CD_{38} expression on peripheral T and B Lymphocytes in newly diagnosed T1DM children

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Abstract

Background: In Type 1 Diabetes Mellitus (T1DM), numerous changes in the cellular as well humoral immune response have been identified. However, it is not known whether both the CD_4^+ and CD_8^+ subpopulation or only one of these or CD_{19}^+contains increased numbers of activated cells.

Objective: The aim was investigate the expression of CD_{38} as activated marker on the peripheral blood lymphocytes of T1DM children at the onset of the disease.

Patients and methods: A total of 60 T1DM patients who were newly onset of the disease (diagnosed less than five months) were included in the present study, all the patients were treated with daily replacement doses of insulin. Fifty apparently healthy control subjects underwent the peripheral blood lymphocytes (PBL) phenotyping. Phenotyping of surface antigens was done by direct Immunoflurocent (IF) technique using mouse antihuman CD_{38}.

Results: Increased percentage of activation marker CD_{38}^+ cells were observed in T1DM patients (24.72%, 23.83%) as compared with the control group (16.86%, 15.97%) in the age group ≤10 years and >10 years old respectively. These differences were highly significant (P_1=0.0001) between the patients and healthy individuals, but failed to reach a significant level (P_2= 0.44) between the patients in both age groups.

Conclusion: A significant elevated percentage of $CD38^+$ activation marker cells were detected in the patients.

Key Words: T1DM, CD_{38}, Immunophenotyping

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Introduction

CD_{38} initially characterized as a protein extensively expressed on human thymocytes, but later is known to be expressed on multiple cell types including monocytes, platelets, natural killer cells, T and B lymphocytes, myeloid cells, vascular endothelium, and in tissues such as brain, cardiac and skeletal muscle, spleen, liver, prostate and kidney [(1)].

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However the surface expression of CD_{38} is under control and varies during lymphocyte development, activation and differentiation, suggesting that CD_{38} may play an important role in lymphocytes function [(2)]. Two functions have been identified for CD_{38} in B and T cells. First, CD_{38} has been demonstrated to be a lymphocyte signaling molecule, and second, it is an ectoenzyme with NAD^+ glucohydrolase, ADP-ribosyl cyclase and cyclic ADP ribose hydrolase activities [(3)]. CD_{38} expression levels on particular cell types can be altered under certain pathological states, and in some orders CD_{38} expression on T cells has prognostic values. This is well established for HIV disease [(4)], and for prostate malignancy [(5)].

It was detected that anti- CD_{38} autoantibodies was found in 9.7% of type II diabetic patients and in 13.1% of type T1DM patients vs. 1.3% in control group [6]. Another report conducted by Mallone *et al.,* found that anti- CD_{38} antibody prevalence among new- onset T1DM patients (3.8%) was lower than previously found in long- standing type 1 diabetics (11.7%) [7], and in a significant number (9-15%) of patients with type II or long- standing type 1 diabetes [8]. Anti- CD_{38} antibodies were found to be associated with anti- GAD antibodies. CD_{38} autoantibodies were found in 8.4% of type II diabetic patients, particularly in anti- GAD positive (14% vs. 6% of anti- GAD negative) [9]. In vitro study conducted by Marchetti, *et al.,* found that prolonged exposure of human pancreatic islets to sera containing CD_{38} antibodies impairs their function and viability [10].

In the present study, we have investigated the expression of CD_{38} on the peripheral blood lymphocytes of T1DM children at the onset of the disease.

Subjects, Materials and Methods

Sixty Iraqi T1DM children (28 males and 32 females) were subjected to this study. The patients were attending to National Diabetes Center at Al-Mustansiriya University during the period May 2004 to October 2005. Their ages range from 3 -17 years, and they were new onset of the disease (diagnosis was from one week up to five months). Diagnosis of Diabetes Mellitus and selection of patients was accomplished with the assistance of the consultant medical staff in the National Diabetes Center. All the patients were treated with daily replacement doses of insulin at the time of blood sampling. The patients were divided into two groups according to their ages in order to assess the aggressive of immune responses: 36 children equal or less than 10 years and 24 children more than 10 and up to 17 years. For the purpose of comparisons, 50 healthy control subjects matched for age (4-17 years old) and sex were selected who have no history or clinical evidence of type 1 diabetes or any chronic diseases and obvious abnormalities as a control group.

Five milliliter of venous blood was drawn from each subject (patients and controls). The collected blood was displaced into glass universal tubes containing heparin (10 IU /ml) as anticoagulant. The mononuclear Lymphocytes were isolated and assayed the same day. Lymphocytes were separated from the whole blood using Ficoll- Isopaque density centrifugation (Flow-Laboratories, UK). This technique was reported by Schendel *et al.,* 1997 [11]. The collected cells were suspended in washing medium (RPMI-1640 free serum cultured media) (Euroclone, UK) and centrifuged three times, then the lymphocytes were resuspended in 2 ml warm RPMI-1640 supplemented with 10% heat inactivated human type AB serum and determined their viability. The viability accepted should be 95% and above. The final lymphocyte concentration was adjusted to 2-3x 10^6 cells/ml [12].

Phenotyping of surface antigens of PBL of both patients and controls was performed by direct Immunoflurocent (IF) technique. In the present study, mouse antihuman CD_{38} monoclonal antibody was used (Serotec, UK). It was purified IgG conjugated to fluorscein isothiocynate isomer-1 (FITC). The method of IF-labeling of fixed cells was done as described by Wigzel and Anderson, 1971. Slides were ready for

examination with IF-microscope immediately or up to 3 days as a maximal duration. The number of the only stained cells was counted. This maneuver was repeated till 200 cells had been counted. Positive cells give green-apple color [13].

The tests which have been used for statistical analysis were Student t-test; the results were expressed as means ± standard error (SE), and also Pearson Correlation (R).

Results

Peripheral blood lymphocytes phenotyping can give an idea of the immunological status in patients with T1DM and it can be considered as a mirror image of the immunity.

Increased percentage of activation marker CD_{38}^+ cells were observed in T1DM patients (24.72%, 23.83%) as compared with the control group (16.86%, 15.97%) in the age group ≤10 years and >10 years old respectively. These differences were highly significant (P_1=0.0001) between the patients and healthy individuals, but failed to reach a significant level (P_2= 0.44) between the patients in both age groups (Table 1) (Figure 1). There was strong direct positive correlation between the mean percentage of CD_{38}^+ cells and CD_4^+ cells (r= 0.808) and CD_{19}^+ cells (r= 0.602) (data was not shown).

Table 1: The differences in mean peripheral CD_{38}^+ lymphocyte percentage between control and T1DM patients groups.

Age	Groups	No.	CD_{38}^+ lymphocyte %				P1	P_2
			Mean	SE	Min.	Max.		
≤10 years	Controls	21	16.86	0.76	13.00	23.00	0.0001 (HS)	0.44 (NS)
	T1DM	36	24.72	0.81	15.00	38.00		
>10 years	Controls	29	15.97	0.63	12.00	23.00	0.0001 (HS)	
	T1DM	24	23.83	0.82	15.00	31.00		

P_1: TIDM Patients vs. controls

P_2: TIDM Patients ≤10 years vs. patients >10 years.

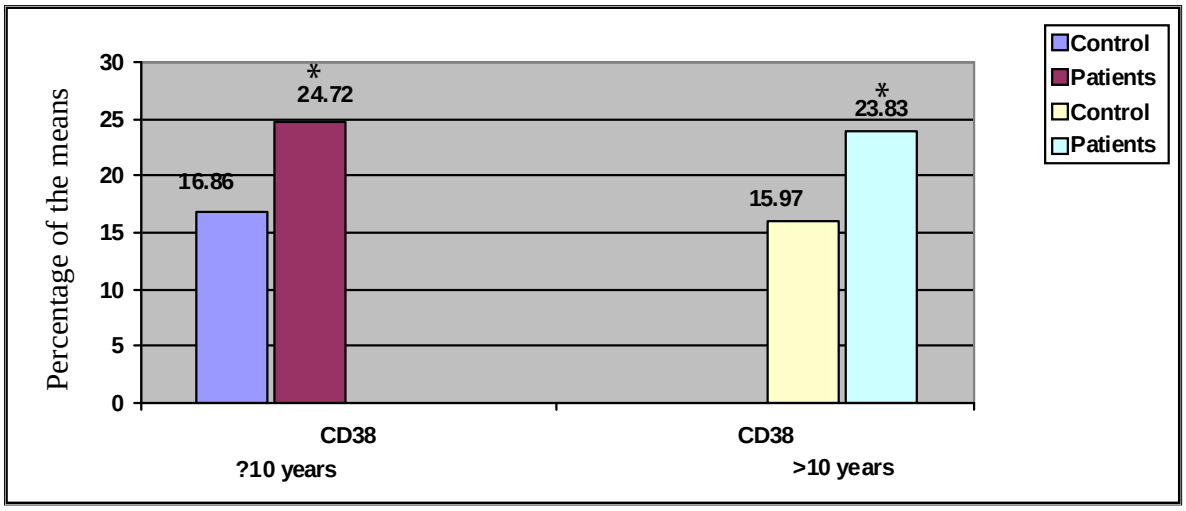

Figure 1: Bar chart of mean percentage of CD_{38}^{+} cell populations for the healthy control and TIDM patients.

Discussion

The results detected a very high significant elevated percentage of activation CD_{38}^{+} antigens in PBL of T1DM patients. CD_{38} is (ADP/ ribosyl cyclase/ ADP ribose hydrolase) an integral membrane glycoprotein. Human CD_{38} is highly expressed on early T-cell precursors migrating to the thymus and on CD_4^{+} CD_8^{+} double positive thymocytes. During the process of negative selection, CD_{38}^{+} expression is decreased and mature single positive T-cells express low levels of CD_{38} [3]. It is present on approximately all pre-B-lymphocytes, in 18% of Th and some Tc cells [14], and in tissues such as human pancreatic islets [15]. In pancreatic beta-cells, this enzyme appears to play a role in glucose induce insulin release via a mechanism involves its cyclase activity which leading to increase cytoplasmic Ca^{+2} concentration and insulin release [6]. Mature T-cells isolated from peripheral blood can acquire CD_{38}^{+} cell surface expression during antigen activation [3].

A strong positive linear relationship is found between CD_{38}^{+} cells and CD_4^{+} cells (r = 0.808), with CD_{19}^{+} cells (r = 0.602). CD_{38}^{+} acts as positive and negative regulator of cell activation and proliferation depending on cellular environment. Thus, mature B-cells proliferate whereas the opposite occurs in immature B-cells in the bone marrow.

The CD_{38} signaling pathway in this environment blocks B-lymophopoiesis, mostly by inducing apoptosis [16]. CD_{38} involved in adhesion between human lymphocytes and endothelial cells. Presence of autoantibodies with anti-CD_{38} specifity in patients with type 1 and type II diabetes has been reported to down regulate CD_{38} expression in lymphoid cells [6]. A study conducted by Pupilli *et al.*, found that CD_{38} autoimmunity increases with time in T1DM children and persist [17]. These

autoantibodies are biologically active, the majority of them (60% displaying agonistic properties i.e. [Ca+2] i-mobilization in lymphocytic cell lines and pancreatic islets [8]. Human anti-CD_{38}^{+} autoantibodies with agonistic properties on the CD_{38}^{+} effector system occur in nature and in human islets, their [Ca+2] i-mobilizing activity is coupled with the ability to stimulate insulin [18].

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