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Percentage of LFA-1$^+$ and ICAM-1$^+$ peripheral blood mononuclear cells in children and adolescents with Type 1 diabetes does not distinguish patients with vascular complications

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Abstract: There are only few studies evaluating lymphocytes activation in the diabetic vascular complications. ICAM-1/LFA-1 adhesion molecules not only participate in the lymphocyte T proliferation but also mediate leukocyte migration to the site of inflammation. We assess a relationship between the percentage of ICAM-1 and LFA-1 expressing PBMCs and the evolution of vascular complications in T1D in children and adolescents. The study was carried out on 60 children and adolescents with T1D (aged 9-20): (a) T1D lasting <5 years (n=20), (b) T1D lasting >5 years (n=20), without complications c) T1D lasting >5 years complicated with microalbuminuria, arterial hypertension, diabetic retinopathy (20 n). 20 healthy volunteers, age and sex matched constituted the control group. The expression of adhesion molecules was evaluated by using three-color flow cytometry. In children and adolescents with T1D <5 years, the percentage of ICAM-1$^+$ and LFA-1$^+$ PBMCs was decreased *vs.* controls ($p<0.05$ and $p<0.001$, respectively). Both in patients with T1D>5 years without vascular complications and in T1D with vascular disease the percentage of LFA-1$^+$ T lymphocytes was significantly reduced in the peripheral blood ($p<0.001$ *vs.* healthy controls). In conclusion the percentage of LFA-1$^+$ and ICAM-1$^+$ PBMCs does not distinguish patients with vascular complications however decreased percentage of LFA-1$^+$ PMBCs could serve as a non-specific marker of the development of local inflammatory process in Type 1 diabetes.

Keywords: type 1 diabetes, intercellular adhesion molecule-1, LFA-1, vascular complications, T cells' subsets

Introduction

It has been documented that the T-cell costimulation and lymphocyte migration play the central role in the inflammatory process [1]. Adhesion molecules, among others are suggested as the inflammatory markers of diabetic micro- and macroangiopathy [2,3]. ICAM-1/LFA-1 adhesion molecules not only participate in the lymphocyte T proliferation but also mediate leukocyte migration to the site of inflammation [1]. In our previous study we have demonstrated that the percentage of PBTLs expressing costimulatory molecules CD28/CTLA-4 was altered in children/adolescents with T1D and with vascular complications [4].

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Abbreviations: CD – cluster of differentiation antigen; CTLA-4 – cytotoxic T lymphocyte antigen 4; PE – phycoerythin; PerCP – peridin chlorophyll protein; FITC – fluorescein isothiocynate; LFA-1 – lymphocyte function-associated antigen-1, ICAM-1 – intercellular adhesion molecule-1; PBTLs – peripheral blood T lymphocytes; PBMCs – peripheral blood mononuclear cells; sVCAM-1 – soluble vascular cell adhesion molecule-1;

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Table 1. Median values of the percentage of PBMCs expressing LFA-1, ICAM-1 in the evaluated groups (*p<0.05 T1D<5 years *vs.* T1D>5 years without vascular complications and *vs.* controls, **p<0.01 T1D>5 years and without vascular complications *vs.* controls, ***p<0.001 T1D<5 years and lasting >5 years without vascular complications *vs.* controls).

Percentage of PBMCs	Type 1 diabetes mellitus			Healthy controls
	Lasting < 5 years	Lasting > 5 years	With vascular complications	
	median	median	median	median
$CD3^+LFA\text{-}1^+$ T lymphocytes [%]	45.2***	50.0**	45.3***	91.3
$CD14^+ICAM\text{-}1^+$ monocytes [%]	34.6*	76.5	60.3	78.0

Recently an increasing number of studies indicate the genetic influence of ICAM-1 polymorphism in development of the T1D and vascular complications [5,6]. Many reports documented an elevated serum concentrations of sICAM-1 and vascular adhesion molecules in patients with diabetes and microvascular disease [7-12]. Nevertheless, data on the expression of $ICAM\text{-}1^+/LFA\text{-}1^+$ peripheral blood mononuclear cells in children/adolescents with Type 1 diabetes with vascular complications is lacking.

In the present study: (a) we examined the expression of LFA-1 on T lymphocytes and the expression of ICAM-1 on monocytes in the course of Type 1 diabetes in children and adolescents; (b) we assessed a relationship between the percentage of PBMCs expressing ICAM-1 and, LFA-1 and the development of diabetic vascular complications (microalbuminuria, diabetic retinopathy and arterial hypertension).

Materials and methods

Patients. Three groups of 60 Caucasian children and adolescents (aged 9-20 years), were randomly selected from the Second Department of Children's Diseases, Medical University of Bialystok. Type 1 diabetes mellitus was diagnosed according to WHO criteria.

1. 20 children (10F and 10M) with T1D<5 years (mean 3±0.97), aged 9-19 (mean 14.9±2.7);
2. 20 children (8F and 12M), with T1D>5 years (mean 8±1.95), aged 11-20 (mean 14.5±2.4); without complications;
3. 20 children (9F and 11M), with T1D>5 years (mean 9.45±2.87), aged 12-20 (mean 17.1±2.5), and with vascular complications: microalbuminuria, arterial hypertension, diabetic retinopathy (9 children had one vascular complication, 8 subjects two complications and 3 subjects had all complications). All patients were under intensive insulin therapy.

Presence of diabetic retinopathy, arterial hypertension and incipient nephropathy-microalbuminuria were diagnosed according to the standards of American Diabetes Association [13].

Presence of diabetic retinopathy was diagnosed after pupil dilation on basis of direct and indirect ophthalmoscopy and confirmed by fluorescein angiography. Arterial hypertension was found when 30% of Ambulatory Blood Pressure Monitoring (ABPM) values were over 95 percentile according to age and sex tables for blood pressure. For a diagnosis of diabetic nephropathy, a urinary albumin excretion rate (UAE) of 20-200 µg/min or >30mg/24h from at least two subsequent specimens was classified as microalbuminuria after exclusion of urinary infection.

Control group consisted of 20 healthy volunteers, (13 F and 7 M), aged 6-17 (mean 14□3.1), with no family history of insulin-dependent diabetes or other autoimmune diseases. Written informed consent was obtained and the protocol for the study was approved by the Local Ethics Committee.

Flow cytometric analysis. Briefly, 100 µL samples of whole blood were stained with 10 µL of the following tree-color mAb: (Simultest, Becton Dickinson Immunocytometry System, San Jose, CA, USA) CD3-PerCP; CD3-PerCP/CD11a-FITC; CD14-FITC/CD54-PE. After incubation at room temperature for 20 min, the samples were than processed with a 35s cycle on rapid no-wash whole blood lysis work station. A minimum of 10^4 cells of the evaluated subset were analysed for each sample on Coulter EPICS XL flow cytometer. The PBMCs' subpopulations were calculated by the software. The total numbers of leukocytes, lymphocytes and monocytes in the peripheral blood were measured by Coulter MAXM haematological counter.

Isotype-identical mAbs served as controls (IgG1-FITC/IgG2b-PE). The percentage of positive cells was determined by setting the lower limit over the non-specific fluorescence with suitable controls.

Assessment of HbA_{1c} concentrations. HbA_{1c} was quantified in diluted and K_2EDTA serum by liquid chromatography technique HPLC – Variant (Bio-Rad).

Statistical analysis. Results are presented as median (range). Kolmogorov-Smirnof' test, Kruskal-Wallis test and Spearman's rank correlation test were used. A *p* value of less than 0.05 was considered statistically significant. All data were performed using Statistica 6.0 (StatSoft, Tulsa, OK, U.S.A.).

Results

Metabolic control: HbA_{1c}

The values of HbA_{1c} were higher in the groups of T1D>5 years with and without vascular complications than in T1D<5 years (8% and 8.2%, *vs.* 7.9%; respectively). However no statistical difference was observed.

The percentage of PBMCs expressing ICAM-1/LFA-1

Subjects with T1D lasting <5 years showed both a decreased percentage of PBMCs expressing ICAM-1 and LFA-1 molecules (p<0.05 and p<0.001 *vs.* con-

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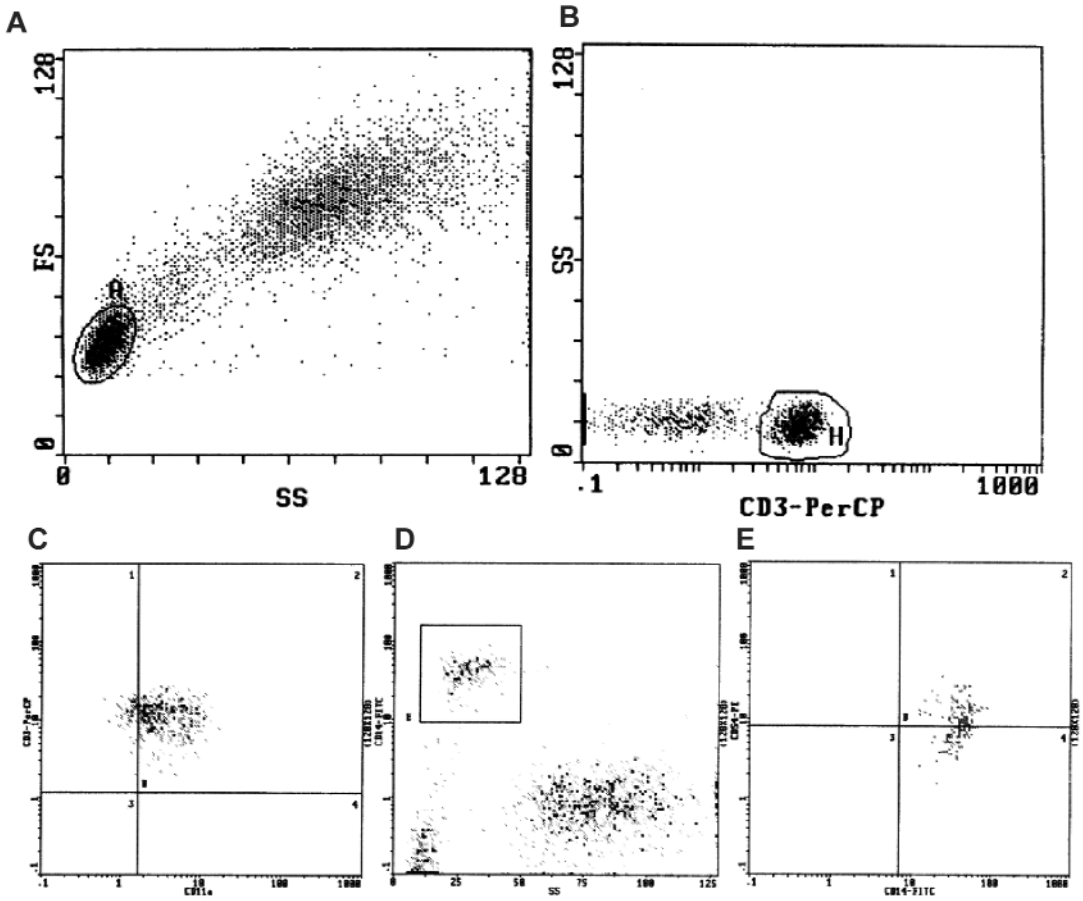

Fig. 1. Flow cytometric dot plots of PBMCs from a representative patient. (**A**) Scatter plot of lymphocytes pregating by light properties; (**B**) gating of CD3+PerCP (T lymphocytes population) and its subsets (CD3+PerCP; CD3+PerCP/CD11a+FITC), (**C**); (**D**) CD14+FITC monocytes SSC gating; (**E**) The subpopulation of CD14+FITC/CD54+PE peripheral blood monocytes. The percentage of positive cells was determined by setting the lower limit over the non-specific fluorescence with suitable controls.

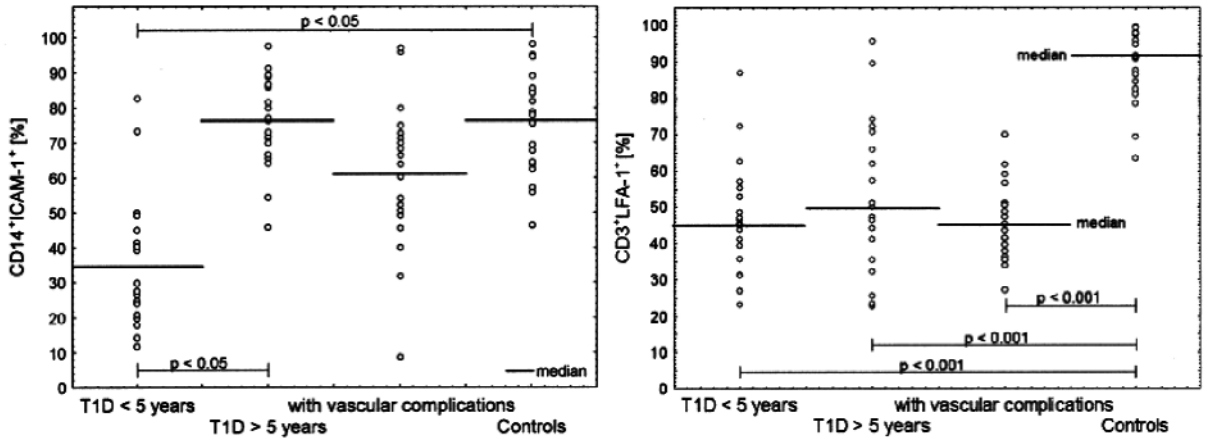

Fig. 2. The expression of ICAM-1 on peripheral blood monocytes and LFA-1 on PBTLs in the evaluated groups with Type 1 diabetes mellitus.

trols respectively) (Table 1). The difference between the median percentage of ICAM-1+ PBMCs in children with T1D<5 years and in children with T1D>5 years without vascular complications was statistically significant ($p<0.05$) (Table 1, Fig. 2).

In subjects suffering from T1D lasting >5 years without complications and with vascular complications disease only a decreased percentage of LFA-1+ PBTLs was observed ($p<0.001$ *vs.* healthy controls respectively) (Table 1, Fig. 2).

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Discussion

In our previous studies, we observed the decreased percentage of VLA-4$^+$ and CD28$^+$ PBTLs in children with T1D lasting <5 years [4-14]. The diminished expression of LFA-1 and ICAM-1 on the PBMCs may be due to the tapping of these cells expressing adhesion molecules into the site of inflammation and hence their lack in the circulation [15]. Both the elevation in serum and the reduced membrane expression can result from increased shedding of LFA-1 and ICAM-1 molecules and reflect the ongoing immune process [4,15].

Earlier studies, documented the elevated serum concentrations of sICAM-1 and sVCAM-1 in the diabetic complications indicating an endothelial dysfunction [9-12].

Many authors have suggested that the vascular disease starts early in the course of childhood diabetes and that the adhesion molecules participate in the diabetic micro- and macroangiopathy [9,11,12].

In our recent report, suggesting PBMCs activation, we have documented that the percentage of PBLTs expressing L-selectin was increased in Type 1 diabetic children, especially in subjects with vascular complications, while the percentage of VLA-4+ PBLTs was decreased only in children without vascular complications [14]. We concluded that the proportion of L-selectin$^+$ and VLA-4$^+$ PBTLs might be considered as an early non-specific marker of diabetic microvascular complications or arterial hypertension.

This notion may be supported by the results of Targer *et al*. indicating that the level of sICAM-1 was significantly increased in T1D without a clinically overt macrovascular disease, moreover it was even higher in the advanced microvascular complications [16].

There are only few studies evaluating lymphocytes activation in the diabetic vascular complications [4,14,17,18]. Shestakova *et al*. found an increased percentage of ICAM-1$^+$ lymphocytes and granulocytes in patients with microalbuminuria and especially neovascularization. These findings showed not only endothelium but also leukocytes activation at early disease stages [17]. Increased counts of T lymphocytes and high expression of ICAM-1 in ocular tissues of diabetics, suggest an important role of these factors in recruitment of this cells at the site of microvascular lesions [3,18-20].

In conclusion, the percentage of ICAM-1$^+$ and LFA-1$^+$ PBMCs does not distinguish patients with vascular complications however decreased LFA-1$^+$ PMBCs percentage could serve as a non-specific marker of the local inflammatory process in T1D. Nevertheless, since for all surface markers there is a substantial overlapping in the evaluated groups the study needs further analysis.

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