



Short Communication

Abnormal Distribution of $\gamma\delta$ T Lymphocytes in Graves' Disease and Insulin-Dependent Diabetes Type 1

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Abstract. There is an increasing evidence that $CD3^+$ cells, bearing $\gamma\delta$ T cell receptors representing a minor subpopulation of T cells in the peripheral blood of humans are involved in the development of autoimmunity. The aim of the present study was determination of the $\gamma\delta$ T cell subpopulation levels in the peripheral blood of subjects with Graves' disease and newly diagnosed type 1 diabetes in comparison to age-matched healthy controls. The percentages of $CD3^+$, $CD8^+$, $\gamma\delta$ TCR $^+$ CD8 $^+$, $\gamma\delta$ TCR $^+$ CD8 $^-$ lymphocyte subsets were measured by flow cytometry. In the peripheral blood of newly diagnosed Graves' disease patients we showed a significant decrease of $\gamma\delta$ TCR $^+$ cells and $\gamma\delta$ TCR $^+$ CD8 $^-$ subset content in comparison to the percentages observed in subjects after methimazole treatment and in healthy controls. We also found a significant increase of $\gamma\delta$ TCR $^+$ CD8 $^+$ cells in the peripheral blood of subjects with insulin-dependent diabetes, treated with insulin for 3–6 months. The present findings confirm our previous hypothesis that $\gamma\delta$ TCR $^+$ CD8 $^+$ lymphocyte subset could play a role in the pathogenesis of diabetes type 1, probably as regulatory T cells and could be induced by delivery of exogenous insulin. Our results suggest that $\gamma\delta$ T cells ($\gamma\delta$ TCR $^+$ CD8 $^-$ subset) could also play an important role in the development of Graves' disease and that their levels are modulated by thyreostatic treatment.

Key words: $\gamma\delta$ TCR $^+$ lymphocytes; Graves' disease; type 1 diabetes mellitus.

Introduction

$CD3^+$ cells, bearing $\gamma\delta$ TCR, (T cell receptor, formed by γ and δ chains) represent the minor subpopulation of T cells in the peripheral blood of humans (5–10%)^{3, 5}. In contrast to $\alpha\beta$ T cells, the recognition of antigens by $\gamma\delta$ T cells is not restricted to MHC class I or II presented molecules^{2, 5}. Human $\gamma\delta$ T cells have been described to recognize lipid derivatives of mycobacteria, heat shock proteins and other non-peptide, low weight antigens⁵. It has been suggested that $\gamma\delta$ T cells have a natural capacity to recognize self antigens^{5, 7} and reveal cytotoxic and natural killer's capabilities¹⁵.

$\gamma\delta$ TCR $^+$ lymphocytes cells have also been recently suggested to play a critical role in the pathogenesis of autoimmune diseases^{4, 12}. An altered proportion of $\gamma\delta$ T cells in the peripheral blood and infiltration of the target organs have been previously described in autoimmune diseases such as: multiple sclerosis, systemic lupus erythematosus and rheumatoid arthritis^{8, 10, 15}.

The aim of the present study was the estimation the $\gamma\delta$ T cell subpopulation levels in the peripheral blood of subjects with newly diagnosed and treated with methimazol Graves' disease and type 1 diabetes in comparison to healthy controls.

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Materials and Methods

The study was carried out in 3 groups of subjects: group I – 18 patients with Graves' disease (13 females and 5 males, mean age 27.7 ± 13.3 years): 10 subjects with clinical and biochemical signs of hyperthyroidism and 8 subjects in euthyrosis state (after 7–24 months therapy with methimazol),

group II – 16 subjects with a recent onset of type 1 diabetes mellitus (< 6 months from diagnosis; 6 females and 10 males, mean age 25.6 ± 6.9 years),

group III – 16 healthy volunteers (control group) age and sex matched to group I and II (9 females and 7 males, aged 25.2 ± 6.6 years, who had no family history of Graves' disease, insulin-dependent diabetes or other autoimmune diseases. None of the subjects had experienced acute infection or illness during 3 weeks prior to the sample collection. An informed consent was obtained. The protocol for the study was approved by the Local Ethical Committee at the Białystok University Medical School.

Fasting venous samples were collected between 7.30 and 8.30 a.m. for measurement of the serum fT₄, TSH and glucose levels, and (in EDTA tube) for morphology parameters, HbA_{1c} and CD phenotyping.

Leukocyte preparation from whole blood was performed within 2 h of storage at room temperature on the Q-Prep EPICS Immunology Workstation (Coulter Corp., Hialeah, USA).

The percentages of CD3⁺, CD8⁺, $\gamma\delta$ TCR⁺CD8⁺, $\gamma\delta$ TCR⁺CD8⁻ lymphocyte T subsets were measured on a Coulter EPICS XL cytometer using combinations of monoclonal antibodies conjugated with fluorescein isothiocyanate or R-phycoerythrin directed against $\gamma\delta$ TCR (Becton Dickinson, San Jose CA), CD3, CD8 and CD14/CD45 (gate check) (Dako A/S, Glostrup, Denmark) lymphocyte surface markers. A minimum of 10 000 lymphocytes were analyzed for each sample. The percentage of positive cells was determined by setting the lower limit over the non-specific fluorescence with a suitable control.

The total number of leukocytes and lymphocytes in

the peripheral blood were measured by hematological counter (MAXM, Coulter Germany) and the absolute cell numbers of studied lymphocyte subpopulations were counted.

Levels of free T₄ and TSH in the serum were determined by EIA method (Abbott). HbA_{1c} was quantified by the liquid chromatography technique (Bio-Rad), glucose concentration was measured by the enzymatic method (Cormay, Lublin).

The results are presented as means $\pm$ standard deviation for percentage and absolute number of lymphocyte subpopulations. The statistical significance of the differences in the percentages/numbers of lymphocyte subsets between the studied and the control groups were estimated by the Mann-Whitney *U*-test. The relationship between the studied parameters were evaluated by Spearman's rank correlation (Statistica 5.0, Stat Soft).

Results

The absolute number/percentage of total leukocytes, CD3⁺ T lymphocytes and CD8⁺ T cells did not differ statistically between patients with Graves' disease, diabetes type 1 and the healthy controls (Table 1). The absolute number of $\gamma\delta$ TCR⁺ lymphocytes was significantly lower at the onset of Graves' disease in comparison to patients with treated Graves' disease, diabetes type 1 and the healthy controls (Table 1). The analysis of $\gamma\delta$ TCR⁺ lymphocyte subsets in the peripheral blood revealed that depletion of $\gamma\delta$ TCR⁺ cells in untreated Graves' disease is restricted mainly to $\gamma\delta$ TCR⁺CD8⁻ cells, whereas the levels of $\gamma\delta$ TCR⁺CD8⁺ T cells were similar to the percentages in the controls (Fig. 1). In the group after methimazole treatment $\gamma\delta$ TCR⁺CD8⁻ T cell levels were significantly higher in comparison to untreated Graves' disease patients (Fig. 1) and were comparable to the percentages in the control group. We did not observe any alterations of $\gamma\delta$ TCR⁺CD8⁺ T cells in the peripheral blood of patients with treated Graves' disease. Moreover, the number of CD8⁺ lymphocytes

Table 1. Mean $\pm$ SD of absolute numbers/percentages of leukocytes, CD3⁺ lymphocytes and $\gamma\delta$ TCR⁺CD3⁺ cells in Graves' disease and newly diagnosed diabetes type 1 patients and the healthy controls

	Graves' disease		IDDM	Controls
	before treatment	after treatment		
Total leukocytes ($\times 10^3$ cells/ml)	6.6 $\pm$ 1.6	6.5 $\pm$ 2.6	6.7 $\pm$ 1.1	6.6 $\pm$ 1.1
CD3 ⁺ lymphocytes T (% of total)	74.2 $\pm$ 7.1	75.1 $\pm$ 8.5	76.6 $\pm$ 4.5	76.3 $\pm$ 5.3
CD3 ⁺ CD8 ⁺ cells (% of total)	31.6 $\pm$ 6.7	28.6 $\pm$ 13.1	33.5 $\pm$ 4.1	30.3 $\pm$ 6.2
$\gamma\delta$ TCR ⁺ CD3 ⁺ (cells/ml)	76 $\pm$ 27*	119 $\pm$ 17	134 $\pm$ 21	124 $\pm$ 23

* $p < 0.01$ – statistically significant vs. controls.

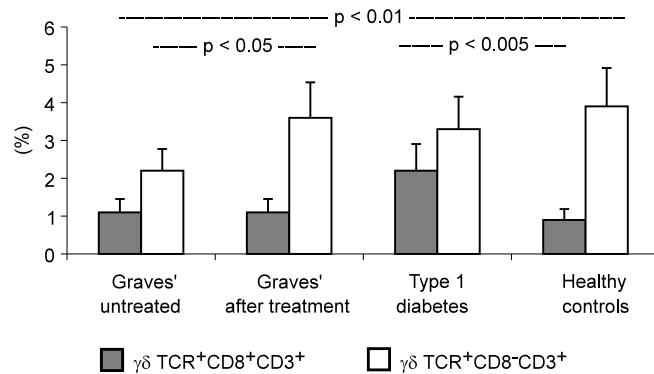


Fig. 1. Mean $\pm$ SD of percentages $\gamma\delta$ TCR⁺ CD3⁺ T cell subpopulations in Graves' disease (before and after methimazole treatment), newly diagnosed diabetes type 1 patients and healthy controls

bearing $\gamma\delta$ TCR, were statistically higher in the patients with recently diagnosed insulin-dependent diabetes mellitus in comparison to the other studied groups (Fig. 1). We did not find any correlation between metabolic status (HbA_{1c}, glucose levels) and the number of studied lymphocyte subsets in diabetic patients. There was also no significant correlation between fT₄ and TSH levels and the percentages of $\gamma\delta$ TCR⁺ cell subsets in the Graves' disease (data not shown). There was, however, a significant correlation between the age and the levels of $\gamma\delta$ TCR⁺ cell in the control group and subjects treated with methimazole ($R = 0.24$, $p < 0.03$).

Discussion

In the present study we have shown a significant decrease of $\gamma\delta$ TCR⁺ in the peripheral blood of patients with newly diagnosed Graves' disease, but normal levels of this lymphocyte subset in subjects after methimazole treatment were registered. Our observation is in agreement with the recently published study of SASIAN et al.¹⁴, who have found lower levels of $\gamma\delta$ TCR⁺ in Graves' disease patients who required treatment for thyrotoxicosis in comparison to subjects maintained in euthyroid state. In previous studies, however, ROURA-MIR et al.¹³ and TENG et al.¹⁶ did not observe any statistically significant changes in the percentage of $\gamma\delta$ TCR⁺ in the peripheral blood of patients with Graves' disease. The subjects they studied were, however, not age-matched to the controls and represented different stages of thyrostatic treatment. It has been recently shown by OSUGI et al.¹¹, and confirmed in our present study, that there is a positive correlation between age and $\gamma\delta$ T cells percentages in the peripheral blood. Interestingly, in spite of normal distribution of $\gamma\delta$ T cells in the peripheral blood ROURA-MIR et al.¹³ found a high

proportion of $\gamma\delta$ T cells among the lymphocytes infiltrating the thyroid gland¹. This observation, together with our and SASIAN's et al.¹⁴ findings, could suggest abnormal distribution of $\gamma\delta$ TCR cells in a different compartment of the immune system. Since the accumulation of $\gamma\delta$ cells has been observed in other affected organs in autoimmune diseases, such as synovium in rheumatoid arthritis or lamina propria in coeliac disease^{8, 17}, it could be suggested that $\gamma\delta$ T cells play an important role in the etiopathogenesis of the autoimmune process. One could hypothesise that decreased levels of these cells in the peripheral blood of subjects with untreated Graves' disease are the result of thyroid infiltration at the early stage of the autoimmune process. On the other hand, it could be suggested that $\gamma\delta$ T cell alterations in the peripheral blood are a primary defect of the immune system leading to autoimmunity development⁵. It has been hypothesised that $\gamma\delta$ T cells are, at least in part, responsible for downregulation of autoimmune disease and could be associated with clinical remission^{5, 17}.

What is worthy of note in our study is that, $\gamma\delta$ TCR⁺ cells depletion observed in Graves' disease patients is the result of $\gamma\delta$ TCR⁺CD8⁻ T cell numbers decrease, while CD8⁺ lymphocyte levels bearing $\gamma\delta$ T cell receptors, remained unchanged. To our knowledge the role of $\gamma\delta$ TCR⁺CD8⁻ subsets is still not well known. It is not excluded that they could have cytotoxic properties or play a role as T helper cells, but these suggestions need further studies.

In the present study we also found a significant increase of $\gamma\delta$ TCR⁺CD8⁺ cells in the peripheral blood of subjects with insulin-dependent diabetes treated with insulin for 3–6 months. In our previous study we showed a decreased percentage and absolute number of $\gamma\delta$ T cells in first degree relatives with a significant depletion of the first phase of insulin in comparison to the healthy controls, newly diagnosed diabetic subjects and first degree relatives with humoral alterations, but still with normal pancreatic B cell function⁹.

The differences in the distribution of $\gamma\delta$ T cell subpopulation in the peripheral blood between the patients with overt Graves' disease and type 1 diabetes seems to be understandable since pathogenesis of Graves' disease is linked to production of TSH receptor antibodies and predominance of Th2 cell clones (humoral immune reactivity), while type 1 diabetes is generally accepted to be mediated by Th1 cells (cell-mediated autoimmunity)⁶.

Our present observation could be explained by the recently published studies of HARRISON et al.⁴ who have shown in animal models of autoimmune diabetes that

aerosol insulin treatment of diabetes prone NOD mice is highly protective and associated with the expansion of $\gamma\delta$ TCR⁺CD8⁺ T cells. They suggested that $\gamma\delta$ TCR⁺CD8⁺ T cells could play a key role in the prevention of autoimmune diabetes in animals. We believe that the increased number of $\gamma\delta$ CD8⁺ T cells in the peripheral blood of diabetes type 1 patients, in comparison to the prediabetics, observed in our present and previous studies, is a result of insulin stimulation. One could speculate, that similarly to the observations in animal models, early insulin treatment of subjects at risk of diabetes type 1 could alter $\gamma\delta$ T cell distribution and hypothetically downregulate the autoimmunity, leading to diabetes type 1 prevention also in humans.

In summary, our findings concerning the levels of $\gamma\delta$ T cells in newly diagnosed type 1 diabetes, are in agreement with our previous hypothesis that $\gamma\delta$ TCR⁺CD8⁺ subset plays a role probably as regulatory T cells and could be induced by delivery of exogenous insulin.

The present study suggests that $\gamma\delta$ T cells (especially $\gamma\delta$ TCR⁺CD8⁺) also play an important role in the development of Graves' disease and their levels could be modulated by thyreostatic treatment.

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