11.2 deletion syndrome (22q11.2DS) is an inborn error of the immunity caused in >90% of cases by a 3 Mbp deletion on the long arm of the 22nd chromosome, which is immunologically characterized by various degrees of thymic dysplasia and impaired development of T cells. The resulting T cell lymphopenia is most pronounced in childhood, with T cells replenishing through homeostatic proliferation during childhood and into adolescence [1,2]. This process results in a predominance of memory T cells compared to healthy age-matched controls [3], but its impact on functional capabilities of the resulting T cells in 22q11.2DS is incompletely understood.

Several previous studies tried to assess this aspect of 22q11.2DS through surrogate markers mapping different lineages of helper T cells. Our group has shown an increase in follicular helper T cells in patients with 22q11.2DS [4], which we hypothesized may correspond to the hypergammaglobulinemia seen in a proportion of 22q11.2DS patients especially since puberty [5]. A study evaluating cytokine production by 22q11.2DS T cells by Pierdominici et al. observed higher production of IFN-γ, IL-2 but not IL-4 in children [6]. This finding was repeated by Zemble et al., who additionally highlighted a shift away from IFN-γ towards IL-4 skew in adults [7]. Nevertheless, whether this was a result of their more mature developmental state was not explored.

Proliferation of peripheral lymphocytes as a mechanism of replenishing their lacking pool has been shown to cause increased expression of the inhibitory receptor PD1 as well as telomere shortening [8], which are some of the main hallmarks of cellular senescence. Increased expression of exhaustion and senescence-associated molecules such as PD1 or CD57 has been shown in 22q11.2DS patients previously [3], but has not been put into context of the functional changes which are borne by highly exhausted or senescent cells. Nevertheless, the accelerated proliferation and cellular division driving features of exhaustion remains a concern.

Therefore, in this study we set out to explore the ontology and delineation of T cells in patients with 22q11.2DS, assess their transcriptional signature and functional capabilities, and evaluate the possible role of IL-7, a potent stimulant of T cell proliferation and survival, in replenishing the 22q11.2DS characteristic T cell lymphopenia.

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2. Methods

2.1. Patients and controls

Patients followed at the Department of Immunology, 2nd Faculty of Medicine, Charles University and University Hospital in Motol who had a verified 22q11.2 deletion [9] and corresponding healthy age and sex-matched donors (HD) were enrolled into the study, which was approved by the Ethical Committee of the Motol University Hospital in Prague, Czech Republic (dated 19.6.2019). All participants or their legal guardians signed a written informed consent in accordance with the Declaration of Helsinki.

2.2. Flow cytometry

Peripheral blood from patients and HDs was collected into EDTA-coated tubes. For extracellular marker panels, 50 μl of full blood was immediately stained with antibody-fluorochrome conjugates for 15 min in the dark at room temperature, followed by red blood cell lysis with a NH4Cl-based lysis buffer for 15 min, centrifuged at 300 *g* for 5 min, resuspended in PBS and measured on a BD LSRFortessa flow cytometer (BD Biosciences, San Diego, CA, USA). For panels including intracellular markers, cells were isolated and stained using previously published protocol [4]. The list of used antibody-fluorochrome conjugates is shown in Supplementary Table S2.

2.3. Cytokine production

For cytokine production, T cells were isolated from full blood using the affinity-based FABian Fab-TACS® system (IBA Lifesciences, Goettingen, Germany). 100,000 T cells were seeded in U-bottom 96-well plate wells in RPMI 1640 medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% FBS, 1% penicillin, 1% GlutaMAX (ThermoFisher Scientific, Waltham, MA, USA) and stimulated with/out 5 ng/ml PMA and 750 ng/ml ionomycin or 2.5 μl DynaBeads (ThermoFisher Scientific) as indicated in the text and figures for 24 h at 37 °C and 5% CO_2. The plates were then centrifuged at 300 *g* for 5 min, supernatant was harvested and cytokine production was measured using the magnetic bead-based Milliplex Human Th17 Magnetic Bead Panel kit according to manufacturer instructions. Data were acquired by LUMINEX 200 and Belisa software (Luminex, Austin, TX, USA) was used for analysis.

2.4. RNA sequencing

One million T cells isolated as described above preserved in −80 °C in 300 μl RLT buffer (Qiagen, Hilden, Germany). Total RNA was isolated using RNeasy Mini Kit following manufacturer's instructions (Qiagen), RNA quality and quantification was determined by TapeStation 4200 (Agilent, St. Clara, CA, United States) following manufacturer's instructions.

Library construction was conducted employing the Illumina True Seq stranded mRNA Poly A protocol and subsequently sequenced using a NovaSeq 6000 (2 × 50 bp pairedend reads, 20 samples per lane) following the manufacturer's guidelines. This resulted in a median of 28 million reads before and 16 million reads per sample after mapping (genome build: GRCh38). The following tools and versions were employed as part of the analysis: nf-core/rnaseq v1.4.2, Nextflow v21.03.0.edge, FastQC v0.11.8, Cutadapt v2.5, Trim Galore! v0.6.4, SortMeRNA v2.1b, STAR vSTAR_2.6.1d, HISAT2 v2.1.0, Picard MarkDuplicates v2.21.1, Samtools v1.9, featureCounts v1.6.4, Salmon v0.14.1, StringTie v2.0, Preseq v2.0.3, deepTools v3.3.1, RSeQC v3.0.1, dupRadar v1.14.0, edgeR v3.26.5, Qualimap v.2.2.2-dev, MultiQC v1.7 [10]. Differential expression was performed using the DESeq2 package in R [11], only genes with at least 5 reads in at least 3 samples were included resulting in 1916 differentially expressed genes (DEGs) with nominal *p*-value of ≤5% and 199 genes with FDR of ≤5%. GSEA of whole transcriptome was performed in GSEA 4.2.0 on Gene sets from GO:Biological process library (Settings: Size of gene set 15–500, number of permutations - 1000, permutation type – gene-set, enrichment statistic - weighted, metric for ranking genes - Signal2Noise) [12]. Leading edge analysis was performed on the same platform. Maps of gene sets were created in Cytoscape [13] using ClusterMaker plugin [14].

2.5. Spectral cytometry

For the spectral cytometry experiments, cryopreserved PBMCs were thawed, counted and plated in flat-bottom 96-well plates at 200 k cells per well in RPMI 1640 medium supplemented with 10% FBS, 1% penicillin and 1% GlutaMAX (see above) for 72 h at 37 °C and 5% CO_2. To test the effect of IL-7, a concentration of 10 ng/ml was used, based on previous studies [15]. For the final 6 h of culture, cells were stimulated with 5 ng/ml PMA, 750 ng/ml ionomycin and exocytosis was blocked with 1:1000 brefeldin A (BioLegend, San Diego, CA, USA). Thereafter, plates were centrifuged at 300 *g* for 5 min and cell pellets were stained as described above, with the addition of 10 μl BD Horizon™ Brilliant Stain Buffer Plus (BD Biosciences) per sample. After intracellular staining, cells were resuspended in FluoroFix™ Buffer (BioLegend) and measured on the Cytek Aurora spectral cytometer (Cytek, Fremont, CA, USA) within 24 h. Acquired data was analyzed in OMIQ (Dotmatic, Boston, MA, USA). Algorithms were used as indicated in the text, including FIt-SNE [16] and FlowSOM [17] (subsampling to 18,000 T cells, number of clusters was set using the elbow method to 16).

2.6. Statistics

Statistical analysis, other than the transcriptome analysis outlined above, was performed using GraphPad Prism 8.0 (San Diego, CA, USA). Values of $p = 0.01$–0.05 (*), $p = 0.001$–0.01 (**) and $p < 0.001$ (***) were considered statistically significant. Tests used described in figure legends.

3. Results

3.1. Memory and Th1 bias of T cells persists into young adulthood in 22q11.2DS

For this study, 12 patients with molecularly verified 22q11.2DS (age 13.5 ± 4.9 years, 6 female) were recruited and compared with 15 healthy donors (age 13.3 ± 1.9 years, 7 female). At time of testing, all subjects were in full health with no ongoing infections. 10/12 patients had a history of CD4 T cell lymphopenia, 8/12 had a history of total T cell lymphopenia. As shown in numerous previous studies, 22q11.2DS patients were markedly T cell lymphopenic, with a depletion of RTE and naive and expansion of memory T cells (Fig. 1A, Supplementary Fig. 1A, B). More information on the patient cohort can be found in Supplementary Table S1.

Comparing the delineation of helper T cells into distinct lineages such as Th1, Th2, Th17 and Th1/17 based on the expression of chemokine receptors CXCR3 and CCR6 [18] we observed a significant expansion of $CXCR3^{+}CCR6^{-}$ Th1 T, but not $CXCR3^{-}CCR6^{-}CCR4^{+}CRTH2^{+}$ Th2 and $CXCR3^{-}CCR6^{+}$ Th17 T cells in 22q11.2DS patients (Fig. 1B, Supplementary Fig. 2). This finding is also recapitulated within the already enlarged compartment of $CD45RA^{-}CXCR5^{+}PD1^{+}$ follicular helper Tfh cells [4], of which $CXCR3^{+}CCR6^{-}$ Tfh1 cells form a disproportionately large part in 22q11.2DS (Fig. 1B).

To test whether the Th1/Tfh1 skew bears functional consequences, we incubated T cells from patients and healthy donors with anti-CD3/CD28 beads or PMA/Ionomycin for 24 h, and measured the concentration of cytokines in the supernatant (Fig. 1C). Patients' T cells had higher levels of IFN-g both natively and following TCR stimulation, while they

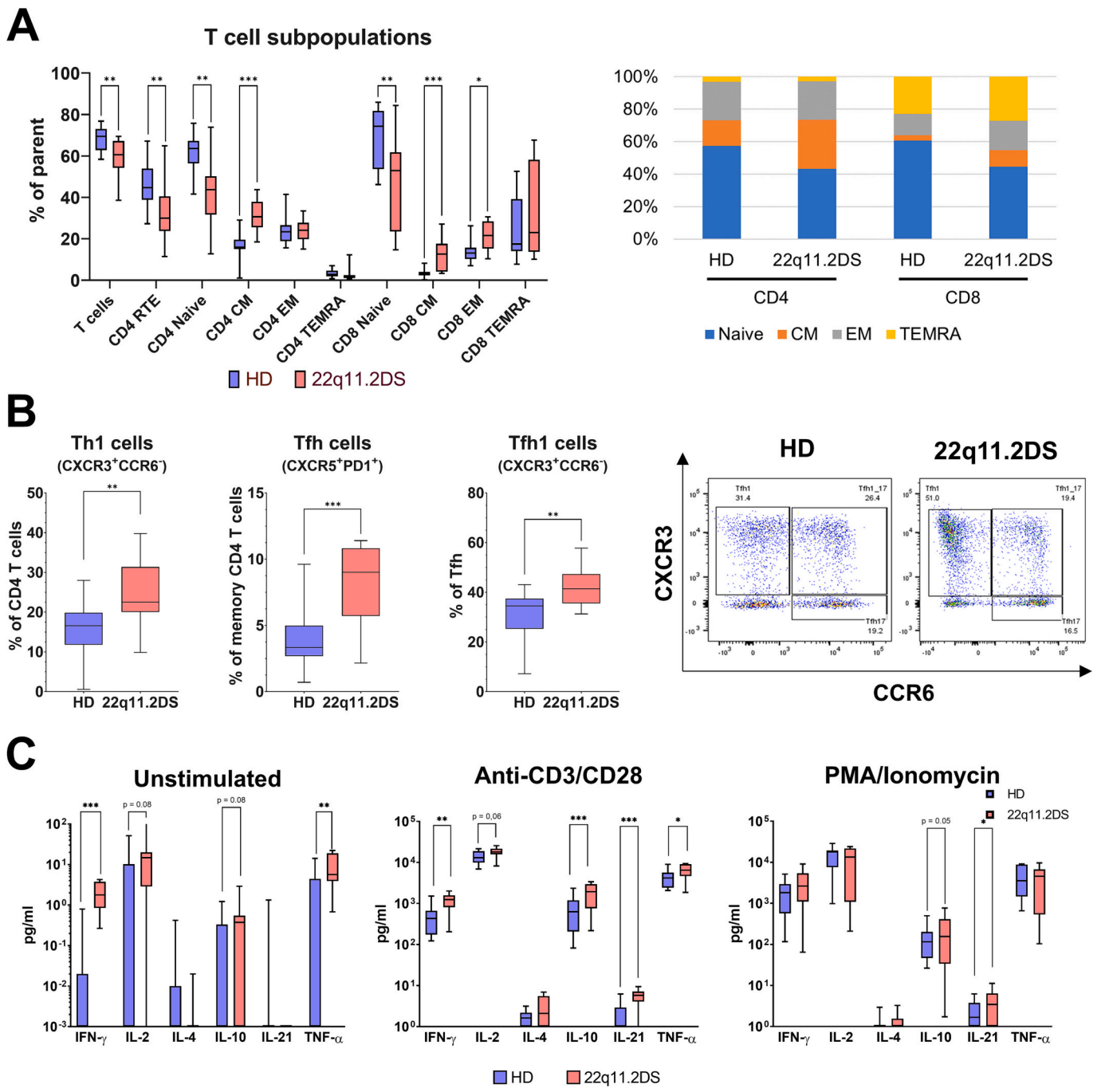

Fig. 1. A) T cell memory subpopulations in 22q11.2DS and HD. B) T helper and follicular helper cell lineages, including a representative dot plot showing CXCR3 and CCR6 expression in Tfh of HD (left) and 22q11.2DS patient (right). C) IFN-γ, IL-10, IL-2, IL-21, IL-4 and TNF-α production in supernatant of T cell culture, unstimulated and after anti-CD3/28 or PMA/ionomycin stimulation. Welch's *t*-test *p* values are shown.

had comparable levels following PMA/Ionomycin stimulation. Patients' T cells also produced higher levels of IL-10, IL-21 and TNF-a following TCR stimulation. Of note, IL-4 production was unchanged.

3.2. *Th1/IFN-γ bias is anchored in T cells on transcriptional level*

In order to confirm the Th1 skew in an unbiased manner and to identify further changes in metabolism we performed RNA-sequencing on Fab-affinity isolated T cells from healthy donors and 22q11.2DS patients, as described in the Methods.

Gene set enrichment analysis (GSEA) identified 34 significantly enriched gene sets with family-wise error rate (FWER) ≤ 5% (Fig. 2A). These gene sets included positive regulation of cell killing (NES = 2.34, FWER = 0.001), T cell mediated cytotoxicity (NES = 2.19, FWER = 0.006), positive regulation of leukocyte mediated immunity (NES = 2.15, FWER = 0.02) and IFN-γ mediated signaling pathway (NES = 2.13, FWER = 0.028)(Fig. 2B).

We then performed leading edge analysis, identifying 107 genes contributing to at least 6 of the enriched pathways (Fig. 2C). The enrichment was most strongly driven by genes of the HLA gene family, proteasome subunit genes, genes related to Th1 response such as *IL12RB* and *IFNG*, genes regulating T cell activation like *CD160*, *LAG3*, *CD226* and *KLRD1* (Fig. 2D).

Meta-analysis of 173 gene sets with FDR ≤ 2% (Fig. 2E) revealed that

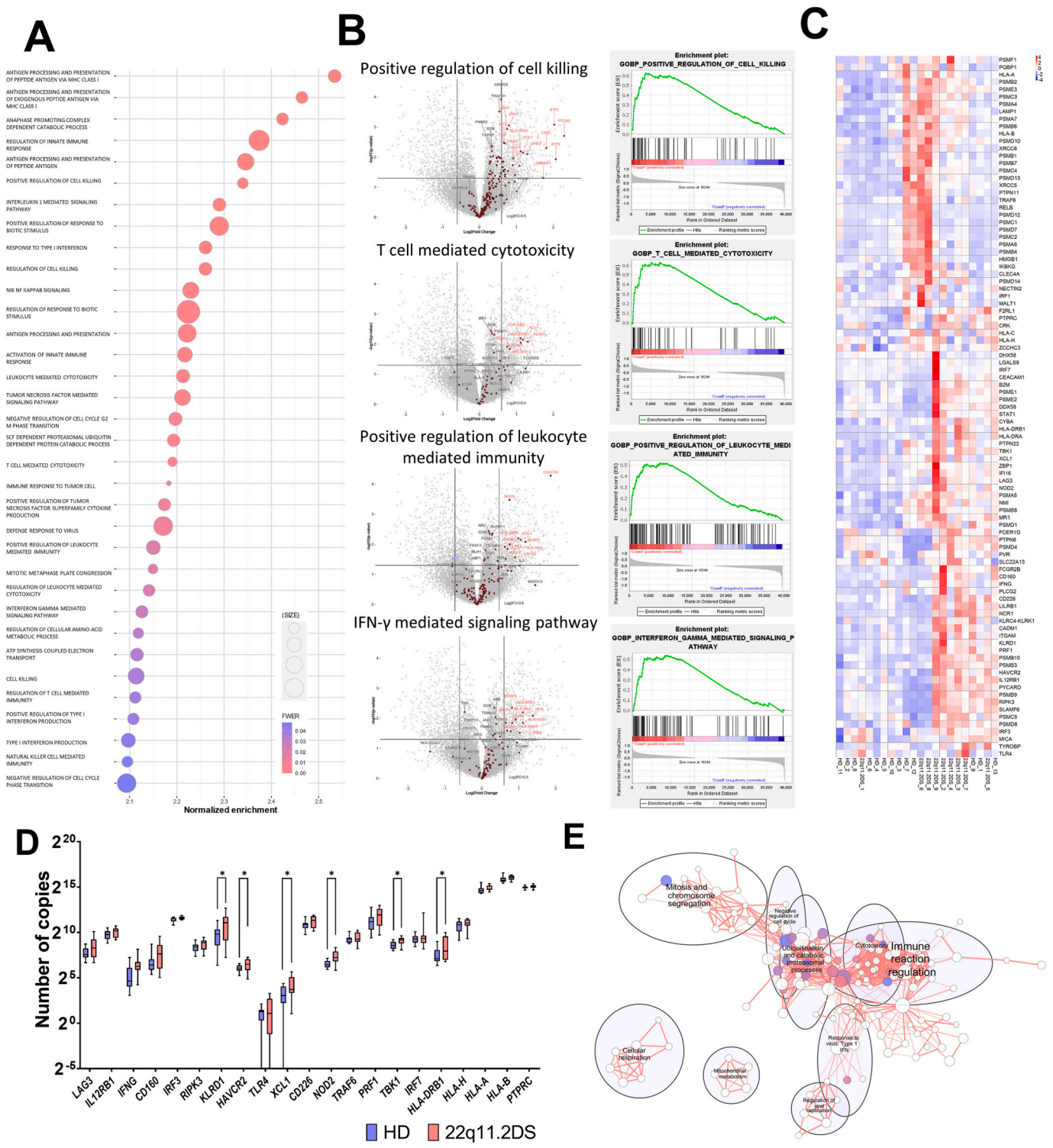

Fig. 2. A) Enriched gene sets from the Gene ontology: Biological process library. B) Volcano plots of select gene sets with respective enrichment plots. C) Leading edge genes contributing to >5 enriched gene sets. D) Selected relevant leading edge genes and their expression levels. Welch's *t*-tests shown. E) Cytoscape map of gene sets enriched at FDR adjusted *p*-value ≤0.02, total number of nodes 173, clustered by Cluster Maker plugin in Cytoscape, coloured nodes mark gene sets from Fig. 2. A, white nodes mark less significant gene sets.

the leading dysregulated processes include the regulation of immune response, catabolism in proteasomes, mitochondrial metabolism, and cellular respiration, as well as negative regulation of cell cycle and response to viral infection. These biological processes correspond well to the skew towards effector and memory stages and to higher proliferation rate of 22q11.2DS cells supported by the cluster of gene sets related to mitosis and chromosome segregation (Fig. 2E).

3.3. *Spectral analysis pinpoints IFN-γ hyperproduction skew into expanded TEMRA T cells*

The maturation of T cells from naive to central, effector and finally

TEMRA stages drastically changes not only their phenotype but also functional capabilities. We therefore designed a 29-color spectral cytometry panel enabling single-cellular mapping of T cell phenotype, with the goal of identifying multi-parametric patterns of cellular differentiation, exhaustion, and senescence. Additionally, this approach would enable us to assess the association of phenotypic and functional changes with specific groups of cells. We used it to characterize PBMCs from 6 patients (patient IDs 2, 3, 7, 9, 11, 12, see Supplementary Table S1)) and 6 healthy donors (3 female, age 12.9 ± 1.8 years) which were cultured for 72 h, including a stimulation with anti-CD3/CD28 ligation for the final 6 h.

Recapitulating previously obtained data in this subcohort we observed we observed an expansion of memory T cells at the expense of naive T cells. (Fig. 3A). Dimensional reduction of high-parametric spectral data into two dimensions with FIt-SNE demonstrated altered T cell landscape in 22q11.2DS compared to HD (Fig. 3B).

Using manual gating methods we showed higher proportion of cells positive for CD45RO, CD95/Fas, CD57, PD1, Tim3, 2B4, CXCR5, IFN-γ, IL-2, Ki67 and CCL3, while CD62L, CD27, CD28, CD127 and TCF1 positive cells were reduced in 22q11.2DS (Fig. 3C). These features corresponded to a more differentiated spectrum of cells, with more exhausted and senescent phenotype.

We then inspected in detail the phenotype of individual memory subpopulations in 22q11.2DS compared to HD (Fig. 3D). Within this more granular analysis, differences were more muted. For example, when limiting the comparison to $CD45RO^{-}CD62L^{-}$ TEMRA T cells, dominant producers of IFN-γ (Fig. 4D, inner rings), phenotype was comparable between 22q11.2DS and HD (Fig. 3D). The loss of CD28 in CD4 TEMRAs suggests that delineation of memory stages based on only two markers (CD45RO and CD62L) may not accurately reflect the entire T cell ontology. $CXCR5^{+}$ cells were expanded in CD4 effector memory (EM) and TEMRA cells, corresponding to the increased population of Tfh (Fig. 1B).

IFN-γ^{+} cells were somewhat higher in 22q11.2DS TEMRA CD4 T cells, but the difference no longer reached significance between patients and controls, supporting the notion that differentiation status and not intrinsic genetic defect is the cause of Th1 bias in patients with thymic dysplasia caused by 22q11.2DS.

Finally, we used unsupervised clustering methods to group cells into phenotypically similar populations with FlowSOM, which identified 16 distinct clusters of T cells (Fig. 3E, F). The most significant difference between 22q11.2DS patients and HD was in the loss of cluster 1, which phenotypically corresponds to naive CD4 T cells, and increase of cluster 16, which phenotypically corresponds to highly matured memory CD8 T cells (Fig. 3G). Using manual gating to replicate the phenotype of cluster 16 we show a highly significant expansion of $CD8^{+}CD62L^{-}CD27^{-}CD28^{-}TCF1^{-}TOX^{+}CD57^{+}$ senescent cells in 22q11.2DS patients (Fig. 3H). This population was almost non-existent in healthy donors, giving it a high specificity for the maturational changes driven by the homeostatic proliferation which repopulates the T cell compartment in 22q11.2DS patients with thymic dysplasia.

3.4. IL-7 exacerbates the memory and IFN-γ bias

IL-7 has been shown to support T cell proliferation in cell cultures and has even been tested as a therapeutic cytokine in patients with idiopathic T cell lymphopenia (https://clinicaltrials.gov/ct2/show/NCT00839436), sepsis-induced T cell lymphopenia (https://clinicaltrials.gov/ct2/show/NCT02640807) and others. We thus tested if the effect of IL-7 may not be suitable to support the restoration of T cell numbers in patients with 22q11.2DS, by adding 10 ng/ml IL-7 into the 72-h PBMC cell-culture analyzed in Fig. 3.

We noted a significant decrease of clusters 2 and 7 (Fig. 4A), which phenotypically and functionally correspond to naive CD4 and CD8 T cells respectively (Fig. 3G), and verified this decrease through manual gating of $CD45RO^{-}CD62L^{+}CD127^{+}TCF1^{+}$ cells (Fig. 4B). Corresponding to the shift away from naivety, $CD45RO^{-}CD62L^{-}$ TEMRA CD4 T cells were significantly upregulated by the addition of IL-7, this effect was more pronounced in 22q11.2DS (Fig. 4C).

As discussed previously, mature CD4 and CD8 T cells are the dominant producers of IFN-γ (Fig. 4D, inner rings), which remains unchanged in the presence of IL-7 (Fig. 4D, outer rings). The changes in differentiation status of T cells driven by IL-7 therefore significantly alter the phenotype and functional capabilities of the total 22q11.2DS bulk T cell compartment, manifesting as upregulation of CD95/Fas, exhaustion markers TIGIT, Tim3 and TOX, loss of TCF1 and upregulation of effector molecules Granzyme B, TNF-α and of course IFN-γ (Fig. 4E).

These changes are mirrored within CD4 TEMRAs, in which IL-7 drives a supra-normal level of production of IFN-γ and other cytokines and forces their phenotype further along the differentiation path in both 22q11.2DS and HD (Fig. 4F).

4. Discussion

In this manuscript we explore the phenotypic and functional characteristics of T cells in 22q11.2DS, finding a strong Th1/IFN-γ signature within CD4 and CD8 T cells, which is predominantly driven by accelerated maturation and shift towards late effector memory stages and augmented by the effect of IL-7.

The impaired thymic function in 22q11.2DS, which is a hallmark of this syndrome [19,20], results in the reduction of naive and expansion of memory T cells. This trend persists from infancy, where the T cell deficiency is most pronounced [4,21], throughout adolescence and into young adulthood, as shown in patients analyzed in this study. T cells divide through homeostatic proliferation in the periphery, diluting their TRECs and leading to a skew of TCR spectra [6]. The process is supported by IL-7, a cytokine vital for the survival and proliferation of T cells, which acts through the IL-7 receptor comprised of IL-7Rα (CD127) and the common γ chain [22]. In this study, the stimulation of T cells with IL-7 skewed T cells further away from naive and into memory stages and massively downregulated the expression of CD127, which corresponded to the process of homeostatic proliferation and mimicked in healthy donors the phenotype seen in native 22q11.2DS patient-derived T cells. Indeed, IL-7 has been previously suggested as a marker of T cell homeostatic proliferation in partial DiGeorge syndrome patients, as it was elevated in patients with lower TRECs and total T cells [23]. Thus, our results further support the notion that IL-7 supported homeostatic proliferation is the driving factor behind the memory skew in 22q11.2DS patients.

In earlier studies, we and others have shown a skew towards follicular helper T cells within the memory CD4 compartment of 22q11.2DS patients [4,24]. Derfalvi et al. have demonstrated higher *IL21* mRNA expression in PBMCs, which we here support by showing an increased production of IL-21 in T-cell culture. We hypothesize that this increased Tfh signature may correspond to the tendency of patients to exhibit hypergammaglobulinemia [5,25]. Nevertheless, B cells display impaired maturation in 22q11.2DS with low switched memory B cells [5], suggesting that the T cell help provided by the expanded Tfh population may be inefficient, or preferential for some B cell clones but not others. Mechanistic insights into this mechanism and the possible role of Th1/IFN-γ and cytokine milieu skew in 22q11.2DS is as of yet missing, but it is feasible to hypothesize that the Tfh1 shift impairs the germinal center reaction, which has been shown previously in common variable immunodeficiency (CVID), and was accompanied by the expansion of $Tbet^{+}CD21^{low}$ B cells [26]. Similarly to CVID, 22q11.2DS patients' B cells show lackluster memory switching [5] and one study has also shown expanded $CD21^{low}$ B cells in 22q11.2DS patients [27].

The Th1/IFN-γ bias of 22q11.2DS-derived T cells is the dominant finding of this study, spanning surface phenotype, transcriptional signature and production of cytokines in cell culture. Recent data on Th1/Th2/Th17 balance in 22q11.2DS is limited - in 2010, Zemble et al. showed a Th1 shift in childhood and Th2 shift in adults [7]. Here we

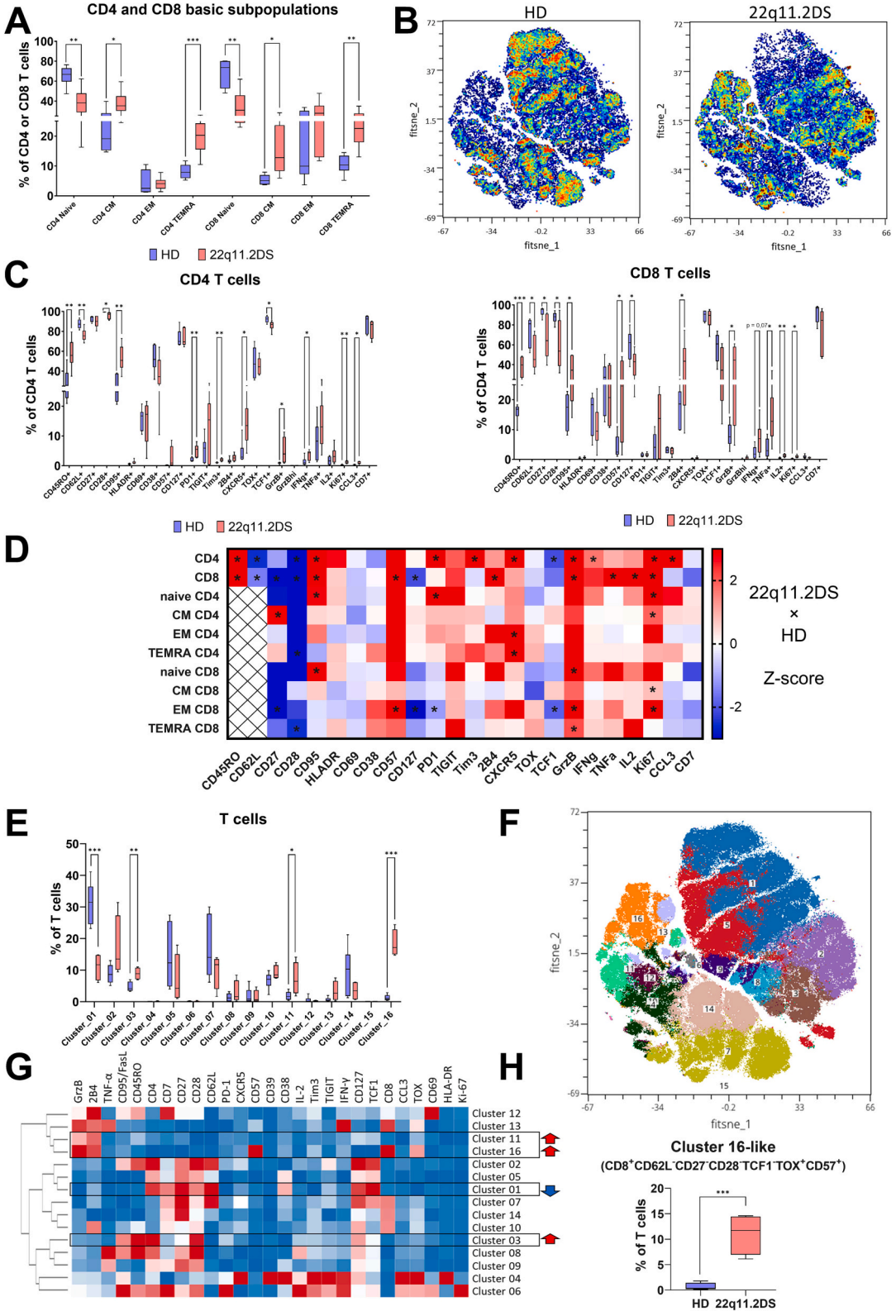

Fig. 3. A) T cell memory subpopulations in 22q11.2DS and HD. B) FIt-SNE plot of 22q11.2DS (right) and HD (left) T cells based on reduction of 27 surface and intracellular markers. C) Percentage of cells positive for each tested marker within CD4 (left) and CD8 (right) T cells, in HD and 22q11.2DS. D) Heatmap showing the z-score (calculated as difference of 22q11.2DS and HD averages divided by HD standard deviation) of cells positive for each marker in 22q11.2DS patients as compared to HD, within bulk CD4/CD8 T cells and all basic memory subpopulations. Asterisks denote Welch's t-test significant difference. E) Percentage of cells belonging to FlowSOM-identified clusters in 22q11.2DS patients and HD. F) Clusters overlaid over FIt-SNE 2D landscape, as shown in B). G) Heatmap showing expression of all markers in each cluster, clustered within both rows and columns. H) Proportion of cluster 16-like cells in HD and 22q11.2DS patients. Welch's t-tests shown.

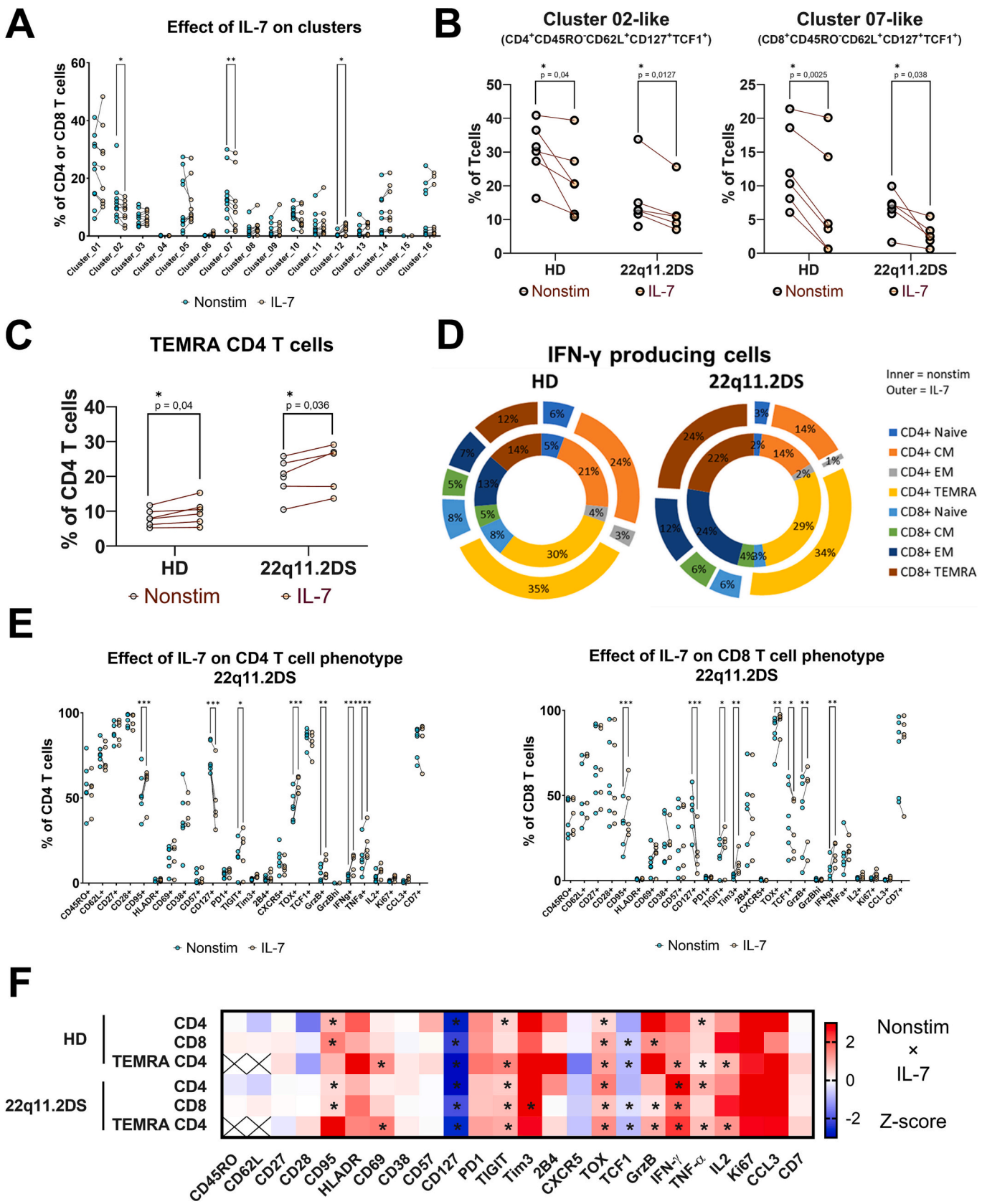

Fig. 4. A) Changes in cluster populations in IL-7 treated cells, compared to cells in media. B) Manually gated naive cluster 2/7-like cells in HD and 22q11.2DS, with/out treatment of IL-7. C) TEMRA CD4 T cells in HD and 22q11.2DS, with/out treatment of IL-7. D) IFN-γ-producing cells in HD and 22q11.2DS divided based on memory phenotype, with/out treatment of IL-7. E) Percentage of cells positive for each tested marker within CD4 (left) and CD8 (right) T cells, in 22q11.2DS, with/out treatment of IL-7. F) Heatmap showing the z-score of cells positive for each marker in cells treated with IL-7 as compared to untreated cells, shown in 22q11.2DS patients and HD, within bulk CD4/CD8 T cells and TEMRA CD4 subpopulations. Paired t-tests shown.

show significantly increased production of several effector cytokines, including IFN-γ, TNF-α, IL-10, IL-21 and IL-2,in pediatric and young adult 22q11.2DS patients after TCR stimulation, but only insignificantly increased levels of these cytokines after non-specific stimulation by PMA/Ionomycin. This discrepancy may be caused by the less physiological stimulus of PMA/Ionomycin stimulation, which activates all cells regardless of their developmental status, and thus does not reflect the increased amount of effector/memory T cells and their altered responsiveness to TCR stimulation. Zemble et al. hypothesized that the shift from IFN-γ/Th1 to IL-4/Th2 is caused by homeostatic proliferation, a mechanism which has been proposed in mice [28]. More recently, Raje et al. performed transcriptional analysis in pediatric 22q11.2DS patients, identifying numerous DEGs related to proinflammatory state, including *IFNG* [29]. Using epigenetic data, Zhang et al. have identified Th2 polarization in adult 22q11.2DS patients [30], further supporting previous findings of the shift from Th1 to Th2. Our data expands on these results by identifying gene sets corresponding to crucial biological pathways related to cell cycle, metabolism, cytotoxicity and again Th1 response. We contextualize the transcriptional changes through cytokine production assays and high-parametric spectral cytometry, which provided us with the high resolution necessary for granular analysis of cytokine production within T cell memory stages. With it we demonstrate that within TEMRA T cells, the chief producers of effector cytokines including IFN-γ, patients have a comparable proportion of IFN-γ^{+} cells to healthy donors. Furthermore, the addition of IL-7 into culture, promoting homeostatic proliferation, significantly upregulated the production of IFN-γ by CD4 TEMRAs in both patients and healthy donors, as expected from studies documenting memory-skew and increased effector cytokine/IFN-γ shift in mice [31]. Although such clinical correlation was not observed in our cohort, this Th1 bias may contribute to the increased prevalence of autoimmune arthritis seen in 22q11.2DS patients [2,32].

This data also points towards an increasing necessity of differentiating effector/memory T cells into tighter and smaller functional subpopulations, as demonstrated by the unbiased clustering algorithms in our study and by the substantial changes even within the CD4 TEMRA stage. For example, here IL-7 upregulated TIGIT and TOX, markers of cellular exhaustion [33,34], and caused loss of TCF1, a progenitor transcription factor [34,35], even when limiting the analysis to the canonically gated population of CD4 TEMRAs. This suggests further developmental stages in the CD4 TEMRA population, such as exhausted or senescent cells.

5. Conclusions

In summary, this work provides novel information on the effector functions and Th1/IFN-γ bias in pediatric and young adult patients with 22q11.2DS, and shows how the activity of IL-7 and homeostatic proliferation augments this bias. Its main limitation is the size of the cohort, which was primarily caused by the depth of investigations in each patient, but may limit the strength of its conclusions. Future studies mapping the impact of this Th1/IFN-γ bias on T-B interaction and providing further increased granularity (such as that provided by single-cell based CITE-Seq methods and others) in clinically well-characterized cohorts are needed.

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Authorship contributions

OV performed the experiments, analyzed data, interpreted results, and co-wrote the manuscript.

PV performed the experiments.

ZP assisted with experiment design.

MN assisted with experiment design.

RH performed RNA sequencing, reviewed the manuscript.

AS participated in the collection of biological material from patients, contributed to experiment design and reviewed the manuscript.

TK secured funding, contributed to experiment design and reviewed the manuscript.

AK designed the study, analyzed data, interpreted results, recruited patients, and co-wrote the manuscript.

Ethics approval

Written informed consent with participation in this study was obtained from all patients and control subjects in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments. The study was approved by Ethical Committee of the Motol University Hospital in Prague, Czech Republic (dated 19.6.2019).

Consent to participate

Written informed consent was obtained from all individual participants included in this study.

Consent to publish

No identifiable information is published as part of this study.

Declaration of Competing Interest

None.

Data availability

Data is available from authors upon reasonable request. Material is not available.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clim.2023.109793.

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