frontiers in Immunology

ORIGINAL RESEARCH
published: 24 April 2019
doi: 10.3389/fimmu.2019.00820

CD4$^+$ T-Cells With High Common γ Chain Expression and Disturbed Cytokine Production Are Enriched in Children With Type-1 Diabetes

Julia Seyfarth[1]*, *Nathalie Mütze*[1], *Jennifer Antony Cruz*[1], *Sebastian Kummer*[1], *Christina Reinauer*[1], *Ertan Mayatepek*[1], *Thomas Meissner*[1,2] *and Marc Jacobsen*[1]

[1] Department of General Pediatrics, Neonatology and Pediatric Cardiology, University Children's Hospital, Medical Faculty, Düsseldorf, Germany, [2] German Center for Diabetes Research (DZD), Partner Düsseldorf, Düsseldorf, Germany

The common gamma chain (γ_C) contributes to the formation of different cytokine receptors [e.g., IL-2 receptor (IL-2R), IL-7R, and IL-15R], which are important for generation of self-reactive T-cells in autoimmune diseases, like in type 1 diabetes (T1D). Whereas, the roles of membrane and soluble IL-2Rα and IL-7Rα variants in T1D disease pathogenesis are well-described, effects of γ_C expression and availability for dependent receptors remain elusive. We investigated expression of the γ_C and dependent receptors on T-cells and soluble γ_C concentrations in serum from patients with T1D ($n = 34$) and healthy controls ($n = 27$). Effector T-cell cytokines as well as IL-2, IL-7, and IL-15 induced STAT5 phosphorylation were analyzed to determine functional implications of differential γ_C expression of CD4$^+$ T-cell subsets classified by t-distributed Stochastic Neighbor Embedding (t-SNE) analyses. We found increased γ_C and IL-7Rα expression of CD4$^+$ T-cells from T1D patients as compared to controls. t-SNE analyses assigned differential expression to subsets of memory T-cells co-expressing γ_C and IL-7Rα. Whereas, γ_C expression was positively correlated with IL-2Rα in memory T-cells from healthy controls, no dependency was found for patients with T1D. Similarly, the effector T-cell cytokine, IL-21, correlated inversely with γ_C expression in healthy controls, but not in T1D patients. Finally, T1D patients with high γ_C expression had increased proportions of IL-2 sensitive pSTAT5$^+$ effector T-cells. These results indicated aberrantly high γ_C expression of T-cells from T1D patients with implications on dependent cytokine receptor signaling and effector T-cell cytokine production.

Keywords: biomarker, immunology, common gamma chain, interleukin-2, interleukin-7, type 1 diabetes, interleukin-15

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***Correspondence:**
Julia Seyfarth
julia.seyfarth@med.uni-duesseldorf.de

Specialty section:
This article was submitted to Autoimmune and Autoinflammatory Disorders, a section of the journal Frontiers in Immunology

Received: *11 December 2018*
Accepted: *28 March 2019*
Published: *24 April 2019*

Citation:
Seyfarth J, Mütze N, Antony Cruz J, Kummer S, Reinauer C, Mayatepek E, Meissner T and Jacobsen M (2019) CD4$^+$ T-Cells With High Common γ Chain Expression and Disturbed Cytokine Production Are Enriched in Children With Type-1 Diabetes. Front. Immunol. 10:820. doi: 10.3389/fimmu.2019.00820

INTRODUCTION

Type 1 diabetes (T1D) is an autoimmune disease characterized by destruction of pancreatic beta islet cells. Self-reactive effector T-cells are found in T1D and contribute to disease pathogenesis. Mainly T helper type (T_H) 1 cells, producing the key cytokines IFN-γ and TNF-α, were detected in T1D-specific pancreas islet inflammation (1), but also other CD4$^+$ T-cell subsets, e.g., producing IL-17 or IL-21, have been found at higher frequencies in peripheral blood from patients with T1D

(2, 3). IL-21 is a key cytokine of T follicular helper (T_{FH}) cells, which are central for B-cell support and may play a role for generation of auto-antibodies in T1D (2).

The T-cell repertoire of patients with T1D is generally prone to increased basic activation (4, 5). Possible explanations for this are increased regulation resistance of effector T-cells and/or impaired regulatory T-cell (T_{reg}) functions (6, 7). Members of the γ_c cytokine family, namely IL-2, IL-7, and IL-15 are crucially involved in T-cell regulation and the generation of effector T-cells. IL-2 and IL-7 exert largely contrary roles with IL-2 promoting T_{reg} cells and IL-7 being essential for generation of effector and memory T-cells (8). Especially the generation of self-reactive T-cells depends on IL-7 potentially by lowering the T-cell activation threshold (9). IL-15 is an IL-7 related cytokine with similar functions for $CD8^+$ T-cells and NK cells and potential relevance for autoimmunity (10).

Membrane IL-2Rα and IL-7Rα expression of T-cells affect their response to respective cytokines and both receptors are strongly regulated during T-cell activation and maturation (8). The relevance of IL-2Rα and IL-7Rα regulation for development of autoimmune diseases including T1D has been clearly shown (11–13). Both, IL-2R and IL-7R, are members of the γ_c cytokine receptor family, characterized by γ_c chain dependency for receptor formation and cytokine signaling. Only limited evidence for regulation of γ_c expression has been found so far. The majority of previous studies reported stable γ_c expression in T-cells and assumed that differences in γ_c availability are functionally irrelevant (14). However, there is some evidence for γ_c upregulation during T-cell activation and differential γ_c expression in T-cell subpopulations (15, 16). In addition, modified availability of γ_c, e.g., due to occupation of individual receptor chains, has been assumed to affect the response against γ_c cytokines (17, 18). In this regard, observations from patients with γ_c gene deficiency demonstrated that IL-7, IL-2 and IL-15 require different levels of γ_c for optimal signaling (19, 20). In support of these findings, Monti et al. showed that disengagement of the IL-2Rα chain with daclizumab enhanced T-cell responses for IL-7 and demonstrated the functional relevance of γ_c availability (21).

Evidence for a potential role of γ_c in T1D pathology comes from the study of Demirci et al. who showed that antibodies against γ_c prevented T1D disease onset in animal models (22). Chronic inflammatory diseases may also be affected by γ_c expression since increased serum concentrations of the soluble (s)γ_c variant were reported in human inflammatory bowel disease (23) and rheumatoid arthritis (24, 25).

In the present study, we compared expression of γ_c cytokine receptor chains between patients with T1D and matched healthy controls. Associations between individual chain expression and phenotype of different T-cell subpopulations were examined by t-distributed Stochastic Neighbor Embedding (t-SNE) analyses. Finally, T-cell activation induced intracellular cytokine expression pattern and cytokine induced STAT5 phosphorylation were characterized.

TABLE 1 | Baseline characteristics of children with T1D and healthy controls.

Characteristic	Healthy controls	T1D patients	*p*-Value
Number (*n*)	27	34	
Age (years)	13.0 [3.5–17.5]	13.9 [4.4–17.7]	0.888
Sex distribution (m/f)	15/12	20/14	0.798
Disease duration	na	5.5 years [0.9–14.7 years]	
HbA1c (%)	nd	7.7 [6.4–9.6]	
HbA1c (mmol mol^{-1})	nd	61 [46–81]	
C-peptide (μg l^{-1})	nd	<0.01 [<0.01–1.96]	

f, female; m, male; na, not applicable; nd, not defined. Median and [range] are given. P-values were based on the Mann-Whitney U-test (for the continuous variable age) and chi-squared test (for the categorical variable sex distribution).

RESULTS

γ_c and IL-7Rα Expression Levels Are Higher in $CD4^+$ Memory T-Cells From T1D Patients as Compared to Healthy Controls

We determined expression of IL-2R, IL-7R, and IL-15R chains on $CD4^+$ T-cells from children with T1D ($n = 34$) as well as healthy controls ("controls"; $n = 27$). Donor characteristics are summarized in **Table 1**. No differences in mean expression were detected for the IL-2Rα, the IL-2Rβ, and the IL-15Rα chain between the study groups (**Figure 1A**, upper graphs; for gating strategy see **Supplementary Figure 1A**). Interestingly, children with T1D had higher mean expression of IL-7Rα ($p = 0.006$) and γ_c ($p = 0.044$) on $CD4^+$ T-cells as compared to healthy controls (**Figure 1A**, bottom graphs). To further characterize affected T-cell subsets, we applied the unbiased approach of t-distributed Stochastic Neighbor Embedding (t-SNE) analysis for two-dimensional visualization of high-dimensional data (26). **Figure 1B** shows combined flowcytometry data of $CD4^+$ T-cells from T1D patients and controls (for gating strategy see **Supplementary Figures 1A,B**). Naïve and memory T-cells were classified by $CD45RA_{high}$ and $CD45RA_{low}$ expression, respectively (**Figure 1B**, left graph). γ_c high T-cells (top 10% according to mean γ_c expression) clustered almost exclusively within the memory $CD4^+$ T-cell subset (**Figure 1B**, right graph). This suggested higher γ_c expression in memory $CD4^+$ T-cells. Hence, we next compared γ_c expression between naïve and memory T-cells from both study groups. As expected, γ_c expression was generally higher in memory T-cells as compared to naïve T-cells (**Figure 1C**, $p < 0.001$, for T1D patients and controls). Study group comparisons revealed that higher γ_c expression was exclusively detected for memory T-cells of T1D patients ($p = 0.036$).

Identification of a γ_c and IL-7Rα High Expressing $CD4^+$ Memory T-Cell Subset Enriched in T1D Patients

To further characterize γ_c high memory T-cell subsets and to compare study groups, we performed t-SNE analyses for subgroups of patients with T1D and controls separately. Three main populations were identified with high γ_c expression (top

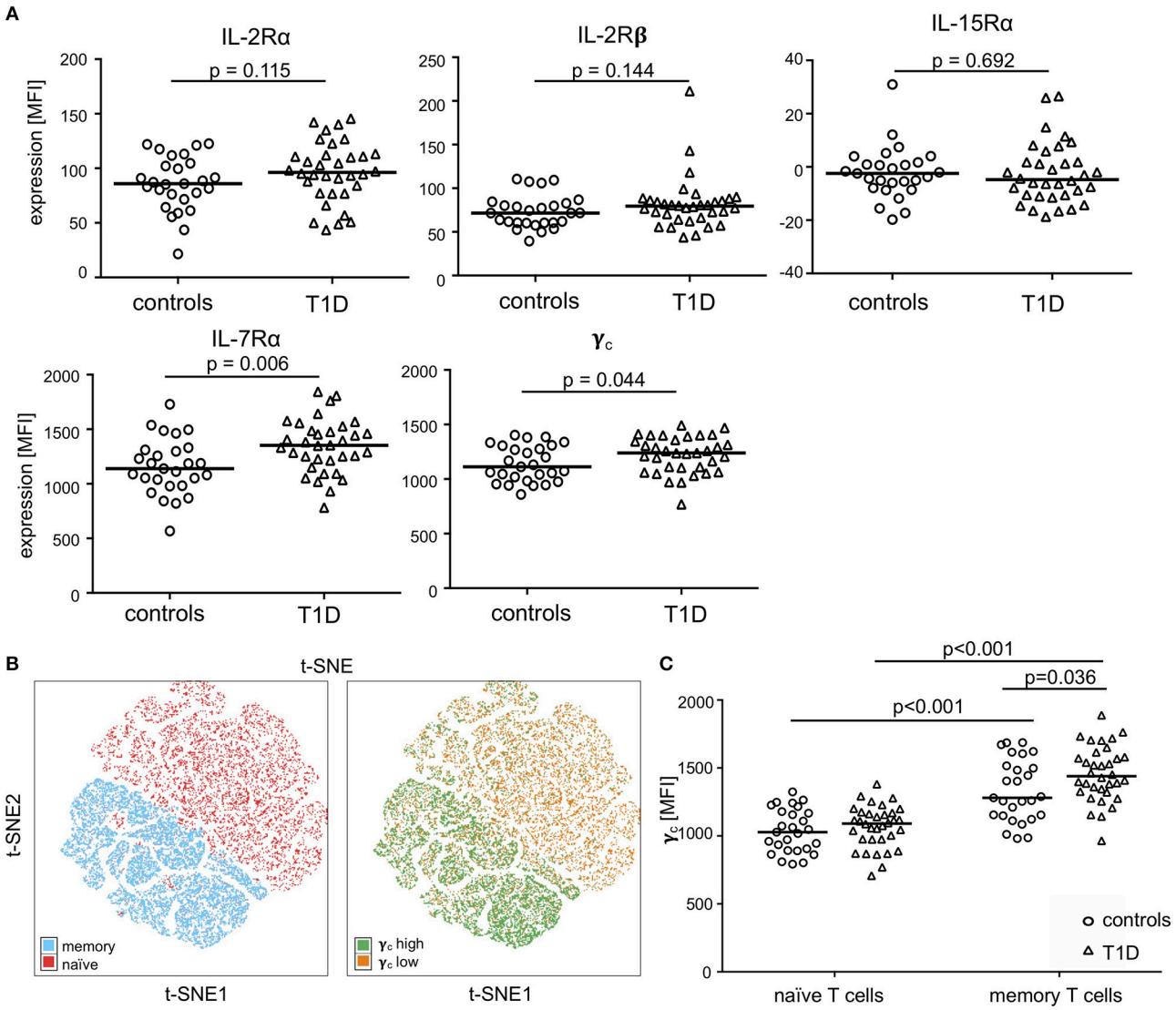

FIGURE 1 | Expression of the γ_c cytokine receptor chains on naïve and memory $CD4^+$ T-cells. **(A)** Expression of γ_c, IL-7Rα, IL-2Rα, IL-2Rß and IL-15Rα on $CD4^+$ T-cells of healthy children (controls, $n = 27$, open circles) and children with T1D (T1D, $n = 34$, open triangles) are shown as (geometric) mean fluorescence intensity (MFI). Each symbol represents the mean of triplicates for an individual donor. Median values of groups are indicated and nominal p-values of the two-tailed Mann-Whitney U-test are provided. **(B)** Unbiased t-distributed Stochastic Neighbor Embedding (t-SNE) analysis of a concatenated subgroup of $CD4^+$ T-cells from healthy controls ($n = 11$) and T1D patients ($n = 19$) illustrate distribution of naïve $CD45RA_{high}$ and memory $CD45RA_{low}$ (red and blue, respectively; left panel) and γ_c high (top 10% mean fluorescence of all $CD4^+$ cells; green) and γ_c low (bottom 90% mean fluorescence; orange) (right panel) $CD4^+$ T-cells. t-SNE calculates two-dimensional depiction of multi-factorial similarity. These two dimensions are characterized by t-SNE1 and t-SNE2 in given graphs. **(C)** γ_c expression of naïve $CD45RA_{high}$ and memory $CD45RA_{low}$ $CD4^+$ T-cells are shown for healthy controls ($n = 27$, open circles) and T1D patients ($n = 34$, open triangles). Median values of groups and statistically significant nominal p-values for the Mann-Whitney U-test (two-tailed) are indicated.

10% according to mean γ_c expression; for gating strategy see **Supplementary Figure 1B**) within $CD4^+$ memory T-cells for both study groups (**Figure 2A**, t-SNE plots). Higher similarity, indicated by distance in t-SNE principal component analyses, was suggested for high γ_c expressing subpopulations 1 and 2 in controls whereas subpopulations 2 and 3 were more similar in T1D patients (**Figure 2A**). In accordance, subpopulation 2 clustered in a region of IL-7Rα high expressing memory T-cells from patients (**Figure 2A**, upper right plot) whereas lower IL-7Rα expression characterized subpopulation 2 in healthy controls (**Figure 2A**, upper left plot; for gating strategy see **Supplementary Figure 1B**). γ_c high subpopulations showed comparable expression of IL-2Rα and IL-2Rβ between both study groups (**Figure 2A**; histograms), whereas higher IL-7Rα mean expression of γ_c high memory T-cells—especially for subpopulation 2—was detected for T1D patients (**Figure 2A**; histograms). Hence, we compared γ_c high and low T-cells for IL-7Rα expression between patients and controls. γ_c low T-cells expressed generally less IL-7Rα in both study groups as compared to γ_c high T-cells ($p < 0.001$ for patients and

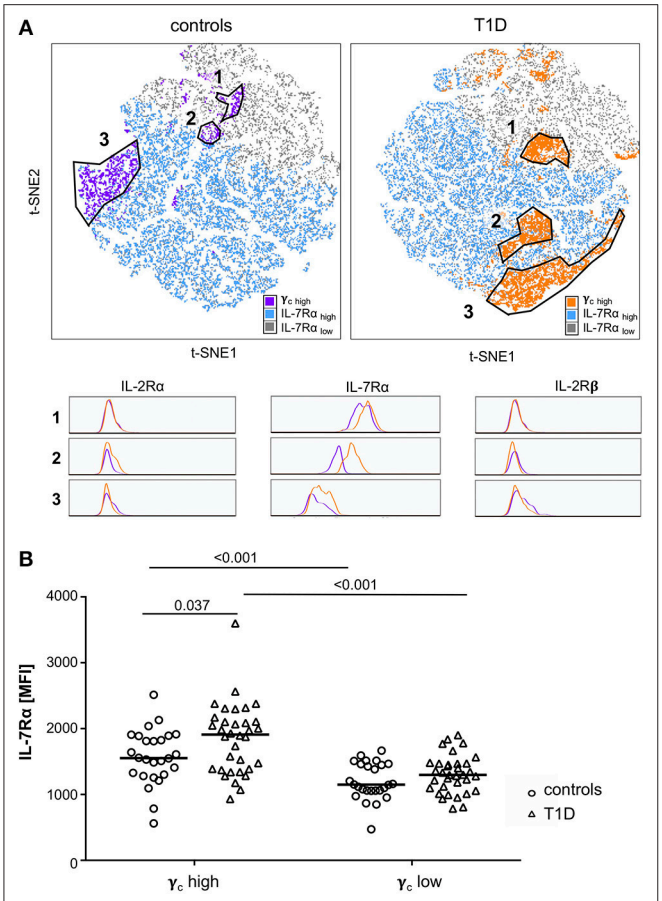

FIGURE 2 | Characterization of γ_c high expressing memory T-cell populations. **(A)** Unbiased t-distributed Stochastic Neighbor Embedding (t-SNE) analysis of memory $CD4^+$ T-cells (i.e., $CD45RA_{low}$) from healthy controls ($n = 20$, left graph) and T1D patients ($n = 25$, right graph). IL-7Rα high cells (blue), IL-7Rα low cells (gray), and γ_c high cells (purple for controls; orange for T1D patients) are illustrated. γ_c high populations (top10% mean fluorescence of all $CD4^+/CD45RA_{low}$ cells) of controls and patients were gated (populations 1, 2, and 3) and compared for the respective IL-2Rα, IL-7Rα and IL-2Rβ expression (histograms). **(B)** IL-7Rα expression of γ_c high and γ_c low cells is shown for healthy controls ($n = 27$, open circles) and T1D patients ($n = 34$, open triangles). Median values of groups and statistically significant nominal p-values for the Mann-Whitney U-test (two-tailed) are indicated.

controls) and no differences were found for γ_c low T-cells between study groups (**Figure 2B**). In contrast γ_c high T-cells from patients with T1D expressed significantly higher IL-7Rα levels as compared to healthy controls ($p = 0.037$; **Figure 2B**). These results indicated that γ_c/IL-7Rα high co-expressing T-cell proportions were enriched in T1D patients.

Loss of Correlation of γ_c With IL-2Rα on Memory $CD4^+$ T-Cells From Patients With T1D

Availability of γ_c has been shown to affect T-cell cytokine signaling for γ_c family members (19, 21). Therefore, we next measured relative expression of γ_c cytokine receptor chains in individual donors. γ_c did not show significant correlation with IL-7Rα expression for $CD4^+$ T-cells from controls or patients with T1D (**Figure 3A**, upper graphs). Similar results were gained for the IL-2Rβ chain (**Supplementary Figure 2**). In contrast, γ_c correlated positively with IL-2Rα chain expression for healthy controls ($r = 0.52$, $p = 0.006$), whereas no correlation between γ_c and IL-2Rα was detectable for patients ($r = 0.16$, $p = 0.379$) (**Figure 3A**, lower graphs). Since differential γ_c expression was only found for memory T-cells, we next compared γ_c and IL-2Rα on $CD4^+$ naïve and memory subsets. We found significant correlation between γ_c and IL-2Rα for both naïve and memory T-cells of healthy controls ($r = 0.47$, $p = 0.015$; $r = 0.54$, $p = 0.004$, respectively) (**Figure 3B**, left graphs). In patients, however, only naive T-cells showed a moderate correlation ($r = 0.35$, $p = 0.044$) whereas no correlation was detectable for memory $CD4^+$ T-cells ($r = 0.10$, $p = 0.591$) (**Figure 3B**, right graphs). We concluded that differential γ_c expression of patients with T1D abrogated positive correlation with IL-2Rα on memory T-cells found in healthy controls.

Negative Correlation of γ_c With Cytokine Expression of $CD4^+$ Memory T-Cells Is Absent in Patients With T1D

To evaluate if dysregulated γ_c expression in memory T-cells from T1D patients affects $CD4^+$ T-cell function, we next assessed *in vitro* T-cell activation induced cytokine production for T1D patients and controls. IFNγ, IL-21, TNFα, and IL-2 expressing $CD4^+$ memory T-cell proportions (see **Supplementary Figure 3A** for gating procedures) were compared with individual γ_c expression on memory $CD4^+$ T-cells. For healthy controls, we detected negative correlation of γ_c expression with IL-21 producing $CD4^+$ memory T-cells ($r = -0.48$, $p = 0.027$) and a tendency for IFNγ ($r = -0.37$, $p = 0.087$) (**Figure 4**, left panel). In contrast, patients showed no correlation of γ_c expression with IL-21 or IFNγ producing $CD4^+$ memory T-cells (**Figure 4**, right panel). No correlations were found for γ_c expression and TNFα/IL-2 expression, neither for controls nor for T1D patients (**Supplementary Figure 3B**). This suggested that the high γ_c expression in T1D abrogated the negative association between γ_c expression of $CD4^+$ memory T-cells and IL-21 production found in controls.

Type 1 Diabetes Patients With High γ_c Expression on $CD4^+$ T-Cells Have Increased Proportions of IL-2 Induced pSTAT5 Positive T-Cells

To address the question of functional impacts on cytokine signaling, we measured IL-2, IL-7, and IL-15 induced STAT5 phosphorylation of $CD4^+$ T-cells from both study groups. The gating procedure of cytokine induced pSTAT5 expression is depicted in **Supplementary Figure 4A**. IL-7 induced pSTAT5 in the vast majority of $CD4^+$ T-cells (median: 92.6%) and no differences between the study groups were detected (**Figure 5A**, left graph). In contrast, IL-2 increased the proportion of pSTAT5 positive T-cells in T1D patients as compared to healthy controls (**Figure 5**, middle graph; $p = 0.045$) whereas proportions were not significantly different after IL-15 stimulation (**Figure 5A**, right graph). Interestingly, mean pSTAT5 expression of positive

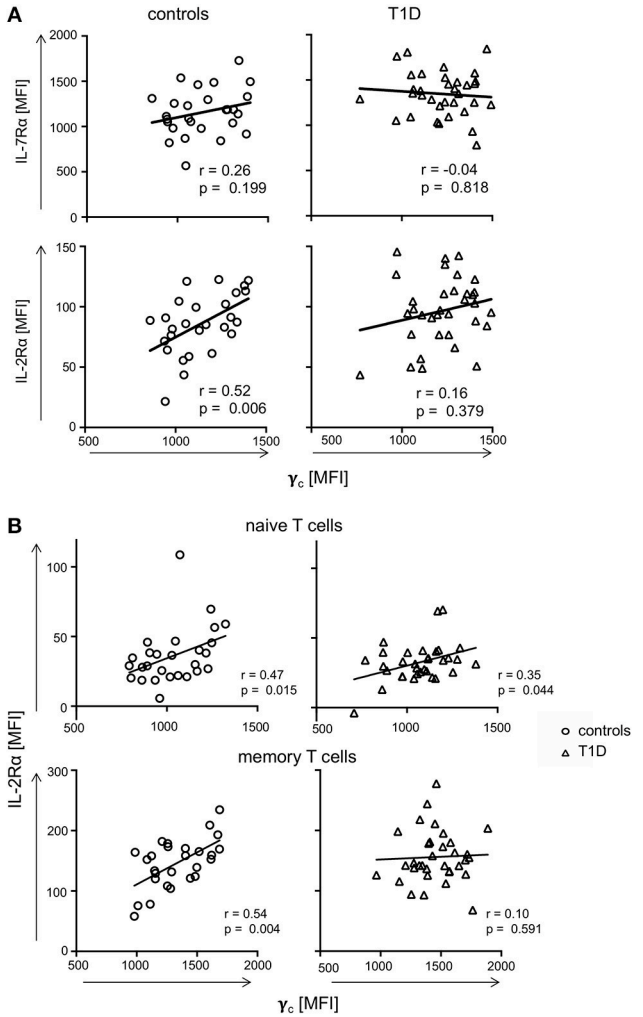

FIGURE 3 | Correlations of γ_c with IL-7Rα and IL-2Rα. **(A)** Correlations of IL-7Rα (upper panels) and IL-2Rα (lower panels) with γ_c on $CD4^+$ T-cells are shown for healthy controls (left graphs, $n = 27$, open circles) and T1D patients (right graphs, $n = 34$, open triangles). **(B)** Correlations of IL-2Rα with γ_c on naïve (i.e., $CD45RA_{high}$ $CCR7_{high}$) (upper panels) and memory $CD45RA_{low}$ (lower panels) $CD4^+$ T-cells are shown for healthy controls (left panels, $n = 27$, open circles) and T1D patients (right panels, $n = 34$, open triangles). **(A,B)** Spearman rank correlation coefficients *r* and *p*-values are indicated. A trend line was fitted by linear regression analysis.

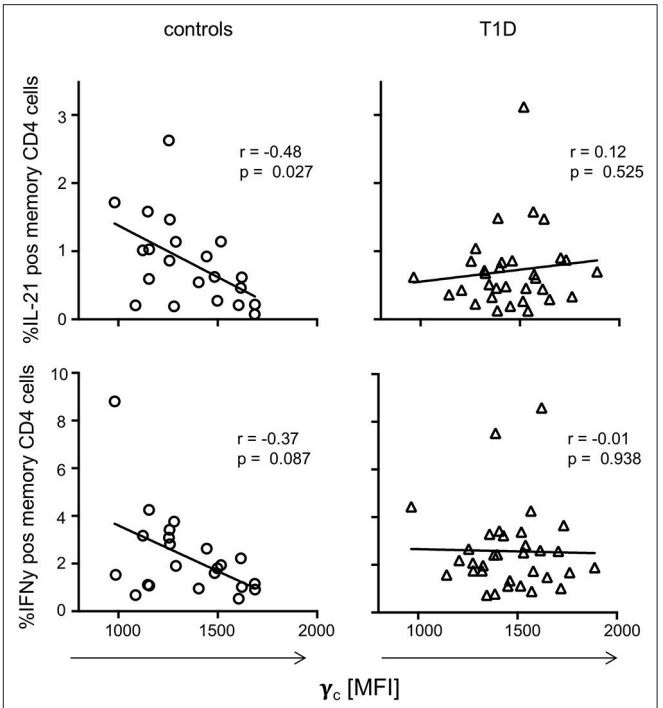

FIGURE 4 | γ_c expression and cytokine production on T cell receptor stimulation. Correlation between γ_c expression on $CD4^+$ memory T-cells and *in vitro* activated cytokine expressing memory $CD4^+$ T-cells (upper graphs: IL-21, bottom graphs: IFNy) are shown for healthy controls ($n = 21$, left graphs) and patients with T1D ($n = 33$, right graphs). Spearman rank correlation coefficients (*r*) and respective *p*-values are indicated. A trend line was fitted by linear regression analysis.

T-cells was similar between the study groups independent of the respective cytokine (**Figure 5B**). This suggested that patients with T1D have more IL-2 sensitive $CD4^+$ T-cells than controls but no differences in the IL-2 induced signaling intensity as compared to control T-cells.

Procedures used for measurement of phosphorylated proteins by flow cytometry rendered concomitant cytokine receptor analyses not feasible. However, to identify potential effects of differential γ_c expression between the study groups on IL-2 induced signaling, we correlated both in patients with T1D patients and controls. Whereas, no correlation of γ_c expression and IL-2 induced signaling was found for healthy controls (**Figure 5C**; left graph), a significant positive correlation was found for γ_c expression and IL-2 induced pSTAT5 proportions for patients ($r = 0.42$; $p = 0.015$; **Figure 5C**, right graph).

These findings may be caused by different proportions of γ_c low or high T-cells. To address this question, we classified T1D patients and healthy controls as γ_c low or high (for details see Methods) and compared subgroups for pSTAT5 positive T-cell proportions. Comparisons of IL-2 induced pSTAT5 positive T-cells showed increased proportions in $CD4^+$ T-cells from γ_c high as compared to γ_c low T1D patients ($p = 0.004$) (**Figure 5D**) and a similar tendency was seen for IL-15 ($p = 0.068$) (**Supplementary Figure 4B**, right graph). Notably, no differences were seen when comparing IL-2/IL-15 induced γ_c high and γ_c low healthy controls (**Figure 5D**). IL-7 induced pSTAT5 proportions were also similar between γ_c high and low individuals as well as between both study groups (**Supplementary Figure 4B**, left graph). Differences between the study groups were not due to differential mean γ_c since γ_c high and γ_c low from the study groups had comparable values (**Supplementary Figure 5A**). Furthermore, regulatory T (T_{reg}) cells were likely not causative for differential IL-2/IL-15 response since T_{reg} proportions (for gating strategy see **Supplementary Figure 5B**) were similar between γ_c high and γ_c low subgroups (**Supplementary Figure 5B**). These results argued for an increased number of $CD4^+$ T-cells with increased

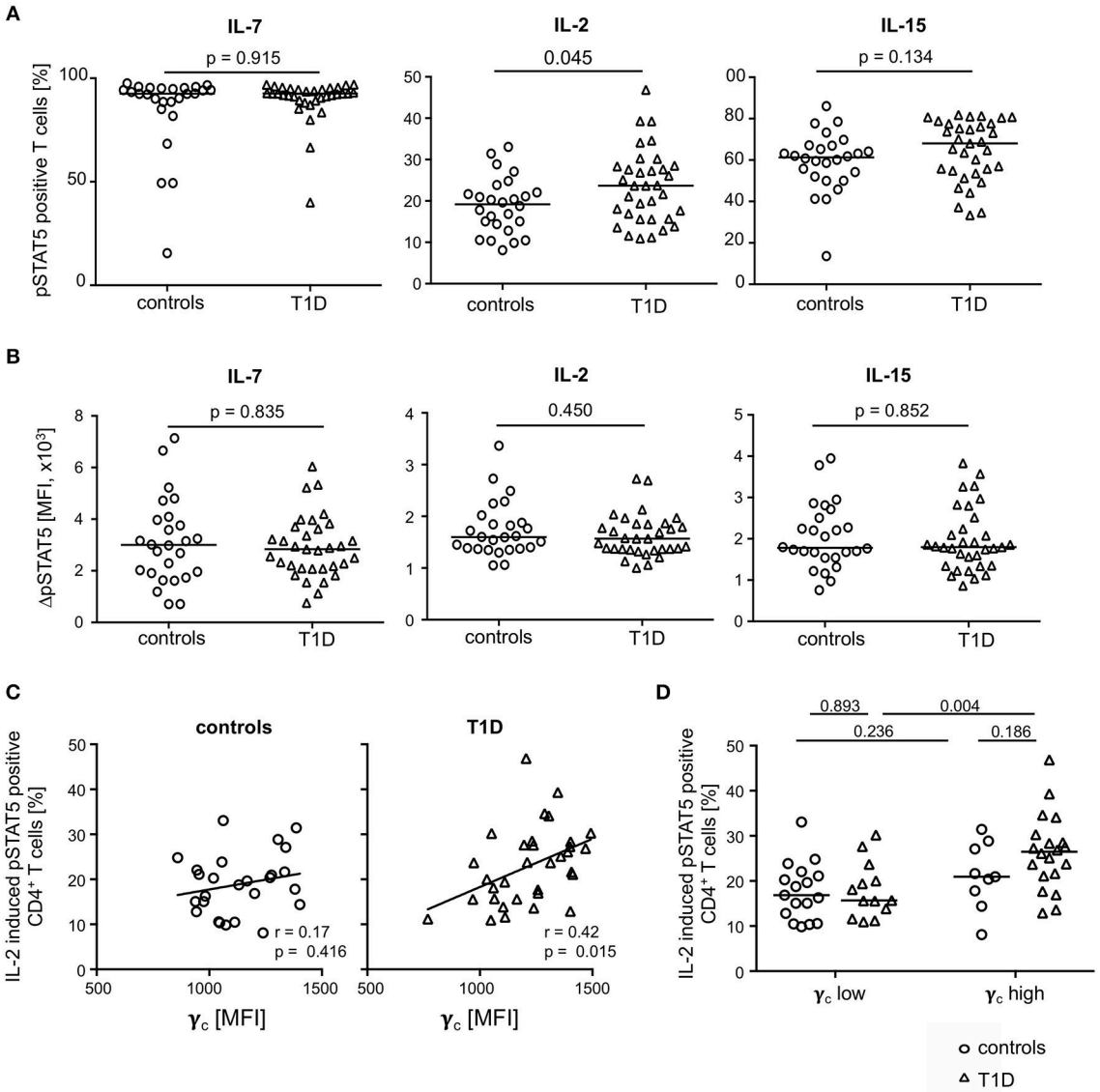

FIGURE 5 | Cytokine induced STAT5 phosphorylation in $CD4^+$ T-cells from healthy controls and patients with T1D. Proportions of pSTAT5 positive $CD4^+$ T-cells **(A)** and ΔpSTAT5 mean fluorescence intensity (MFI) of $CD4^+$ T-cells **(B)** induced by IL-7 (left graph), IL-2 (middle graph) and IL-15 (right graph) are shown for healthy controls ($n = 26$) and T1D patients ($n = 34$). ΔpSTAT5 MFI was calculated as follows: MFI (pSTAT5 positive cells)—MFI (unstimulated cells). Every symbol represents the mean of duplicates measured for an individual donor. Median values of groups and nominal *p*-values for the Mann-Whitney U-test (two-tailed) are indicated. **(C)** Correlations between γ_c expression on $CD4^+$ T-cells and proportions of IL-2 induced pSTAT5 positive $CD4^+$ T-cells are shown for controls (left panel) and children with T1D (right panel). Spearman rank correlation coefficients (*r*) and respective *p*-values are indicated. A trend line was fitted by linear regression analysis. **(D)** Study participants were stratified into a "γ_c low" and a "γ_c high" subgroup according to γ_c expression (below or above the median γ_c MFI expression) on $CD4^+$ T-cells. Proportions of pSTAT5 positive $CD4^+$ T-cells were compared between healthy controls and T1D and between both subgroups. Median values of groups and nominal *p*-values for the Mann-Whitney U-test (two-tailed) are indicated.

sensitivity for IL-2 (partly IL-15) in type 1 diabetes patients with higher γ_c expression.

No Differences of Soluble γ_c Serum Levels Between T1D Patients and Controls and No Correlation With Membrane-Associated γ_c Expression

In previous studies, soluble γ_c levels in serum were shown to be affected in autoimmune pathologies (23, 24). Hence, we compared soluble γ_c concentrations in serum from T1D patients and healthy controls. Soluble γ_c concentrations differed strongly between individuals and the vast majority of individuals from both study groups (16/27 = 59.3%, 16/33 = 48.5% of patients and controls, respectively) had no detectable soluble γ_c concentrations in serum (**Figure 6A**). No significant differences between study groups were found ($p = 0.357$) (**Figure 6A**). Finally, membranous γ_c expression was not correlated with soluble γ_c concentrations ($r = 0.21$, $p = 0.115$) (**Figure 6B**).

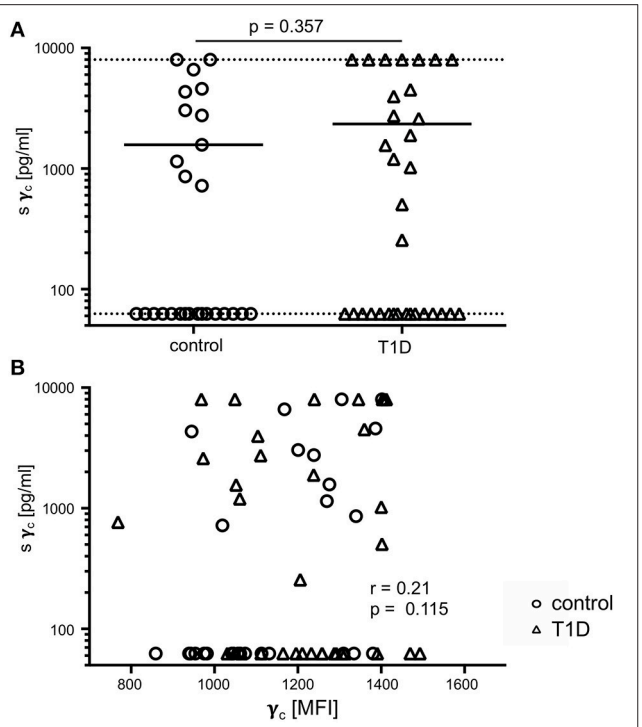

FIGURE 6 | Soluble (s) and membrane-associated γ_c expression. **(A)** Serum concentrations of sγ_c measured by ELISA are shown for healthy controls (n = 27, open circles) and T1D patients (n = 33, open triangles). The dotted lines indicate the upper and lower detection limit. Every symbol represents the mean of duplicates measured for an individual donor. Mean values of groups and the nominal p-value for the Mann-Whitney U-test (two-tailed) are indicated. **(B)** Correlation of serum concentrations of sγ_c with membrane-associated γ_c on $CD4^+$ T-cells are shown (open circles: healthy controls, open triangles: patients with T1D). Spearman rank correlation coefficient (r) and p-value are indicated.

These results indicated no association between membranous γ_c expression and soluble γ_c serum levels.

DISCUSSION

T1D-specific differences in cytokine expression and activation pattern of memory T-cell populations have been described and a role of γ_c cytokines IL-2 as well as IL-7 is likely. T-cell sensitivity for IL-2 and IL-7 depends on IL-2Rα and IL-7Rα receptor expression levels (8). Furthermore, initial studies indicated a role of the shared γ_c receptor and its soluble variant in autoimmune pathogenesis (22, 25, 27). Therefore, we performed a case-control study and identified higher γ_c and IL-7Rα chain expression on $CD4^+$ T-cells from T1D patients. Notably, γ_c expression of memory $CD4^+$ T-cells from patients with T1D was accompanied by the absence of positive correlation with the IL-2Rα found for healthy controls. Since this positive correlation was preserved in naïve T-cells from patients with T1D, we concluded that T1D-specific changes of γ_c expression occur during effector and memory T-cell generation. The absence of correlation between γ_c and IL-2Rα together with generally increased γ_c expression suggested T1D-specific γ_c up-regulation in IL-2Rα low or medium T-cells. In accordance, t-SNE analysis pointed toward γ_c up-regulation in memory T-cells with moderate IL-2Rα expression and concomitantly high IL-7Rα and γ_c expression of memory T-cells from T1D patients.

So far, only few studies focused on γ_c expression of T-cells in autoimmune and inflammatory diseases. This may be explained by the prevailing assumption that γ_c expression is constitutive and not transcriptionally regulated (14). However, several studies report upregulation of γ_c expression after cytokine and T-cell receptor activation (15, 16, 27) or during infections (16, 28). Mechanistically, γ_c was shown to be stored intracellularly and to be translocated to the plasma membrane after T-cell activation (16, 29). Causative mechanisms underlying higher γ_c expression of memory T-cells from patients with T1D could not be addressed in the present study since cytokine receptor chains were only measured on the plasma membrane. Comparison of intracellular γ_c expression levels as well as on the mRNA level would be needed to reveal potential causes.

To elucidate the impact of increased γ_c expression on T-cell function, we correlated γ_c expression with memory $CD4^+$ T-cell cytokine production in T1D patients and healthy controls. We found a negative association of γ_c expression with IL-21 production exclusively in healthy controls whereas no correlation was found for children with T1D. Although not significant, similar results were gained for IFN-γ positive T-cells. These results suggested that, under "healthy" conditions, high γ_c expression levels are found in donors with low IL-21 expression, indicating negative effects of γ_c high expressing cells on IL-21 cytokine production. Abrogation of γ_c negative correlation could indicate that γ_c high IL7Rα co-expressing T-cells produce IL-21 or promote IL-21 producing T-cells in T1D patients. Accordingly, previous studies suggested that IL-21 significantly contributes to T1D development (30). Direct proof for IL-21 promoting effects of γ_c high T-cells from T1D patients would have required FACS-based sorting of γ_c high and low T-cell subsets from children with T1D and healthy controls. This approach and additional experiments to elucidate underlying mechanisms were not feasible because of the limited blood sample volume available from participating children.

Indication of a potential functional impact of differential γ_c expression is provided by pSTAT5 analyses and identification of higher IL-2 (partly IL-15) sensitivity in a subset of $CD4^+$ T-cells from T1D patients characterized by high γ_c expression. Own previous studies had indicated higher IL-7 sensitivity of effector memory T-cells from T1D patients leading to increased T-cell activation in the presence of IL-7 (31). Hence, we assumed that enhanced IL-7 mediated T-cell responses in T1D patients could be due to higher γ_c levels. However, no differences in IL-7 induced STAT5 phosphorylation were found between the study groups and similar pSTAT5 levels were seen for subgroups classified for differential γ_c within T1D patients and controls. Although IL-7Rα was increased in T cells from T1D patients, these results did not suggest an association between γ_c expression and IL-7 mediated T-cell responses. In contrast, we detected increased IL-2 (and a tendency for IL-15) sensitive γ_c high $CD4^+$ T-cell proportions in T1D patients. Although direct association analyses were not possible in STAT5 phosphorylation

assays, we concluded that cytokine sensitivity of γ_c high T-cell proportions promoted IL-2 (IL-15) rather than IL-7. Evidence for a role of differential γ_c-dependent γ_c cytokine signaling come from several animal models including experimental autoimmune encephalitis (EAE) and rheumatoid arthritis (25, 27, 32–34). Hong et al. assessed implications of the sγ_c chain on cytokine signaling and found that this inhibitory variant especially blocked IL-2R signaling probably by binding to the IL-2Rß chain and preventing the association with membrane γ_c proteins (27). Sγ_c mediated impaired IL-2 response of T cells caused increased IL-17 production and worsening of EAE and arthritis symptoms (25, 27). Here we detected a promoted IL-2 response in T1D patients with high membrane γ_c expression. Since Th17 differentiation is negatively regulated by IL-2 signaling (35), future studies will address the question if IL-17 expression is impaired in high γ_c expressing T cells. This may contribute to the recent assumption that Th17 cells may be increased, but also decreased in the context of T1D (36).

Previous studies focused on limiting membrane γ_c expression levels and competition of γ_c family cytokines under such circumstances. Smyth et al. showed, that under conditions where the availability of γ_c is limiting (in patients with x-SCID and trace amounts of correctly spliced γ_c), IL-2 and IL-15 stimulation was preserved. In contrast, IL-7 stimulation did not induce pSTAT5, suggesting that IL-2/IL-15 signaling needs less γ_c expression as compared to IL-7R-mediated signaling (19). Hierarchies of cytokine responses may be due to differential capacity to bind γ_c and thereby reduce γ_c availability for other family members (18). Future studies are necessary to address the question, how increased γ_c expression differentially favors γ_c cytokine signaling.

Since previous reports indicated a role of soluble γ_c in autoimmunity, we measured soluble γ_c concentrations in the serum of patients with T1D and healthy controls. In accordance with previous studies, marked variability in soluble γ_c serum concentrations was found (23), however, no differences between T1D patients and controls were detected.

In summary, our study suggests a potential role of membranous γ_c expression in memory T-cells for T1D pathophysiological mechanisms. Future studies will have to shed light on the question, how dysregulated γ_c expression is involved in T1D development and maintenance.

METHODS

Donor Characteristics

We recruited children and adolescents with T1D ($n = 34$) and healthy controls ($n = 27$) at the University Children's Hospital, Duesseldorf, Germany. Children with T1D had clinical manifestation more than 11 months ago. The control group consisted of children with negative history for autoimmune and systemic inflammatory diseases. Study group characteristics are given in **Table 1**.

Surface Staining of γ_c Receptor Chains

For *ex vivo* surface staining, 100 µl of blood was immediately diluted in equal parts with DPBS. After centrifugation, the cell pellet was stained with an antibody mixture containing the following antibodies: For identification of T helper cells, we included CD4-BV510 (OKT4, Biolegend) and CD8-BV785 (RPA-T8, Biolegend). For identification of naïve, central and effector memory T-cells, we used CD45RA-FITC (HI100, Biolegend) and CCR7-PE-Cy7 (3D12, BD). In addition, the γ_c cytokine receptors IL-7Rα AF700 (clone A019D5, Biolegend), IL-2Rα-PerCP/Cy5.5 (BC96, Biolegend), IL-2Rβ (CD122)-PE (TU27, Biolegend), IL-15Rα (CD215)-APC (JM7A4, Biolegend), and γ_c (CD132)-PE-CF594 (TUGh4, BD) were included. Fixable viability dye eFluor780 (Thermo Fisher Scientific) was used to exclude dead cells. Staining was performed in triplicates. Sample measurement was performed on a LSR Fortessa flow cytometer (BD Biosciences). For data analysis FlowJo software (Miltenyi Biotech) was used. The gating procedure is depicted as **Supplementary Figures 1A,B**. Dead cells (viability marker positive cells) were excluded.

t-SNE Analysis

t-distributed Stochastic Neighbor Embedding (t-SNE) analysis (26) was done by using a plugin in FlowJo v10.4. t-SNE calculations were performed with 1,000 iterations, a perplexity of 20, an Eta (learning rate) of 200 and a Theta of 0.5. t-SNE visualizes similarities of cells in a 2D plot, illustrating their proximity by their distances in the t-SNE map. This method has been previously used to visualize different cellular subpopulations (24). In other words, each dot in the t-SNE plot represents a cell from an individual study participant and the distance between two dots/cells indicates their similarity. E.g., each T-cell population marked in **Figure 2A** because of high γ_c expression contains memory $CD4^+$ T-cells with high similarity. In contrast, different populations (i.e., 1, 2, and 3) share features (here high γ_c expression) but are different in other parameters (here IL-7Rα) expression.

t-SNE calculates two-dimensional depiction of multi-factorial similarity. These two dimensions are characterized by t-SNE1 and t-SNE2 in given graphs. γ_c expression was classified as 'high' or 'low' by an arbitrary threshold of top 10% or bottom 90% of γ_c mean fluorescence expressing $CD4^+$ (**Figures 1B,C**) or $CD4^+/CD45RA_{low}$ (**Figure 2**) cells (for gating strategy see **Supplementary Figure 1B**).

For t-SNE analysis of a concatenated subgroup of $CD4^+$ T-cells from healthy controls ($n = 11$) and T1D patients ($n = 19$) (**Figure 1B**) the following parameters were included: CD45RA and CCR7 (to identify naïve and memory T-cell subsets); IL-2Rα, IL-7Rα, IL-2Rβ, γ_c (to assess γ_c cytokine receptor expression and classify regulatory and effector T-cells). In a second step, t-SNE analysis was performed separately for $CD45RA_{low}$ memory $CD4^+$ T-cells from healthy controls ($n = 20$) and T1D patients ($n = 25$) (**Figure 2A**, left and right graph) and the following parameters were included: IL-2Rα, IL-7Rα, IL-2Rβ, γ_c (to assess γ_c cytokine receptor expression); CCR7 (to distinguish central memory and effector memory subpopulations).

T-Cell Restimulation and Intracellular Cytokine Analysis

PBMC were immediately isolated by density gradient centrifugation with Biocoll (Biochrom AG) according to

manufacturer's instructions. Without cryopreservation or batching, 2×10^5 PBMC were cultured for a period of 14 h to harmonize experimental conditions between donors. This allowed sample processing on the next day independent of the respective bleeding time point and avoided suboptimal long *in vitro* restimulation for intracellular cytokine detection. Indirect effects of non-T cells during the pre-incubation period cannot be excluded, but since both groups were treated the same, a bias between T1D patients and controls is not likely. PBMC were then stimulated with 1µl/well human T-activator CD3/CD28 Dynabeads (Gibco) for 6 h. Golgi inhibitor Brefeldin A was added after 1 h. Thereafter cells were harvested and stained with Viability Dye dFluor e780 (eBioscience) following manufacturer's instructions. For intracellular cytokine analysis, cells were fixed and permeabilized using Cytokix/Cytoperm Buffer (BD Biosciences) according to manufacturer's instruction. Cells were stained using the following antibodies: CD4-BV510 (OKT4, Biolegend), CD8-BV785 (RPA-T8, Biolegend), CD45RA-FITC (HI100, Biolegend), CCR7-PeCy7 (3D12, BD), IFNγ-V450 (B27, BD), IL-21-PE (3A3-N2, Biolegend), TNFα-AF700 (Mab11, BD), and IL-2-PerCPCy5.5 (MQ1-17H12, Biolegend). Staining was performed in triplicates. Proportions of cytokine positive $CD45RA_{neg}$ $CD4^+$ memory T-cells were determined. Naïve $CD4^+$ T-cells hardly produced cytokines after CD3/CD28 re-stimulation (data not shown). For data analysis FlowJo software (Miltenyi Biotech) was used. The gating procedure is depicted as **Supplementary Figure 3A**. Viability dye positive T-cells were excluded from further analyses. However, the proportion of these dead cells were low indicating no negative effects of the 14 h pre-incubation on cellular viability. Generally, T-cell proportions are calculated. Cytokine-producing T-cells in the present study were based on considerable T-cell numbers per well (median $CD4^+$ T cell count: 9,577 cells) and CD3/28 induced cytokine-producing T-cell proportions ranged from 0.6% (median for IL-21) to 7.5% (median for IL-2). Therefore, cell numbers used for frequency calculations are sufficient to exclude overestimation of differences. The investigator was blinded to the group allocation when analyzing the data.

STAT5 Phosphorylation

2×10^5 PBMC were cultured for a period of 14 h. Afterwards, PBMC were stimulated with IL-2 (10IU/ml) or IL-7 (1ng/ml) for 15 min at 37°C and 5%CO_2. Then, cells were fixed using true nuclear fixation buffer and permeabilized with methanol as described previously (37). Samples were then centrifuged, washed and stained with the following antibodies: CD4-AF700 (clone RPA-T4, Biolegend) and pSTAT5-PE (eBioscience). Stimulation and staining were performed in duplicates. For data analysis FlowJo software (Miltenyi Biotech) was used. After gating on $CD4^+$ T-cells, the following gating procedure is depicted as **Figure 4A**. ΔpSTAT5 MFI was calculated as follows: MFI (pSTAT5 positive cells)—MFI (unstimulated cells). To assess STAT phosphorylation against the background of γ_c expression, study participants were stratified into "γ_c low" and a "γ_c high" subgroups according to γ_c expression on $CD4^+$ T-cells: γ_c low (below the median γ_c MFI expression), γ_c high: (≥median γ_c MFI expression of all study participants).

Measurement of Soluble γ_c

Study participants serum was harvested and immediately stored at −80°C. After simultaneous thawing, soluble γ_c was measured using the Human Common gamma Chain/IL-2R gamma Duo Set ELISA kit (R&D) according to the manufacturer's instructions. All samples were analyzed in duplicates using an Infinite M200 ELISA reader (Tecan). Concentrations were calculated from respective standard curves on every plate by applying 4-parametric logistic regression. Samples outside the detection range were set to the corresponding lower (62.5 pg/ml) or upper range (8,000 pg/ml) value.

Statistical Analysis

Graph Pad Prism 7 (Version 7.0a, GraphPad Software, La Jolla, CA) software was used for statistical analyses and figure preparation. Because of moderate study group sizes non-parametric distributions were assumed and statistical tests were chosen accordingly. The non-parametric Mann-Whitney U-test (two-tailed) was used to compare continuous characteristics of two study groups, the chi-squared test was used for categorical variables. For correlation analyzes, Spearman's correlation was used. *P*-values below 0.05 were considered statistically significant.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the Ethical Committee of the Medical Faculty of the Heinrich-Heine-University Duesseldorf, Germany (ID 4844) with written informed consent from all subjects. All subjects (older than 14 years) and their legal guardians gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Ethical Committee of the Medical Faculty of the Heinrich-Heine-University Duesseldorf, Germany.

AUTHOR CONTRIBUTIONS

JS designed the study, performed experiments, analyzed and interpreted data, and wrote the manuscript. NM performed experiments, analyzed data, reviewed, and edited the manuscript. JAC, SK, and CR recruited patients and revised the manuscript. TM and EM contributed to the conceptualization and reviewed/edited the manuscript. MJ designed the study, analyzed and interpreted data, and wrote the manuscript. All of the contributing authors approved the final version of the manuscript. JS is the guarantor of this work, and as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

FUNDING

This work was supported in part by a grant from the German Federal Ministry of Education

and Research (BMBF) to the DZD (Pediatric diabetes biomaterial bank 2.0, grant number 82DZD01802). This study was supported in part by a grant from the Elternitiative Kinderkrebsklinik e.V. to JS.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2019.00820/full#supplementary-material

REFERENCES

1. Kent SC, Chen Y, Bregoli L, Clemmings SM, Kenyon NS, Ricordi C, et al. Expanded T cells from pancreatic lymph nodes of type 1 diabetic subjects recognize an insulin epitope. *Nature.* (2005) 435:224–8. doi: 10.1038/nature03625
2. Liu SM, King C. IL-21-producing Th cells in immunity and autoimmunity. *J Immunol.* (2013) 191:3501–6. doi: 10.4049/jimmunol.1301454
3. Baharlou R, Ahmadi-Vasmehjani A, Davami MH, Faraji F, Atashzar MR, Karimipour F, et al. Elevated levels of T-helper 17-associated cytokines in diabetes type i patients: indicators for following the course of disease. *Immunol Invest.* (2016) 45:641–51. doi: 10.1080/08820139.2016.1197243
4. Petersen LD, Duinkerken G, Bruining GJ, van Lier RA, de Vries RR, Roep BO. Increased numbers of *in vivo* activated T cells in patients with recent onset insulin-dependent diabetes mellitus. *J Autoimmun.* (1996) 9:731–7. doi: 10.1006/jaut.1996.0095
5. Bian ML, Haigh O, Munster D, Harris M, Cotterill A, Miles JJ, et al. Reactivated CD4+Tm cells of T1D patients and siblings display an exaggerated effector phenotype with heightened sensitivity to activation-induced cell death. *Diabetes.* (2015) 64:2161–71. doi: 10.2337/db14-1151
6. Schneider A, Rieck M, Sanda S, Pihoker C, Greenbaum C, Buckner JH. The effector T cells of diabetic subjects are resistant to regulation via CD4+ FOXP3+ regulatory T cells. *J Immunol.* (2008) 181:7350–5. doi: 10.4049/jimmunol.181.10.7350
7. Long SA, Cerosaletti K, Bollyky PL, Tatum M, Shilling H, Zhang S, et al. Defects in IL-2R signaling contribute to diminished maintenance of FOXP3 expression in CD4(+)CD25(+) regulatory T-cells of type 1 diabetic subjects. *Diabetes.* (2010) 59:407–15. doi: 10.2337/db09-0694
8. Read KA, Powell MD, McDonald PW, Oestreich KJ. IL-2, IL-7, and IL-15: multistage regulators of CD4(+) T helper cell differentiation. *Exp Hematol.* (2016) 44:799–808. doi: 10.1016/j.exphem.2016.06.003
9. Deshpande P, Cavanagh MM, Le Saux S, Singh K, Weyand CM, Goronzy JJ. IL-7- and IL-15-mediated TCR sensitization enables T cell responses to self-antigens. *J Immunol.* (2013) 190:1416–23. doi: 10.4049/jimmunol.1201620
10. Kim HR, Hwang KA, Park SH, Kang I. IL-7 and IL-15: biology and roles in T-Cell immunity in health and disease. *Crit Rev Immunol.* (2008) 28:325–39. doi: 10.1615/CritRevImmunol.v28.i4.40
11. Todd JA, Walker NM, Cooper JD, Smyth DJ, Downes K, Plagnol V, et al. Robust associations of four new chromosome regions from genome-wide analyses of type 1 diabetes. *Nat Genet.* (2007) 39:857–64. doi: 10.1038/ng2068
12. Svejgaard A. The immunogenetics of multiple sclerosis. *Immunogenetics.* (2008) 60:275–86. doi: 10.1007/s00251-008-0295-1
13. Tang W, Cui D, Jiang L, Zhao L, Qian W, Long SA, et al. Association of common polymorphisms in the IL2RA gene with type 1 diabetes: evidence of 32,646 individuals from 10 independent studies. *J Cell Mol Med.* (2015) 19:2481–8. doi: 10.1111/jcmm.12642
14. Waickman AT, Park JY, Park JH. The common gamma-chain cytokine receptor: tricks-and-treats for T cells. *Cell Mol Life Sci.* (2016) 73:253–69. doi: 10.1007/s00018-015-2062-4
15. Nakarai T, Robertson MJ, Streuli M, Wu Z, Ciardelli TL, Smith KA, et al. Interleukin 2 receptor gamma chain expression on resting and activated lymphoid cells. *J Exp Med.* (1994) 180:241–51. doi: 10.1084/jem.180.1.241
16. Sasson SC, Zaunders JJ, Zanetti G, King EM, Merlin KM, Smith DE, et al. Increased plasma interleukin-7 level correlates with decreased CD127 and increased CD132 extracellular expression on T cell subsets in patients with HIV-1 infection. *J Infect Dis.* (2006) 193:505–14. doi: 10.1086/499309
17. Vignali D, Gurth CM, Pellegrini S, Sordi V, Sizzano F, Piemonti L, et al. IL-7 Mediated homeostatic expansion of human CD4+CD25+FOXP3+ regulatory T cells after depletion with anti-CD25 monoclonal antibody. *Transplantation.* (2016) 100:1853–61. doi: 10.1097/TP.0000000000001276
18. Gonnord P, Angermann BR, Sadtler K, Gombos E, Chappert P, Meier-Schellersheim M, et al. A hierarchy of affinities between cytokine receptors and the common gamma chain leads to pathway cross-talk. *Sci Signal.* (2018) 11:eaal1253. doi: 10.1126/scisignal.aal1253.
19. Smyth CM, Ginn SL, Deakin CT, Logan GJ, Alexander IE. Limiting {gamma}c expression differentially affects signaling via the interleukin (IL)-7 and IL-15 receptors. *Blood.* (2007) 110:91–8. doi: 10.1182/blood-2006-11-055442
20. Orr SJ, Roessler S, Quigley L, Chan T, Ford JW, O'Connor GM, et al. Implications for gene therapy-limiting expression of IL-2R gamma c delineate differences in signaling thresholds required for lymphocyte development and maintenance. *J Immunol.* (2010) 185:1393–403. doi: 10.4049/jimmunol.0903528
21. Monti P, Brigatti C, Heninger AK, Scirpoli M, Bonifacio E. Disengaging the IL-2 receptor with daclizumab enhances IL-7-mediated proliferation of CD4(+) and CD8(+) T cells. *Am J Transplant.* (2009) 9:2727–35. doi: 10.1111/j.1600-6143.2009.02825.x
22. Demirci G, Strom TB, Li XC. Islet allograft rejection in nonobese diabetic mice involves the common gamma-chain and CD28/CD154-dependent and -independent mechanisms. *J Immunol.* (2003) 171:3878–85. doi: 10.4049/jimmunol.171.7.3878
23. Nielsen OH, Kirman I, Johnson K, Giedlin M, Ciardelli T. The circulating common gamma chain (CD132) in inflammatory bowel disease. *Am J Gastroenterol.* (1998) 93:323–8. doi: 10.1111/j.1572-0241.1998.00323.x
24. Nishio J, Kohsaka H, Shimamura T, Hamuro J, Miyasaka N. Abundant expression of common cytokine receptor gamma chain (CD132) in rheumatoid joints. *J Rheumatol.* (2001) 28:240–4.
25. Lee B, Jo Y, Kim G, Ali LA, Sohn DH, Lee SG, et al. Specific inhibition of soluble gammac receptor attenuates collagen-induced arthritis by modulating the inflammatory T Cell responses. *Front Immunol.* (2019) 10:209. doi: 10.3389/fimmu.2019.00209
26. Mair F, Hartmann FJ, Mrdjen D, Tosevski V, Krieg C, Becher B. The end of gating? An introduction to automated analysis of high dimensional cytometry data. *Eur J Immunol.* (2016) 46:34–43. doi: 10.1002/eji.201545774
27. Hong C, Luckey MA, Ligons DL, Waickman AT, Park JY, Kim GY, et al. Activated T cells secrete an alternatively spliced form of common gamma-chain that inhibits cytokine signaling and exacerbates inflammation. *Immunity.* (2014) 40:910–23. doi: 10.1016/j.immuni.2014.04.020
28. Kalia V, Sarkar S, Subramaniam S, Haining WN, Smith KA, Ahmed R. Prolonged interleukin-2Ralpha expression on virus-specific CD8+ T cells favors terminal-effector differentiation *in vivo*. *Immunity.* (2010) 32:91–103. doi: 10.1016/j.immuni.2009.11.010
29. Bani L, David D, Moreau JL, Cayota A, Nakarai T, Ritz J, et al. Expression of the IL-2 receptor gamma subunit in resting human CD4 T lymphocytes: mRNA is constitutively transcribed and the protein stored as an intracellular component. *Int Immunol.* (1997) 9:573–80. doi: 10.1093/intimm/9.4.573
30. Walker LS, von Herrath M. CD4 T cell differentiation in type 1 diabetes. *Clin Exp Immunol.* (2016) 183:16–29. doi: 10.1111/cei.12672
31. Seyfarth J, Fortsch K, Ahlert H, Laws HJ, Karges B, Deenen R, et al. Dominant TNFalpha and impaired IL-2 cytokine profiles of CD4+ T cells from children with type-1 diabetes. *Immunol Cell Biol.* (2017) 95:630–9. doi: 10.1038/icb.2017.24
32. Lee B, Ko E, Lee J, Jo Y, Hwang H, Goh TS, et al. Soluble common gamma chain exacerbates COPD progress through the regulation of inflammatory T

cell response in mice. *Int J Chron Obstruct Pulmon Dis.* (2017) 12:817–27. doi: 10.2147/COPD.S123405
33. Kim G, Hwang H, Jo Y, Lee B, Lee YH, Kim CH, et al. Soluble gammac receptor attenuates anti-tumor responses of CD8(+) T cells in T cell immunotherapy (2018). *Int J Cancer* 143:1212–23. doi: 10.1002/ijc.31402
34. Kim G, Jo Y, Lee B, Ali LA, Lee B, Hong C. The potential role of a soluble gamma-chain cytokine receptor as a regulator of IL-7-induced lymphoproliferative disorders. *Int J Mol Sci.* (2018) 19:3375. doi: 10.3390/ijms19113375
35. Laurence A, Tato CM, Davidson TS, Kanno Y, Chen Z, Yao Z, et al. Interleukin-2 signaling via STAT5 constrains T helper 17 cell generation. *Immunity.* (2007) 26:371–81. doi: 10.1016/j.immuni.2007.02.009
36. Fabbri M, Frixou M, Degano M, Fousteri G. Type 1 diabetes in STAT protein family mutations: regulating the Th17/Treg equilibrium and beyond. *Diabetes.* (2019) 68:258–65. doi: 10.2337/db18-0627
37. Lundtoft C, Afum-Adjei Awuah A, Rimpler J, Harling K, Nausch N, Kohns M, et al. Aberrant plasma IL-7 and soluble IL-7 receptor levels indicate impaired T-cell response to IL-7 in human tuberculosis. *PLoS Pathog.* (2017) 13:e1006425. doi: 10.1371/journal.ppat.1006425

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.