Autoimmunity Reviews 11 (2012) 723–730

Contents lists available at SciVerse ScienceDirect

Autoimmunity Reviews

journal homepage: www.elsevier.com/locate/autrev

Review

Thymic functions and gene expression profile distinct double-negative cells from single positive cells in the autoimmune lymphoproliferative syndrome

Atar Lev [a,b], Amos J. Simon [a,c], Ninette Amariglio [a,c], Gideon Rechavi [a], Raz Somech [a,b,*]

[a] Cancer Research Center, Sheba Medical Center, Tel Hashomer affiliated to the Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel
[b] Pediatric Department B North and Immunology Service, Edmond and Lily Safra Children's Hospital, Sheba Medical Center, Tel Hashomer affiliated to the Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel
[c] Hematology Laboratory, Sheba Medical Center, Tel Hashomer affiliated to the Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

ARTICLE INFO

Article history:
Accepted 2 January 2012
Available online 18 January 2012

Keywords:
Autoimmune lymphoproliferative syndrome (ALPS)
Thymus
Autoimmunity
Immunodeficiency
Oligoclonal
T-cell receptor (TCR)
TCR excision circles (TRECs)

ABSTRACT

Objective: Autoimmune lymphoproliferative syndrome is characterized by autoimmunity, enlarged lymph nodes and spleen as well as the presence of a rare T-cell population expressing TCRαβ^{+}CD3^{+}CD4^{-}CD8^{-}, known as double-negative (DN) cells, in the peripheral blood. The origin and function of the double-negative cells are incompletely understood. Here we studied the origin of DN T cells found in patients with autoimmune lymphoproliferative syndrome.
Methods: T-cell receptor diversity, T-cell receptor excision circles and ordered V(D)J DNA gene rearrangement levels were determined in DN as well as CD4^{+}CD8^{-} and CD4^{-}CD8^{+} (single-positive, SP) cells isolated from peripheral blood of two patients with typical autoimmune lymphoproliferative syndrome. In addition, expression of genes associated with autoimmune lymphoproliferative syndrome was determined in these cells by the TaqMan Low-Density Array.
Results: We found that T cell receptor diversity in patients with autoimmune lymphoproliferative syndrome was similar to that of normal controls. In contrast, DN cells of patients contained significantly less T-cell receptor excision circles than SP cells. Similarly, certain DNA rearrangements were markedly reduced in DN cells. The transcriptional profile of the patients' DN cells revealed significant changes in 37.7% of the tested genes, some closely related to the pathophysiology associated with the syndrome. Their gene expression signature was unlike that of both SP cells.
Conclusion: We hypothesize that the double-negative T cells in autoimmune lymphoproliferative syndrome display features that differ, in both thymic function and gene expression profile, from SP T cells. These findings may explain some of the patients' chronic lymphoproliferation and breakdown of self-tolerance checkpoints.

Contents

Abbreviations: ALPS, Autoimmune lympho-proliferative syndrome; DN, Double-negative; tDN, Thymic double-negative; PBMCs, peripheral blood mononuclear cells; SP, Single-positive; TCR, T-cell receptor; TRECs, T-cell receptor excision circles; Ct, Cycle threshold; qRT-PCR, quantitative real-time polymerase chain reaction; TECs, thymus epithelial cells (TECs); AIRE, autoimmune regulator.

* Corresponding author at: Pediatric Department B North, Pediatric Immunology Service, Edmond and Lily Safra Children's Hospital, Chaim Sheba Medical Center, Tel Hashomer, 52621, Israel. Tel.: +972 3 5303477; fax: +972 3 5302557.
E-mail address: raz.somech@sheba.health.gov.il (R. Somech).

1568-9972/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.autrev.2012.01.002

1. Introduction

Autoimmune lymphoproliferative syndrome (ALPS) is a rare disorder of disrupted lymphocyte homeostasis characterized by impaired Fas/CD95-mediated apoptosis. In most patients mutations affecting the *FAS* gene have been found. Mutations of the *FAS* ligand gene and the caspase-10 gene have been also described in some patients. Patients without identifiable mutations in these genes are characterized as ALPS-U (Unknown) [1].

ALPS is characterized by chronic, non-malignant lymphoproliferation, autoimmunity often manifesting as multilineage cytopenias, and an increased risk of lymphoma [2]. It has been hypothesized that the mechanism for elimination of dysfunctional and autoreactive lymphocytes is defective and contributes to the pathogenesis of this disorder [3]. Typically, patients with ALPS also have significantly increased percentage of $TCR\alpha\beta^+CD3^+CD4^-CD8^-$ T-cells known as double-negative (DN) T cells. In normal individuals, DN T cells account for less than 1.5% of total lymphocytes or 2.5% of $TCR\alpha\beta^+$ T cells, while in patients with ALPS, up to 40% of T cells may be DN [4, 5]. The function and origin of the DN cells are not clearly understood. The expression of activation markers, such as HLA-DR and CD69 suggests that the DN cells are mature cells that lost the expression of CD4 or CD8. However these cells also exhibit a phenotype of naïve T cells, expressing CD45RA but not CD25 [6–8]. It was recently shown that both DN and $CD8^+$ T cells of ALPS patients have a skewed TCR repertoire with oligoclonal expansions of similar CDR3 sequencings that strongly argue for a CD8 origin of DN T cells in that syndrome [9]. Identifying somatic FAS mutations in the DN cells but not in SP cells [10] might indicate that these cells do not originate from SP cells. Indeed, another possible origin of these cells suggested that they are a unique T lineage that had selectively expanded due to a defect in Fas signaling, and that the proportion of these cells varies as a function of ongoing, in vivo cell proliferation. However, functional analysis showed that DN cells are unable to proliferate in vitro and they produce only low amounts of interleukin-2 and interferon-γ, suggesting that these cells are anergic [11]. Another example reflecting the obscure phenotype of these DN cells is that while the expression of granzyme A, a marker of cytotoxic T cells, is high, the expression of perforin, another cytolytic granule, is controversial [12].

Normally, T cell maturation in the thymus progresses through distinct stages which are defined phenotypically by the expression of T-cell receptors (TCRs). The TCR is created through a sequential order of recombination events between the different TCR genes, initiated with TCRD, followed by TCRG and TCRB and culminating with TCRA rearrangements. These sequential orders also occur within every individual gene and therefore are correlated with stages of T cell maturation [13]. The DNA fragments excised during V(D)J rearrangement of the TCR α and β chain in the thymus form stable by-products known as TCR excision circles (TRECs), that remain in the cells released into the peripheral blood, allowing indirect quantification of thymocyte production and development [14]. Indeed, enumeration of TRECs in peripheral blood is frequently used to estimate thymocyte production [15]. We hypothesized that DN cells of patients with ALPS have reduced thymic activity and distinct gene expression profile, different from SP cells. Therefore, we analyzed TCR diversity, TRECs, and the sequential DNA rearrangement as well as the gene expression profile in sorted DN and SP T cells from patients with ALPS.

2. Methods

2.1. Study population

Blood samples from 2 patients with clinical and immunological features typical of ALPS were used for this study. Both patients were not on any immunosuppressive treatment when blood was obtained. The study was approved by the Institutional Review Board of Sheba Medical Center, Tel Hashomer, Israel and the patients' guardians provided written informed consent.

2.2. Lymphocyte markers and T-cell proliferative responses

Peripheral blood mononuclear cells were isolated from freshly drawn heparin-treated blood by Ficoll–Hypaque density gradient centrifugation. Cell surface markers of peripheral blood mononuclear cells (PBMCs) were determined by immunofluorescent staining and flow cytometry (Epics V; Coulter Electronics, Hialeah, FL) with antibodies purchased from Coulter Diagnostics. The proportion of DN T cells was determined by flow cytometry on PBMCs following staining with mAbs (CD3-PerCP, CD4-FITC, CD8-APC) purchased from BD Biosciences. Lymphocyte proliferation in response to phytohemagglutinin (PHA) and anti-CD3 stimulation was assessed by titrated thymidine incorporation, while serum concentration of immunoglobulins was measured by nephelometry as previously described [15].

2.3. Isolation of DN and SP T cells

DN and SP T cells from freshly isolated PBMCs of the two patients were isolated using CD3-PerCP, CD4-FITC, CD8-APC mAbs (BD Biosciences) with a FACS-Aria cell sorter system (98% purity).

Table 1
List of primers used by qRT-PCR analysis to detect the amount of TRECs and the various TCR rearrangements.

Taqman probe (FAM-TAMRA) (5′–3′)	Reverse primer (5′–3′)	Forward primer (5′–3′)	
ATACGCACAGTGCTACAAAACCTACAGAGACCT	TTGCCCCTGCAGTTTTTGTAC	CAAGGAAAGGGAAAAAGGAAGAA	TCRD-δ/D2-D3
CCCGTGTGACTGTGGAACCAAGTAAGTAACTC	TTAGATGGAGGATGCCTTAACCTTA	AGCGGGTGGTGATGGCAAAGT	TCRD-δ/D2-J1
CCCGTGTGACTGTGGAACCAAGTAAGTAACTC	TTAGATGGAGGATGCCTTAACCTTA	ATGCAAAAAGTGGTCGCTATT	TCRD-δ/V1-J1
CCAGAGGTGCGGGCCCCA	GGCACATTAGAATCTCTCACTGA	AAAAAGCAACATCACTCTGTGTCT	TCRA-REC
ACACCTCTGGTTTTTGTAAAGGTGCCCACT	GCCAGCTGCAGGGTTTAGG	CACATCCCTTTCAACCATGCT	TREC