THE CYTOTOXIC ABILITY OF NK CELLS IN CHILDREN WITH AUTOIMMUNE THYROIDITIS.

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INTRODUCTION

The perforin protein is responsible for target cells permabilization, and is a constituent of lytic granules of cells with the ability to cytotoxic reaction. It plays an essential role in cytotoxicity of NK cells and CD8+ lymphocytes. Hashimoto disease belongs to the organ specific autoimmune diseases with a preponderance of cell's mechanisms in auto-antigens destruction. Spontaneous cytotoxicity as well as phagocytosis and cytokines release seems to be very important for disease initiation and development.In autoimmune thyroiditis type Hashimoto the key role in thyrocytes destruction plays the spontaneous cytotoxic activity of T cells, and antibodies dependent mechanisms are of a less value. A spontaneous cytotoxicity is associated with the number and degree of activity of NK cells. An important role in this process plays perforin contributed in permabilization of target cells.

OBJECTIVE AND HYPOTHESES:

The aim of the study was to evaluate the number of NK cells, their cytotoxic ability and the perforin expression in peripheral CD56 cells in children with Hashimoto's thyroiditis.

METHOD:

10 children at the age 10-17 years diagnosed with Hashimoto's thyroiditis were enrolled and 9 healthy children as the control group.

The studied group consisted of 10 children with autoimmune Hashimoto's thyroiditis aged 4-18 years including 8 females and 2 males. The patients were qualified to the studied group on the basis of hypoechogeneity of thyroid gland in ultrasound and detection of elevated levels of antityroid antibodies (anti-TPO and/or anti-Tg antibodies) in the serum . **Control group** consisted of patients aged 4-18 years (median: 12, lower quartile: 6, upper quartile: 16), including 7 females and 2 males. Healthy children were referred to the Children's Hospital of Medical University of Warsaw and qualified for routine health screen or minor surgical procedures. Blood collection was performed at least three weeks after last episode of infection, vaccination and any medication and at least 3 months after last episode of viral disease as mononucleosis, smallpox. No cases of autoimmune thyroiditis or any other immune abnormalities were observed in this group. Each individual was assessed clinically by a pediatrician or by a research physician for eligibility to take part in the study.

For all analyses 3 ml of venous blood was taken from the patients to the tubes with heparin anticoagulant. In every child were evaluated: the number of NK cells (CD56+), cytometric test of cytotoxic ability of NK cells and perforin expression in CD56+cells.

Cytometric assessment of perforin expression in NK cells and CD4+ and CD8+ T cells

Peripheral blood mononuclear cells (PBMC) isolated from whole blood were stained with monoclonal anti-CD4, -CD8 and –CD56 antibodies (Becton Dickinson) to identify surface expression of population specific antigens. Cells were permeabilized with IntraPrep 1 and next with IntraPrep 2 permabilization reagent (Beckman Coulter) After incubation 10 µl of intracellular mouse anti-Human Perforin antibodies (Becton Dickinson) were added and incubated. The final probes were analyzed using flow cytometer Cytomix FC 500 Beckman Coulter (Figure 1).

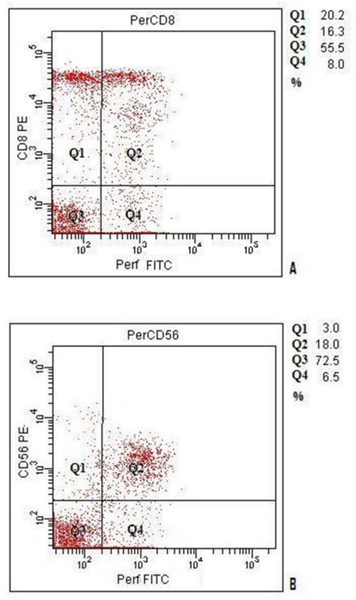

Figure 1. The example of flow cytometric histogram:
A. Perforin expression in CD8+PBMC
B. Perforin expression in CD56+NK)cells

Flow cytometric cytotoxicity test

PBMC from heparinized blood samples were separated and suspended in RPMI media at final concentration $4x10^6$/ml. Target cells (K562) were harvested from the culture, washed and suspended in RPMI media in a final concentration of $1x10^6$/ml. Separated PBMC were cultured with target K562 cells in 3 sterile tubes: tube 1- effector (E) to target (T) cells ratio (E:T) 13:1, tube 2 - control with effector PBMC without target K562 cells, tube 3- control with target K562 without PBMC. The culture was incubated for 4 hour and after were stained with 5 µl of propidium iodide (PI) (50µg/ml stock) and left for another 30 min incubation. Afterwards, the samples were acquired to flow cytometer to the final number of 5000 events presenting DiO fluorescence. Live target (K562) cells showed only DiO_{18} fluorescence (D4), dead target cells showed both DiO_{18} and PI fluorescence (D2) (Fig.2). The percentage of dead target cells was calculated according to the following formula: dead cells x 100% / total number of target cells. Specific lysis index (% of cytotoxic activity) was calculated as follows: [the percentage of dead target cells (including effector cells)] - [dead target cells (without effector cells)].

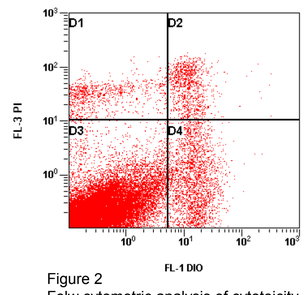

Figure 2
Folw cytometric analysis of cytotoicity.

RESULTS

In cytometric test of cytolysis with K 562 cells the values of spontaneous cytotoxicity of NK cells were significantly higher in children with Hashimoto's thyroiditis in comparison to healthy children (p=0,04) (Fig.6), whereas the percentage of circulating NK cells in both groups was comparable. Simultaneously in children with Hashimoto's thyroiditis the expression of perforin in CD56+ cells and CD8+ cells was significantly lower than that observed in healthy children (p=0,04) (Fig.3, 4). There was a strong positive correlation between the perforin expression in NK cells and CD8+ cells in choldren with Hashimoto's thyroiditis (r=0,74).(Fig.5)

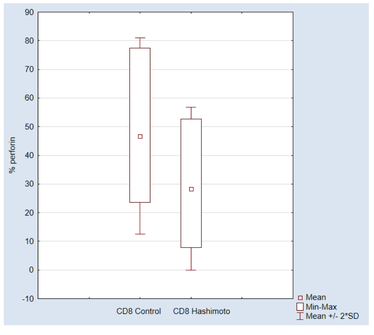

Figure 3. The comparison between perforin expression in CD8+ lymphocytes of children with Hashimoto disease and in control group (p=0,01).

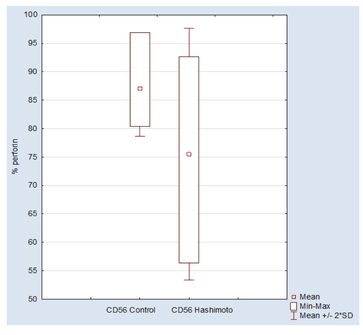

Fig.4. The comparison between perforin expression in NK (CD56) cells of children with Hashimoto disease and in control group (p=0,004).

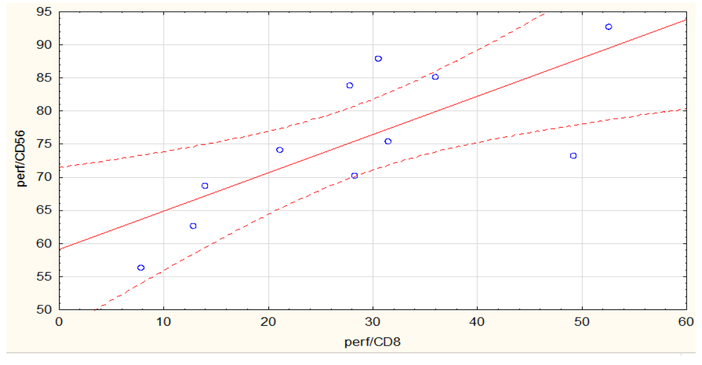

Figure 5. Correlation between perforin expression in CD8+ (perf/CD8) cells and in NK cells (perf/CD56) in children with Hashimoto disease (r=0,74)

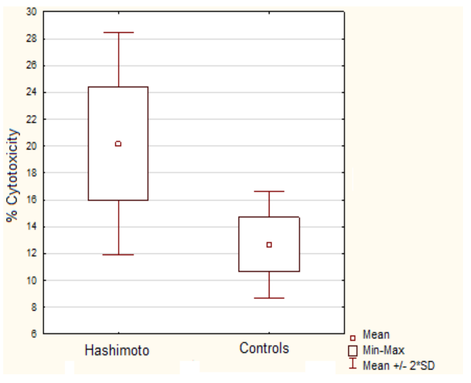

Figure 6. The comparison of the values of spontaneous cytotoxicity inchildren with Hashimoto's disease and in control group.

CONCLUSION:

In children with Hashimoto's thyroiditis in comparison to healthy children a higher cytotoxic activity of T cells is observed with simultaneously decreased perforin expression in NK and CD8+ cells. Probably this apparently paradoxical effect might be a consequence of hyperactivity of this cells and results from exhausting secretion of perforin.

Authors have no conflict of interest.