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Defective IL-4 signaling in T cells defines severe common variable immunodeficiency

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ABSTRACT

Common variable immunodeficiency (CVID) is defined by hypogammaglobulinemia and B-cell dysfunction, with significant clinical and immunological heterogeneity. Severe non-infectious complications, such as autoimmunity, granulomatous disease and splenomegaly, constitute a major cause of morbidity in CVID patients. T cells are generally regarded important for development of these clinical features. However, while T-cell abnormalities have been found in CVID patients, functional characteristics of T cells corresponding to well-defined clinical subtypes have not been identified. As common γ-chain cytokines play important roles in survival and differentiation of T cells, characterization of their signaling pathways could reveal functional differences of clinical relevance. We characterized CVID T cells functionally by studies of cytokine-induced signaling, and correlated the findings to defined clinical subtypes. Peripheral blood T cells from 29 CVID patients and 19 healthy donors were analyzed for i) phenotype, ii) cytokine-induced (interleukin (IL)-2, IL-4, IL-7 and IL-21) phosphorylation of signal transducer and activator of transcription (STAT) 3, STAT5 and STAT6, and iii) T-helper (Th)1/Th2 polarization. Expression of IL-4 receptor and downstream signaling molecules was measured.

A subgroup of CVID patients (n = 7) was identified by impaired IL-4-induced p-STAT6 in naive and memory CD4 and CD8 T cells. This corresponded to patients with the largest accumulation of severe (non-infectious) complications. The signaling defect persisted over years and was not due to constitutively activated p-STAT6. The CD4 T cells were strongly Th1-skewed, but IL-4 signaling was impaired independently of Th status. However, IL-4Rα and Janus kinase (JAK) 1 mRNA levels were significantly lower than in normal donors, providing a likely mechanism for the defective IL-4-induced p-STAT6 and Th1-bias. In conclusion, we identified a subgroup of CVID patients with defective IL-4 signaling in T cells, with severe clinical features of inflammation and autoimmunity.

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1. Introduction

Although common variable immunodeficiency (CVID), hallmarked by recurrent airway infections with encapsulated bacteria, is defined by hypogammaglobulinemia and B-cell dysfunction, clinical and immunological heterogeneity is significant [1]. Identified monogenic mutations can only explain a minor fraction of the cases [2], and the mechanism of B-cell abnormality remains

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Abbreviations

BFA	brefeldin A
γc	common γ-chain
IFN-γ	interferon-γ
Ig	Immunoglobulin
IL	Interleukin
IL-4R	IL-4 receptor
IVIG	intravenous immunoglobulin therapy
JAK	Janus kinase
MFI	median fluorescence intensity
PBMC	peripheral blood mononuclear cells
PMA	Phorbol-12-Myristate-13-acetate
SCIG	subcutaneous immunoglobulin therapy
STAT	Signal transducer and activator of transcription

elusive for the large majority of patients. While the antibody defect is effectively treated with immunoglobulin (Ig)-substitutions, reducing the incidence of sinopulmonary- and systemic bacterial infections [3], other therapeutic regimens are required for disease control in patients suffering from non-infectious complications [4]. These comprise distinct clinical phenotypes [5], including most commonly (i) autoimmune diseases, such as autoimmune cytopenia, thyroiditis, arthritis, (ii) polyclonal lymphocytic infiltration, such as splenomegaly, granuloma, interstitial pneumonitis, granulomatous disease, enteropathy, and more rarely (iii) malignancies, such as lymphoma and cancers of the gastrointestinal tract [5–7]. Patients with non-infectious complications have more severe disease and a worsened prognosis [8], and T cell pathophysiology seems to be involved in the development of several of these complications [9]. T cell immunodeficiency, resulting in frequent viral and fungal infections is not a characteristic of CVID [9,10]. In contrast, abnormalities of the T cell compartment occur in a significant proportion of the patients [9]. The abnormalities include among others, skewed subsets of naïve and memory CD4 and CD8 T cells [11–13], suggesting a dysregulated peripheral T cell differentiation. The cytokines interleukin (IL)-2, IL-4, IL-7 and IL-21 play an important role in differentiation, survival and activation of T cells [14] by signaling through the common γ-chain (γc)-JAK-STAT pathways [15]. The γc-cytokines use heterodimeric or trimeric receptors consisting of a specific α-chain, a β-chain (IL-2), and the γc to initiate signaling by activation of JAK1 and JAK3, which further phosphorylate the respective signal transducer and activator of transcription (STAT) transcription factors. IL-2 and IL-7 mainly activate STAT5, while IL-4 activates STAT6 and IL-21 activates STAT3 [15]. These signaling pathways have not been previously characterized in CVID T cells [16]. Here, we studied the early cytokine-induced signaling events in T cells in peripheral blood T-cells from 29 CVID patients and 19 healthy donors, and correlated the findings to defined clinical subtypes.

2. Materials and methods

2.1. Patients and healthy donors

Peripheral blood was collected from 29 CVID patients, diagnosed based on the criteria from the ESID Registry [10], and from 19 sex- and age-matched healthy donors. The study was conducted according to the Declaration of Helsinki and approved by the Regional Ethical Committee.

2.2. Activation of cell signalling and phospho -specific flow cytometry

Cryopreserved PBMC samples were thawed, resuspended in RPMI-1640 (Gibco Laboratories, Grand Island, NY, USA) with 10% heat-inactivated Fetal Bovine Serum (PAN-Biotech GmbH, Aidenbach, Germany), Fig. E1. PBMC viability after thawing was the same in patients and controls (median = 95% versus median = 94%, p = 0.6). The PBMCs were then rested at 37 °C for 30 min before distribution into v-bottomed 96-well polystyrene plates at 200 μL per well, and rested additional 20 min before start of activation. Cells were left unstimulated or activated with interleukin (IL)-2, IL-4, IL-7 or IL-21 (eBioscience) at 20 ng/mL for 15 min before fixation with paraformaldehyde (PFA) (Electron microscopy Service, Hatfield, PA, USA) at a final concentration of 1.6% for 5 min to stop signaling [17]. The PBMCs were then permeabilized by adding >90% freezer-cold methanol, and stored at −80 C° before further processing. Rehydration of the cells was performed by washing twice with PBS by centrifugation. In experiments with multiple stimuli, cells were barcoded as previously described [18,19] using the fluorescent esters Pacific Blue and Pacific Orange (Life Technologies, Molecular Probes) at the following final concentrations: Pacific Blue: L4: 300 pg/μL; L3: 80 pg/μL; L2: 20 pg/μL; L1: 2 pg/μL and Pacific Orange: L1: 9 pg/μL; L2: 90 pg/μL; L3: 500 pg/μL. Some of the activation conditions were designed for B-cell stimulation and not reported here [20]. After incubation with barcoding dyes for 30 min at RT in the dark, the samples were washed twice, and all the different stimulation conditions from one patient were combined into one tube before splitting into new tubes for different antibody staining panels (8-colour panels), using the following antibodies: p-STAT3 PE (Y705), p-STAT5 Alexa 647 (Y694), p-STAT6 Alexa 488 (Y641), CD20 PerCPCy5.5 (clone: H1), CD4 Alexa 700 (RPA-T4), STAT3 PE (M59-50), STAT6 Alexa 647 (23/Stat6), and PD-1 Brilliant violet 711 (EH12.1), all from BD Biosciences; and CD3 PECy7 (UCHT1), CD8 Brilliant violet 785 (RPA-T8) and CD45RO Brilliant violet 711 (UCHL1) from Biolegend, San Diego, CA, USA. The cells were then stained for 30 min in the dark at RT, washed once with PBS 1% BSA, pelleted by centrifugation at 710g and resuspended in the wash buffer. The samples were collected on an LSR II flow cytometer, and data were analyzed using Cytobank software (www.community.Cytobank.org). T cells were identified by gating on singlets, lymphocytes (by scatter properties), $CD3^+$, $CD20^-$ cells and then gated into different subsets based on $CD45RO^+$ or $CD45RO^-$ subpopulations of $CD4^+$ or $CD8^+$ cells. Further, the samples were deconvoluted according to barcode fluorescence, corresponding to the respective activation condition. Relative phosphorylation changes were calculated using arcsinh transformation of median fluorescence intensity (MFI) of the cell population of interest. Activation-induced phosphorylation was calculated as relative change = arcsinh (MFI of phospho-protein in activated T cells/scale argument) - arcsinh (MFI of phospho-protein in unstimulated T cells/scale argument). The scale argument numbers are channel-specific (ranged between 150 and 400). Basal phosphorylation levels were calculated relative to healthy donor T cells as follows: Basal levels = arcsinh (MFI of phospho-protein in untreated T cells/scale argument) - arcsinh (MFI of phospho-protein in a control donor T cells/scale argument). One healthy blood donor sample was included in each run of the assay and used for normalization of the data.

2.3. Immunophenotyping

Cryopreserved PBMCs were thawed and stained with Alexa 750 (Molecular Probes, Waltham, Thermo Fisher Scientific, M, USA) as

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