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Brief communication

Increased proportions of γδ T lymphocytes in atypical SCID associate with disease manifestations

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ABSTRACT

Severe combined immunodeficiencies (SCID) comprise a group of genetic diseases characterized by abrogated development of T lymphocytes. In some case reports of atypical SCID patients elevated proportions of γδ T lymphocytes have been reported. However, it is unknown whether these γδ T cells modulate or reflect the patient's clinical phenotype. We investigated the frequency of elevated γδ T cell proportions and associations with clinical disease manifestations in a cohort of 76 atypical SCID patients. Increased proportions of γδ T lymphocytes were present in approximately 60% of these patients. Furthermore, we identified positive correlations between elevated proportions of γδ T cells and the occurrence of CMV infections and autoimmune cytopenias. We discuss that CMV infections might trigger an expansion of γδ T lymphocytes, which could drive the development of autoimmune cytopenias. We advocate that atypical SCID patients should be screened for elevated proportions of γδ T lymphocytes, CMV infection and autoimmune cytopenias.

Abbreviations: SCID, severe combined immunodeficiency; CMV, Cytomegalovirus; VZV, Varicella Zoster virus; EBV, Epstein Barr virus; IBD, inflammatory bowel disease; CRS, cytokine receptor defect; DR, DNA recombination; ADA, adenosine deaminase

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1. Introduction

Severe combined immunodeficiencies (SCID) are disorders characterized by genetic mutations abrogating T lymphocyte development [1]. SCID patients usually succumb to severe infections within the first year of life unless treated by hematopoietic stem cell transplantation or gene therapy. Milder or atypical disease variants emerge from hypomorphic mutations in SCID-associated genes. These mutations allow for residual protein function and, consecutively, partial T lymphocyte development, which usually permits survival beyond the first year of life. In addition to an increased susceptibility to infections, atypical SCID frequently associates with immune dysregulation such as autoimmune cytopenias and lymphoproliferation. Elevated proportions of T lymphocytes expressing γδ T cell receptors have been reported in some atypical SCID patients [2,3]. However, it has not been investigated whether elevated proportions of γδ T lymphocytes are associated with particular genetic SCID variants or disease manifestations. Here, based on a systematic, retrospective analysis of a large cohort of atypical SCID patients, we describe the prevalence of elevated γδ T cell proportions and their association with clinical manifestations.

2. Methods

2.1. Inclusion criteria

Study inclusion required the fulfillment of each of the following 4 criteria: (1) a diagnosis of atypical SCID based on identification of mutation(s) in a SCID-associated gene(s) (*IL2RG, IL7R, JAK3, RAG1, RAG2, ARTEMIS, ADA, LIG4)* as the underlying disease cause, (2) documentation of peripheral blood γδ T lymphocytes on at least one occasion, (3) survival beyond the first year of life without hematopoietic stem cell transplantation or gene therapy and (4) peripheral blood $CD3^+$ T lymphocyte count above 100/μl. This last criterion was chosen to reflect relevant residual function of the affected gene product based on a publication by Felgentreff et al. [4]. Patients included were further classified based on the genes mutated into the following groups: DNA recombination defects ("DR", genes: *RAG1, RAG2, DCLRE1C, LIG4*), cytokine receptor signaling defects ("CRS", genes: *IL2RG, IL7R, JAK3*) and adenosine deaminase defect ("ADA", gene: *ADA1*).

2.2. Ethics statement

This study was conducted in accordance with the Declaration of Helsinki. Data acquisition for all unpublished patients was performed under local institutional review board approval. All results presented in this study were obtained as part of the routine medical attendance the patient received.

2.3. Data sources

Patients fulfilling the above inclusion criteria were identified by screening published literature or the database of the observational P-CID study or *via* personal communication. For identification of published patients an NCBI Pubmed database search was performed on

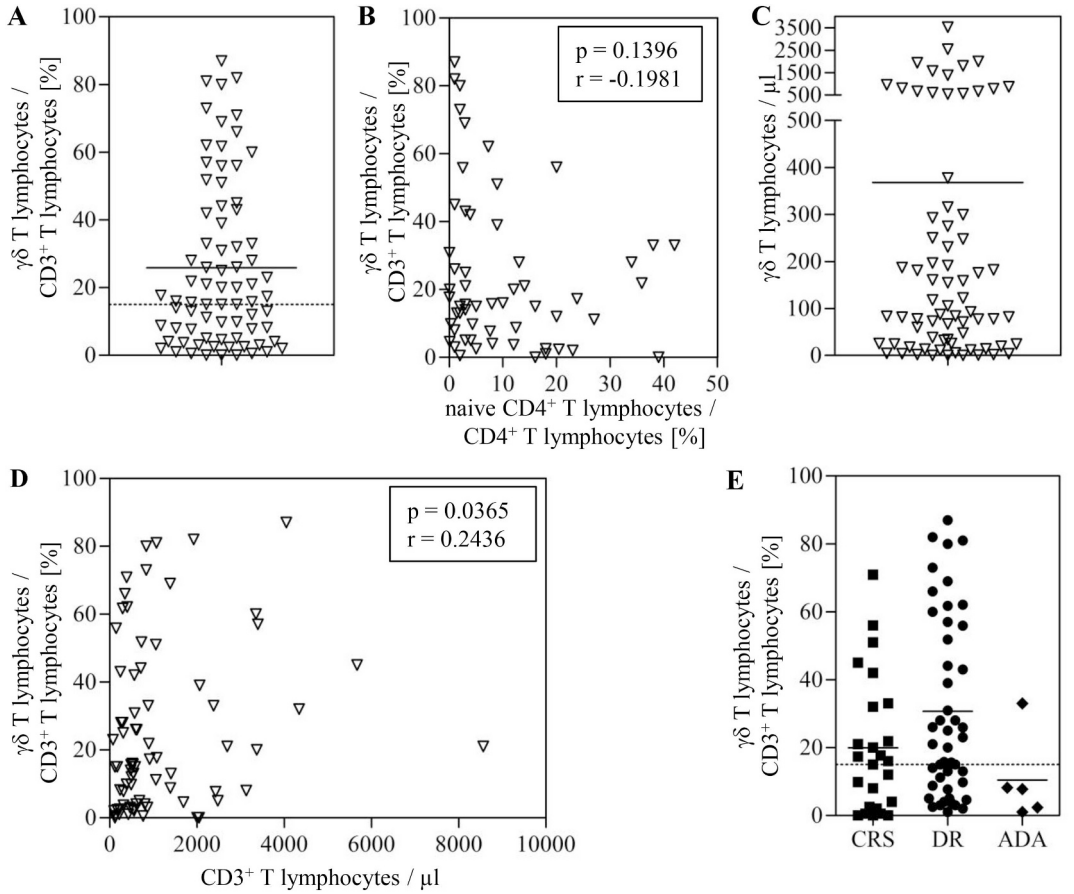

Fig. 1. Correlation between immunological manifestation and proportion of γδ T lymphocytes.
(A) Proportion of γδ T lymphocytes of $CD3^+$ lymphocytes and (B) Correlation between proportion of γδ T lymphocytes and percentage of naive $CD4^+$ T lymphocytes. (C) Absolute count of γδ T lymphocytes in blood. (D) Correlation between proportion of γδ T lymphocytes and counts of $CD3^+$ lymphocytes in blood. (E) Proportion of γδ lymphocytes of $CD3^+$ lymphocytes depicted separately for patients harboring mutations in genes affecting cytokine receptor signaling (CRS), DNA recombination (DR) or in adenosine deaminase (ADA).

25.02.2018 using the following keywords: “atypical severe combined immunodeficiency”, “leaky severe combined immunodeficiency”, “mild combined immunodeficiency”, “late-onset severe combined immunodeficiency”, “RAG mutation, case report”, “DLRE1C (Artemis) mutation, case report”, “Ligase IV mutation, case report”, “IL2RG, case report”, “IL7R, case report”, “JAK3 mutation, case report”, “ADA mutation, case report”, “ADA deficiency, case report”. The observational P-CID study is a prospective outcome study on patients with profound combined immunodeficiency (registered under DRKS-ID: DRKS00000497, http://www.drks.de/drks_web/setLocale_EN.do)[E1]. Furthermore, anonymized data of unpublished patients was provided by Stephan Ehl (Center for Chronic Immunodeficiency, Freiburg, Germany), Luigi Notarangelo (Children's Hospital, Boston, USA; IRB-approved protocol number: 04-09-113R), Alessia Scarselli and Caterina

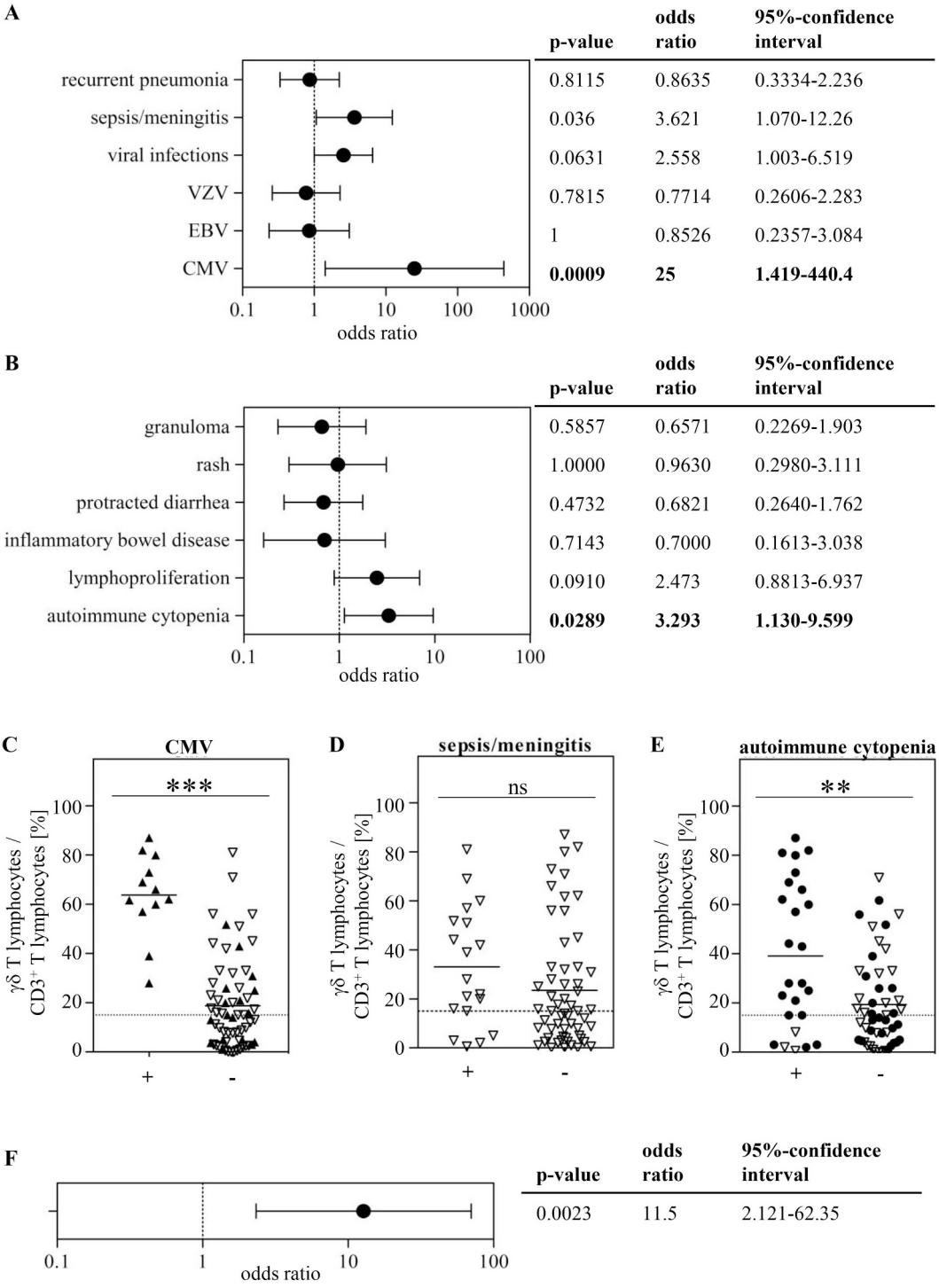

Fig. 2. Correlation between clinical manifestation and proportion of γδ T lymphocytes.
(A, B) Odds ratios of indicated clinical manifestations for patients with γδ T lymphocytes > or < 15%. (C, D, E) Proportion of γδ T lymphocytes in patients with (+) or without (−) the indicated clinical manifestations. In (C) filled triangles denote patients harboring RAG mutations. In (E) filled circles denote patients harboring mutations in genes affecting DNA recombination. (F) Odds ratio for autoimmune cytopenia in case of CMV infection among patients with γδ T lymphocytes > 15%.

Cancrini (Children's Hospital Bambino Gesú, Rome, Italy) and Kimberly Gilmour (Great Ormond Street Hospital for Children, London, UK) after informed consent had been obtained.

2.4. Immunological and clinical parameters

Peripheral blood lymphocyte populations were enumerated by flow cytometry. The percentage of $CD45RA^+$ cells of $CD4^+$ T lymphocytes was used to define the proportion of naïve $CD4^+$ T lymphocytes. Atypical SCID patients were grouped using a cut-off value of 15% γδ T cells of CD3+ T cells. This cut-off was defined conservatively to minimize the risk of falsely assigning normal proportions of γδ T cells as elevated based on published reference values for proportions of γδ T cells in blood across different pediatric age groups [5]. Within our cohort of atypical SCID patients, we performed a receiver-operating-characteristics (ROC)-analysis for each clinical manifestation. These analyses did not reveal a superior performance of cut-off-values other than 15%. Data analysis was based on single cross-sectional observations. If data of several independent analyses was available, data from the point in time corresponding to the highest proportion of γδ T lymphocytes was used for analysis. If values were reported as "below detection limit", the value of the detection limit was used for analysis. Information regarding clinical manifestations were obtained from patient documentation of the treating physician or, for published patients, according to the information provided in the publication. The following clinical manifestations were interrogated: autoimmune cytopenia (encompassing autoimmune hemolytic anemia, autoimmune neutropenia, immune thrombocytopenic purpura), lymphoproliferation (encompassing hepatosplenomegaly, lymphadenopathy), inflammatory bowel disease, protracted diarrhea, granulomas, rash, bacterial sepsis and/or meningitis, recurrent pneumonia, viral infections (encompassing: CMV, EBV, VZV or undisclosed viral infection).

2.5. Statistics

Statistical analysis was performed using GraphPad Prism software using the following tests: Spearman correlation test (Fig. 1B, D), Mann-Whitney test (Fig. 1A, C, Fig. 2C-E, Fig. E1), Fisher's Exact test (Fig. 2A-B, F). Statistical significance was denoted as follows: $p \leq 0{,}05$ "*", $p \leq 0{,}01$ "**" $p \leq 0{,}001$ "***", $p \leq 0{,}0001$ "****".

3. Results and discussion

We compiled a cohort of 76 atypical SCID patients using anonymized datasets from patient registries of different immunodeficiency centers as well as published literature (Table E1). Within this cohort 44 of 76 patients (58%) displayed proportions of γδ T lymphocytes above 15% of $CD3^+$ T lymphocytes (Fig. 1A). There was no correlation between the proportions of γδ T lymphocytes and naive $CD4^+$ T cells suggesting that elevated proportions of γδ T lymphocytes were not merely a reflection of a developmental defect of αβ T lymphocytes (Fig. 1B). Notably, in 9% of patients' blood γδ T cell counts were above 1000/μl (Fig. 1C). There was a weak positive correlation between the proportion of γδ T lymphocytes and the total $CD3^+$ T cell count (Fig. 1D). This is of clinical relevance as it illustrates that an expansion of γδ T lymphocytes can mask a paucity of αβ T lymphocytes unless $CD3^+$ T lymphocytes are subdivided into both populations. There was no association between the proportion of γδ T lymphocytes and defects in DNA recombination, cytokine receptor signaling or adenosine deaminase, indicating that elevated proportions of γδ T lymphocytes are a general feature of atypical SCID (Fig. 1E).

Since TCR γδ T lymphocytes contribute to host defense against infections, we asked whether elevated proportions of γδ T lymphocytes correlated with the patient's history of infection. Grouping patients based on γδ T cell proportions above or below 15% there was no significant difference regarding the presence of recurrent pneumonias or viral infections in general and VZV- and EBV-infections in particular (Fig. 2A). However, in patients with elevated proportions of γδ T lymphocytes there was a significantly higher prevalence of documented CMV infection and sepsis/meningitis (Fig. 2A). Immune dysregulation is a second major clinical manifestation in atypical SCID patients. We did not observe differences in the occurrence of granulomas, skin rashes, protracted diarrhea, inflammatory bowel disease or lymphoproliferation in patients with or without elevated proportions of γδ T cells. In contrast, the odds ratio for autoimmune cytopenias was significantly increased for patients with elevated proportions of γδ T lymphocytes (Fig. 2B). Conversely, in patients with documented CMV infection or cytopenias, but not sepsis/meningitis, the proportions of γδ T lymphocytes were significantly higher than in patients without these manifestations (Fig. 2C, D, E, online repository Fig. E1). Notably, all 12 patients with documented CMV infection belonged to the group of patients with *RAG* deficiency. Furthermore, the correlation between elevated proportions of γδ T cells and autoimmune cytopenias derived mainly from patients harboring mutations in genes involved in DNA recombination. As DNA recombination is required for antigen receptor diversification this could indicate that gaps in the T cell receptor repertoire confer a particular susceptibility to these disease manifestations. Analyses of the γδ TCR repertoire in atypical SCID patients with CMV infection and autoimmune cytopenias could help to investigate this hypothesis.

The associations identified raise the question whether the clinical manifestations trigger an expansion of γδ T lymphocytes or whether elevated proportion of γδ T lymphocytes render patients more susceptible to disease manifestations. None of the patients presenting with CMV infection displayed γδ T cell proportions below 15%, suggesting that this infection triggers the expansion of γδ T lymphocytes. This hypothesis implies that the proportion of γδ T cells should mirror disease activity over time. While our study was based on single cross-sectional observations, consistent with this hypothesis CMV-infection upon kidney transplantation has been found to trigger a long-lasting increase in the proportion of γδ T cells [6–8]. Furthermore, the expanded γδ T lymphocytes of these patients proliferated to CMV antigen *in vitro* [7].

The fact that not all patients with elevated proportions of γδ T lymphocytes had a documented CMV infection suggests that other, unidentified factors may elicit this phenomenon in the context of immunodeficiency. Whereas γδ T cell expansion may be a consequence of CMV infection, it could be a potential cause of autoimmune cytopenias. In mice lacking αβ T lymphocytes, γδ T lymphocytes were found to stimulate autoantibody production upon recurrent infections [9]. This suggests that elevated proportions of γδ T lymphocytes may facilitate the development of autoimmune cytopenias in atypical SCID patients. CMV has been found to activate γδ T lymphocytes which are cross-reactive to self-antigens [10]. Interestingly, among patients with elevated γδ T lymphocytes, there was a significantly increased chance of patients with CMV infection to also exhibit autoimmune cytopenias (Fig. 2F) – 84% of which were autoimmune hemolytic anemias (Table E1). It remains to be determined whether antiviral treatment of CMV infection normalizes the proportion of γδ T lymphocytes and reduces the prevalence or severity of autoimmune cytopenias in atypical SCID patients.

In summary, our dataset documents that elevated proportions of γδ T lymphocytes are frequently present in atypical SCID patients. Furthermore, in these patients they are associated with CMV-infection and autoimmune cytopenia. Based on these observations we recommend investigating atypical SCID patients for elevated proportions of γδ T lymphocytes, for CMV infection and for cytopenias – particularly in the context of RAG deficiency. Furthermore, these patient observations stimulate further studies to determine whether γδ T cells can provide significant microbial protection and whether they

contribute to immune dysregulation under conditions of αβ T cell lymphopenia.

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Declaration of potential conflicts of interest

Dr. Felgentreff reports grants from German Research Foundation (DFG), and grants from Else-Kroener-Fresenius Foundation, during the conduct of the study. Dr. Hoenig reports personal fees from Commercial Sponsor, grants from Foundation, outside the submitted work. Dr. Albert reports grants and other from GSK, other from medac, other from CSL, other from MSD, outside the submitted work. Dr. Worth reports personal fees from BioTest UK, personal fees from British Journal of Hematology, outside the submitted work. Dr. Soler-Palacin reports grants and personal fees from Shire SL, grants and personal fees from CSL Behring, grants from Grifols, personal fees from MSD, outside the submitted work. Dr. Gilmour reports grants from NIHR, during the conduct of the study. Dr. Ehl reports grants from BMBF, grants from Chaim Roifman Scholarship, during the conduct of the study; grants from UCB, outside the submitted work. Dr. Rohr reports grants from Deutsche Forschungsgemeinschaft (DFG), during the conduct of the study. All other authors declare no potential conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clim.2018.11.006.

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