Cellular Immunology 262 (2010) 75–79

Contents lists available at ScienceDirect

Cellular Immunology

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

Rapid Communication

Numerical defects in $CD8^+CD28^-$ T-suppressor lymphocyte population in patients with type 1 diabetes mellitus and multiple sclerosis

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ARTICLE INFO

Article history:
Received 8 December 2009
Accepted 1 February 2010
Available online 6 February 2010

Keywords:
Type 1 diabetes mellitus
Multiple sclerosis
Autoimmune diseases
T-lymphocytes
Regulatory T-cells
Suppressor cells

ABSTRACT

Type 1 diabetes mellitus (T1D) and multiple sclerosis (MS) are organ-specific autoimmune diseases leading to an attack of auto-aggressive lymphocytes against the pancreatic β-cells and central nervous system, respectively. Using four-colour flow cytometry, T-lymphocyte populations having an important function in autoimmune processes were analyzed. T-regulatory cells (Treg) $CD4^+CD25^+CD127^{low}$, T-suppressor cells (Ts) $CD8^+CD28^-$, activated helper $CD4^+CD25^+CD127^+$ and cytotoxic $CD8^+CD25^+$ T-cells and also naive $CD4^+CD45RA^+$ and memory T-cells $CD4^+CD45RO^+$ were compared in the group of patients with T1D ($n = 30$), MS ($n = 31$) and in the group of healthy controls ($n = 29$). Significant differences in Ts cells, activated helper and cytotoxic cells and also memory T-cells were recognized in the group of T1D patients compared to healthy controls. Ts population was significantly lowered in MS patients as well. However, no significant differences were noticed in Treg population. The observed data demonstrate significant differences among patients with T1D and MS in comparison to healthy individuals.

1. Introduction

Type 1 diabetes mellitus (T1D) and multiple sclerosis (MS) represent Th1 cells-mediated autoimmune diseases with not entirely known triggers of the self-reactive processes. T1D is characterized by targeted destruction of insulin-producing pancreatic β-cells; many different autoantigens are involved in T1D pathogenesis, including insulin, glutamate decarboxylase (GAD) and tyrosine phosphatase (IA-2A) [1–3]. Multiple sclerosis (MS) is the most common autoimmune disorder of the central nervous system (CNS) and is caused by devastation of myelin sheath induced by autoreactive populations of T-cells recognizing myelin basic protein (MBP) and other antigens [4].

Impairments or imbalance in the regulatory functions of the immune system [5–7] and the pathogenic role of Th17 cells [8,9] appear to be important mechanisms of initiation of these autoimmune diseases. Over the past decade, increasing attention has been focused on T-regulatory (Treg) and T-suppressor (Ts) cells as key players in the control of self-reactive T-cells as well as their role in the induction of peripheral tolerance *in vivo*. In 1995, Sakaguchi demonstrated immune regulatory role and suppression functions of $CD4^+CD25^+$ Treg cells in peripheral blood [10]. Naturally occurring Treg cells are characterized by the expression of high levels of cytotoxic T-lymphocyte antigen-4 (CTLA-4), glucocorticoid-induced TNF receptor (GITR), inducible co-stimulatory molecule (ICOS) and especially FoxP3 (an intracellular transcription factor that controls development and function of Treg cells) [11] which inversely correlates with low expression of CD127 molecule (surface IL-7 receptor) [12]. In MS and T1D, as well as in other autoimmune disorders, naturally occurring Treg cells exhibit reduced suppressive properties [7,13]. Another T-cell population that plays an important role in the autoimmune pathogenesis is $CD8^+CD28^-$ termed T-suppressor cells [14]. Existence and regulatory functions of Ts cells has been demonstrated in an animal model of MS, in experimental autoimmune encephalomyelitis (EAE) [15]. Ts cells are MHC class I restricted and operate in an antigen-dependent manner [16]. They restrain antigen-presenting cell (APC) function, and thus control further activation of T-helper cells [17]. In MS, it seems that those activated cells could be

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doi:10.1016/j.cellimm.2010.02.002

MBP-reactive T-cells with decreased dependence on CD28-mediated co-stimulation [18]; however, not enough information about the CD28-co-stimulation and suppression mechanisms is available in T1D.

Naive and memory T-cell populations are distinguishable by expression of CD45RA and CD45RO surface molecules. Naive T-cells are able to travel to the secondary lymphoid organs, where they encounter antigen-presenting cells (APC). Being contacted by APC loaded with an antigen, naive cells become activated and start to proliferate. Conversely, memory T-cells are immune system responders affected by previously recognized antigen [19]. These cells are considered the executive part of autoimmune processes [20,21].

Numerous studies on T-cell populations demonstrating important immune functions in T1D and MS were published. Particularly, role of $CD4^+CD25^+FoxP3^+$ Treg cells in T1D and $CD8^+CD28^-$ Ts cells in MS were studied [12,14,22]. But none of these studies were able to compare both regulatory populations with other T-cell subsets including naive and memory populations in patients with T1D, MS and healthy controls.

2. Materials and methods

2.1. Patients and controls

Thirty patients with a history of T1D treated at the Department of Internal Medicine and Hepatogastroenterology of the University Hospital Brno were enrolled into the study. All of T1D patients were well-compensated individuals without metabolic acidosis at the time of study examination. Thirty-one patients with relapsing-remitting MS (RRMS) were included in the study. All MS patients were diagnosed at the Department of Neurology, University Hospital Brno, according to the International McDonald Diagnostic Criteria [23]. None of the T1D or RRMS patients suffered from any other autoimmune disease or inflammation or received any immunomodulatory therapy for at least 2 months preceding the study examination. A control group consisting of 29 age-matched healthy volunteers was consecutively recruited from healthy blood donors at the Transfusion Department and Blood Bank, University Hospital Brno. Blood samples of all subjects were taken exclusively after signing the informed consent form approved by the local Ethical Committee.

2.2. Flow cytometry of T-cell populations

EDTA-anticoagulated venous blood samples were taken from each study subject. One hundred microliters of the sample were incubated with appropriate amounts of monoclonal antibodies. After 20 min at room temperature, erythrocytes were lysed and samples were fixed with 1% paraformaldehyde in PBS. Flow cytometric analysis of T-cell populations was determined using the following markers: anti-CD3, anti-CD4, anti-CD8, anti-CD25, anti-CD127, anti-CD28, anti-CD45RA and anti-CD45RO labelled with fluorescein isothiocyanate (FITC), phycoerythrin (PE), phycoerythrin-cyanin 5 (PC5), or phycoerythrin-cyanin 7 (PC7) (all antibodies from Immunotech, Marseille, France). Immunophenotyping of T-lymphocytes was performed by four-colour flow cytometry on a CytomicsTM FC 500 cytometer (Beckman Coulter, Miami, FL, USA). Data were analyzed using the CXP Software (Beckman Coulter).

2.3. Statistical analysis

Data obtained during the analysis were evaluated using non-parametric Mann–Whitney test for a comparison of patients and healthy controls. Age-associated measurements were assessed using Spearman's correlation analysis. Significance level $P < 0.05$ was adjusted for multiple testing by the step-down Bonferroni–Holm correction. Statistical analyses were performed using the Statistica for Windows package version 8.0 (StatSoft Inc., Tulsa, OK, USA).

3. Results

3.1. T-suppressor cells are distinctly decreased in patients with T1D and RRMS

Flow cytometry was used to analyse populations of T-lymphocytes obtained from peripheral blood of the groups of T1D patients, RRMS patients and healthy controls. Characteristics of all examined groups are shown in Table 1. Different T-lymphocyte subsets were precisely evaluated using the following markers – $CD3^+CD4^+CD25^+CD127^{low}$ Treg cells, $CD3^+CD8^+CD28^-$ Ts cells, activated $CD3^+CD4^+CD25^+CD127^+$ and $CD3^+CD8^+CD25^+$ cells, naive $CD3^+CD4^+CD45RA^+$ and memory $CD3^+CD4^+CD45RO^+$ cells. Lymphocyte population was gated using forward and side scatter. The relative percentages of studied subpopulations were determined within the $CD3^+CD4^+$ or $CD3^+CD8^+$ T-cells, respectively. Gating strategy of $CD4^+CD25^+$, $CD4^+CD25^+CD127^+$ and $CD4^+CD25^+CD127^{low}$ populations is displayed in Fig. 1. Evaluation of T-cell populations was performed for each studied group (Fig. 2). No difference in frequency of T-regulatory population was detected among the groups of patients and healthy controls (Fig. 2a). T-suppressor population was significantly decreased in T1D patients ($P = 0.0008$) as well as in RRMS patients ($P = 0.0008$) when compared to the group of healthy controls (Fig. 2b). Remarkably increased frequencies of activated $CD4^+CD25^+CD127^+$ and $CD8^+CD25^+$ cells were noticed in T1D patients in comparison to healthy controls and RRMS patients, respectively (Fig. 2c and d). Frequencies of naive T-cells demonstrated decreasing trend in patients with T1D as well as RRMS, but they did not reach statistical significance (Fig. 2e). On the other hand, memory T-cells were significantly increased in the group of T1D patients and a similar trend was noticed in RRMS patients (Fig. 2f).

Dependence of T-lymphocyte populations on gender within healthy controls and RRMS patient groups were analyzed, however no significant relation was determined (data not shown).

3.2. T-suppressor cells, naive and memory cells are not age-dependent in T1D and RRMS patients

Correlations of T-lymphocyte populations with age in groups of patients and healthy controls were performed (Table 2). Negative age-dependent trend within the Treg population was detected in all studied groups of subjects; but without any statistical significance. $CD8^+CD28^-$ T-suppressor population was considerably related to age in healthy controls ($P = 0.0082$); however, no such correlations were detected in groups of RRMS and T1D patients. Decreased frequency of activated $CD4^+CD25^+CD127^+$ population with increasing age in the group of healthy controls was noticed.

Table 1
Basic characteristic of healthy controls group and patients with T1D and RRMS.

	Healthy controls	T1D patients	RRMS patients
Number of subjects	29	30	31
Male/female	17/12	17/13	10/21
Age (min.–max.; median)	18–62; 30	18–49; 26	22–58; 34
Median age at diagnosis (years)		22	33