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INTRATHYROID LYMPHOCYTE SUBSETS IN PATIENTS WITH AUTOIMMUNE THYROID DISEASE

K. Hristozov, L. Koeva, S. Balev*, Ts. Odzhakova*

*Department of Endocrinology and Gastroenterology and *Department of Pulmology, Clinical Immunology, Physiotherapy and Occupational Diseases, Medical University of Varna, Varna*

Thyroid gland is an easily approachable for direct examination by aspiration biopsy organ. This fact gives a possibility for serial examination of liable disturbances in the immune regulation, connected with autoimmune thyroid disease and their response to the relevant therapy. Twenty-six patients with Basedow's disease (BD) and 12 patients with Hashimoto's thyroiditis (HT) - 34 women and 4 men (mean age 47,3 years) were studied. For determination of superficial markers of intrathyroidal mononuclear cells (ITMC) were used monoclonal antibodies for identification of total T-lymphocytes (CD3+), T-helper/inducer cells (CD4+), T-suppressor/cytotoxic cels (CD8+), B-lymphocytes (CD 19+), NK-lymphocytes (CD16+56+), activated T-lymphocytes (CD3+/HLA-DR+), interleukin-2 receptors (CD 25+), and the adhesion molecule ICAM-1 (CD54+). In the patients with BD the main lymphocyte subset was CD3+ with increased number of activated T-lymphocytes and CD4/CD8 ratio (2,22) due to the decreased number of T-suppressor/cytotoxic cells. After the thyreostatic therapy there was a normalization of B-lymphocytes, activated T-lymphocytes and reduction of CD4/CD8 ratio, due to the increased number of T-suppressor/cytotoxic cells. In patients with HT and hypothyroidism the main cell population consisted of CD3+cells and increased number of CD16+56+ cells as the CD4/CD8 ratio was decreased (0,98). The presence of increased percentage of IL-2 receptors and of adhesion molecules ICAM-1 in these patients suggests a more pronounced activity of the autoimmune process.

Key-words: Thyroid gland diseases, T-lymphocytes, B-lymphocytes, ICAM-1 adhaesion molecules, intrathyroidal mononuclear cells

The thyroid gland is suitable organ for direct examinination by fine aspiration biopsy and thus it is an appropriated model for serial trials of underlying disturbances of immune regulation connected to autoimmune thyroid diseases and their response to corresponding treatment (3). It was previously established on mononuclear cells of intrathyroidal materials and their phenotyping that most cells are T-lym-

Address for correspondence:
K. Hristozov, Dept. of Endocrinology and Gastroenterology, Medical University, 55 Marin Drinov St, BG-9002 Varna, BULGARIA
E-mail: endocrin@asclep.muvar.acad.bg

phocytes (1,2,4). Nevertheless the reported changes of T- and B-cells in Basedow's disease (BD) and Hashimoto's thyroiditis (HT) always are under discussion so that similar studies have been carried out extremely on surgical materials of patients preliminary treated by thionamide, levothyroxin, or jodide that can change the quantity and quality ratio of T- and B-lymphocytes (5,6,8). Otherwise the tests of untreated patients do not cover peripheral blood (PB) because the changes of peripheral T-and B-cells can not provide a full picture of processes that take place in intrathyroidal (IT) T- and B-population as well as their subsets although there is a risk of interference with peripheral cells as the materials are taken by fine needle aspiration biopsy (FNAB) (7).

In this trial we evaluate the possibility of using laser, two-colour fiow cytometry to define the lymphocyte subsets of cells obtained by FNA in patients with Grave's disease (GD) and HT. The aim of the study is to establish the changes of cell immunity of intrathyroidal as well as peripheral blood in patients with BD and HT before and after treatment.

MATERIAL AND METHODS

Twenty-six patients with active BD (23 women and 3 men, mean age 42,34 years) were studied for a period of 16,84 months of treatment. These patients were followed-up during 24 months as they finished their Metizol therapy. For a 5-year period 12 patients with HT were studied (11 female and 1 male) at mean age of 45,3 years. The patients were clinically euthyroid at the start of the study. Six patients at the end of the observation period developed hypothyroidism.

The trial comprised patient's histories, clinical data, hormonal levels (TSH, FT4 and FT3 were measured radioimmunologically). Microsomal autoantibodies (MsAb) and thyroglobulin autoantibodies (TgAb) were measure by haemoagglutinaton method, the percentages of lymphocyte subsets in peripheral mononuclear blood cells (PMBC), and intrathyroidal mononuclear cells (ITMC) were evaluated by flow-cytometry with monoclonal antibodies - IMK Plus kit Becton-Dickinson - (CD3+ - total T-lymphocytes; CD19+ - total B lymphocytes; CD4+ - T helper/inducer lymphocytes; CD8+ - T- suppressor/cytotoxic lymphocytes; CD3+/HLA-DR+ - activated T lymphocytes; CD 16+ /56+ - natural killer cells; CD25+- Interleukin-2 receptors and CD54+ - adhesion molecule ICAM-1). Ultrasound examination by Aloka SSD 920 apparatus with a 7,5 MHz sector transducer was done. ITMC were obtained by FNAB of the thyroid gland.

RESULTS AND DISCUSSION

Our results showed lack of any significant differences between lymphocyte subsets in peripheral blood in

healthy control group and patients at active stage of BD, with exception of minimal differences contributing CD3+/HLA-DR+ lymphocytes and level of CD8+ lymphocytes with slight increased of CD4/CD8 ratio, respectively.

In contrast to peripheral lymphocyte markers of the IT changes were expressively enhanced especially in patients in active BD where twice increased level of CD19+ lymphocytes than in controls was found. On second place the level of CD3+/HLA-DR+ lymphocytes was increased compatibly to controls and in PB. Significantly lower was the level of CD8+ cells and this changed the ratio between CD4+ and CD8+ lymphocytes over 1,4 (established in PB of the controls). Our results did not confirm other data that thyrostatic treatment could lead to insignificant redestribution of activating CD4+ and CD8+ subsets and to insignificant changes of CD3+/HLA-DR+ lymphocytes.

Retrospective analysis of lymphocyte markers in 3 patients with GD relapse one year past after thyreostatic treatment showed that there were no changes in the level of CD19+ cells and in the ratio CD3+/CD19+ lymphocytes - initially 1,56:1 and after treatment 2,0:1. No changes were found in the level of CD3+/HLA-DR+ cells and in CD4/CD8 ratio, too. It remained higher because of low level of CD8+ lymphocytes. Our study confirmed that in the inactive BD there was an abnormal ratio of T-lymphocyte subsets and these disturbances could normalize after Methymazole treatment. We proved the possibility of using flow cytometry model in the examination of FNAB specimens of the thyroid gland in order to establish the disturbances of immune regulation not only as a proof but as underlying monitoring of intrathyroidal immune response to thyreostatic treatment.

The main cells' proportion of PMBC in patients with HT consists basically of CD3+cells with insignificant differences between hypothyroid (72 ± 1,2 %) and euthyroid patients (72,7 ±2,1 %). Such statistically insignificant differences are noticed in CD 19+(10,6 vs.10,4 %), CD3+/HLA-DR+(9,6 vs. 9,8 %) and CD16+/56+ (18 vs.17,5 %) cells, too. Higher CD4/CD8 ratio was found in hypothyroid patients (2,0) than in the euthyroid ones (1,6) due to increase of CD4+ cells. CD 19+cells were statistically lower in hypothyroid patients (8,0 ± 2,1%) vs in euthyroid ones (29 ± 1,1%). A significantly higher percentage of CD25+ (32 ± 2,1), CD54+ (70 ± 2,3), CD16+/56 (30 ± 1,8) and decreasing CD4/CD8 ratio (1,2) in hypothyroid patients was found. The changes of ITMC showed more signifficant immunological disturbances in HT. These changes in the hypothyroid patients represented a stronger cell cytotoxicity and activity of autoimmune response.

CONCLUSIONS

1. The proportions of intrathyroidal CD19+ and CD3+/HLA-DR+ lymphocytes in patients with BD are significantly increased in comparison with PBMC of the same patients.

2. After thyreostatic therapy there is normalization of CD19+, CD3+/HLA-DR+ lymphocytes and reduction of CD4/CD8 ratio due to the increased number of T-suppressor/cytotoxic cells.

3. There is no decrease of CD19+ and CD3+/HLA-DR+ lymphocytes and redistribution of CD4/CD8 ratio in patients with BD relapse.

4. In patients with HT and hypothyroidism there is an increased number of CD16+56+ cells, decreased CD4/CD8 ratio, and increased percentage of IL-2 receptors and of ICAM-1 adhesion molecules in comparison with euthyroid patients with HT.

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Интратиреоидни лимфоцитни подкласове при болни с автоимунни тиреоидни заболявания

К. Христозов, Л. Коева, С. Балев*, Ц. Оджакова*

*Катедра по ендокринология и гастроентерология и *Катедра по пневмология, клинична имунология, физиотерапия и професионални болести, Медицински университет-Варна*

Резюме: Щитовидната жлеза е лесно достъпен орган за директни изследвания посредством аспирационна биопсия, което позволява серийно изучаване на подлежащите нарушения в имунната регулация, свързани с автоимунните тиреоидни заболявания и техния отговор към съответното лечение. Бяха изследвани и проследени 26 болни с Базедова болест (ББ) и 12 - с тиреоидит на Хашимото (ТХ) - общо 34 жени и 4 мъже, на средна възраст 47,3 г. За определяне на повърхностните маркери на

интратиреоидните мононуклеарни клетки (ИТМК) бяха използвани антитела за идентифициране на Т-лимфоцити (CD3+), Т-хелпери (CD4+), Т-супресори (CD8+), В-лимфоцити (CD19+), НК-клетки (CD16+/56), активирани Т-лимфоцити (HLA-DR+), рецептори за интерлевкин-2 (CD25+) и адхезионни молекули от типа ICAM-1 (CD54+). При болните с ББ основната лимфоцитна популация бе CD3+, с увеличен брой активирани Т-лимфоцити и повишено CD4+/CD8+ отношение (2,22), дължащо се на намаление на Т-супресорите. Проведеното тиреостатично лечение води до нормализиране на В-лимфоцитите, на активираните Т-лимфоцити и снижение на отношението CD4+/CD8+ за сметка на увеличението на Т-супресорите. При болните с ТХ и настъпил хипотиреоидизъм основната клетъчна популация също се състои от CD3+ клетки, както и от по-голям брой CD16+/56 клетки и намалено CD4+/CD8+ отношение (0,98). Наличието на увеличен процент на рецептори за интерлевкин-2 и адхезионни молекули от типа ICAM-1 при тези болни подсказва по-изразена активност на автоимунния процес.