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Original

The effects of radioiodine therapy on peripheral blood lymphocyte subpopulations in patients with Graves' disease. Preliminary report

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Abstract

BACKGROUND: Treatment of Graves' disease patients with radioactive iodide (^{131}I) is becoming the standard therapy in an increasing group of cases but can induce alterations in immune response, like increasing levels of thyroid autoantibodies, and, in part, exacerbation of ophthalmopathy.

The aim of this study was to assess the changes in peripheral blood (PB) lymphocyte subpopulations after ^{131}I treatment of patients with Graves' disease.

MATERIAL AND METHODS: The study was carried out in a group of 30 patients with Graves' disease (23 f; 7 m) 49.5 ± ± 10.0 years of age, 26 with different subjective ocular signs like gritty sensation, increased lacrimation, orbital pain, and exophthalmos. PB lymphocyte subsets were analysed by cytofluorometry, serum concentration of TSH and fT4 were evaluated before and 6 weeks after radioiodine treatment.

RESULTS: After ^{131}I treatment a significant increase in $CD3^+$, $CD4^+$, $CD3^+HLA\text{-}DR^+$ and a decrease in $CD19^+$ percentages of lymphocyte subsets were found in comparison with the initial evaluation. No significant changes in percentage of $CD8^+$ and NK ($CD3^-CD16^+$ $CD56^+$) cells were observed during this study. A significant increase in TSH and a slight decrease in fT4 concentration took place in the 6th week after ^{131}I application. The patients without subjective improvement of ocular signs during the therapy initially had a percentage of $CD3^+$, $CD8^+$ lymphocytes which was significantly lower compared with those with regression of ocular signs observed after ^{131}I treatment.

CONCLUSIONS: The changes in PB lymphocyte subsets caused by ^{131}I treatment of Graves' disease confirm the involvement of acquired cellular immunity after radiation damage of the thyroid gland. The decreased initial percentage of $CD8^+$ and $CD3^+$ lymphocytes could help make a prediction of ocular symptoms persisting after radioiodine treatment in some patients with ophthalmopathy.

Key words: ^{131}I, lymphocytes, Graves' disease

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Introduction

Mechanisms of acquired immunological response play a dominant role in the pathogenesis of Graves' disease (GD). The autoimmune process is probably initiated by activation of autoreactive T lymphocytes by antigens derived from thyrocytes, such as TSH receptor (TSHR) and thyrocyte peroxidase (TPO). Intrathyroidal T lymphocytes seem to be especially involved in this process through thyroid antigen recognition and release of cytokines. Some patients with Graves' disease develop orbital infiltrative disease and infiltrative dermathopathy. Extrathyroidal manifestations of GD have been explained by cell-mediated effector mechanisms involving cross-reacting antigens between the thyroid and orbital connective tissue, like TSHR protein expression in orbital fibroblastic preadipocytes [1]. However the spreading of autoimmunological reactivity beyond the thyroid gland and the association of Graves' disease with other autoimmune diseases suggest a generalised immune disregulation and genetic background of the GD.

T helper cells ($CD4^+$) activate B lymphocytes to autoantibodies production by interleukin-4 (IL-4) and stimulate cytotoxic T

cells ($CD8^+$) to locally cytotoxic effects by interferon-γ (IFNγ) production. Some clones of $CD8^+$ T cells resembling Th2 lymphocytes can exert suppressor effects on immunological response [2, 3]. The cytokine profiles of thyroid-specific T cells indicate that development of GD could be associated with immune deviation toward Th2 lymphocytes and IL-4 predominance [1]. Immune response expressed by alterations in the pattern of lymphocyte subpopulations has been studied either in circulating or infiltrating lymphocyte pools in different stages of the disease activity. So these varying clinical conditions can often give divergent results [4, 5].

The treatment of hyperthyroidism in Graves' disease has most commonly been performed by one or more of these treatments: antithyroid drug therapy, total thyroidectomy and radioiodine therapy.

In recent years treatment of Graves' disease patients with ^{131}I radioactive iodine (RAI) has become the standard therapy in an increasing group of cases because of its simplicity, low cost and relatively high effectiveness.

Radioactive iodine kills thyroid cells by β radiation and leads to the release of antigens into the circulation; at this time a dramatic increase in cell–mediated reactivity (T cells) and in humoral-mediated reactivity (B cells and autoantibodies) to different thyroid antigens is observed. Therefore RAI treatment of hyperthyroidism could be associated with secondary immunological disturbances sharing primary autoimmunological response [6].

Recent discussions have focused on the possible association of ^{131}I therapy of Graves' disease with the development or progression of thyroid-associated ophthalmopathy (TAO) [7, 8].

Several large retrospective studies have found no effects of radioiodine therapy on the clinical course of eye disease [9,10], whereas other investigators observed a worsening (exacerbation) of TAO after RAI therapy [11]. These latter reports suggest a role of circulating autoreactive activated T cells in the development of TAO in the course of radioiodine treatment of Graves' disease patients. But whether it is a primary (associated with immunological imbalance in Graves' disease) or secondary (influence of RAI treatment) event has not yet been resolved.

The main goal of this study was to assess the changes in peripheral blood lymphocyte (PBL) subpopulations after ^{131}I treatment of patients with Graves' disease as manifestations of immunological response activation.

The additional aim was to find prognostic factors of TAO based on the above immunological parameters by comparing initial (before ^{131}I treatment) values of PBL subpopulations, depending on the regression or progression of subjective ocular symptoms after RAI treatment.

Material and methods

The study was carried out on a group of 30 patients with Graves' disease (23 f, 7 m; 49.5 ± 10.0 years of age) recognised from typical clinical and laboratory symptoms. 23 of them had different subjective ocular signs like gritty sensation, increased lacrimation, orbital pain and exophthalmos suggesting ophthalmopathy development before ^{131}I treatment.

7 patients had not presented any subjective ophthalmopathy symptoms before the study. In 3 of them symptoms of TAO appeared after ^{131}I treatment. So finally 26 examined persons had subjective signs of TAO during the study.

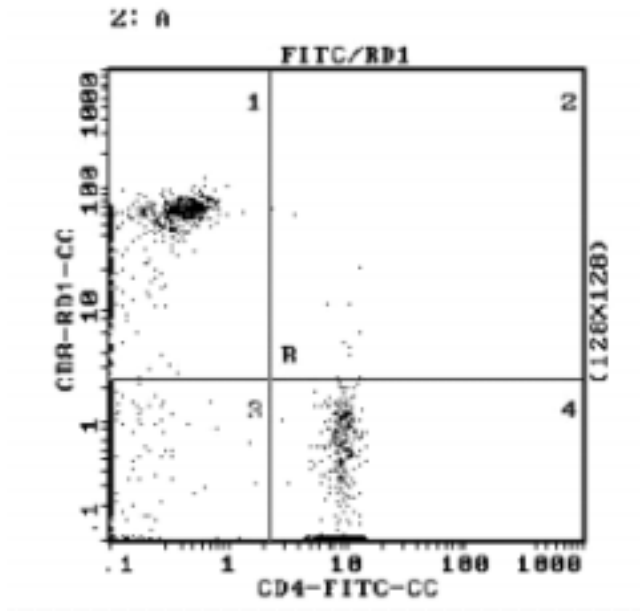

Figure 1. Subsets of $CD4^+$ and $CD8^+$ peripheral blood lymphocytes in cytofluorymetry: B1 – $CD4^-CD8^+$, B4 – $CD4^+CD8^-$.

Peripheral blood lymphocyte subsets were analysed by cytofluorometry (Epics XL Coulter).They were identified by monoclonal antibodies directed against lymphocyte surface antigens conjugated with fluorescent dyes — fluorescein isothiocyanate (FITC) or phycoerythrin (PE) — using IMK Simultest Kit (Becton-Dickinson): CD3 for T cells, CD4 for T helper and CD8 for T cytotoxic lymphocytes, CD19 for B lymphocytes, CD16/CD56 for NK (natural killer) cells and HLA-DR as T cell activation marker (Fig. 1).

Hormonal thyroid function was assessed by measure of serum TSH and fT4 concentration by IRMA and RIA kits respectively (Polatom Świerk, Poland).

Percentages of lymphocyte subpopulations and hormone concentrations were evaluated before and 6 weeks after radioiodine treatment (average dose 505.3 ± 113.9 MBq of ^{131}I calculated according to the Marinelli et al. formula [12]).

The data for all measured parameters in the whole group of patients before and after RAI were expressed as median quartiles range and processed using Wilcoxon's test procedures. In the group of 26 patients with TAO, the differences between lymphocyte values before ^{131}I treatment depending on varying output of RAI on subjective ophthalmopathy were processed using Mann-Whitney's test. Statistical significance was set at $p < 0.05$.

The study was accepted by the Ethical Committee of the Medical University in Białystok, Poland.

Results

Hyperthyroidism was reduced by radioiodine treatment in all patients. We observed a significant increase in TSH and slight decrease in fT4 concentration in 6th week after ^{131}I application: 0.15 (0.002–0.41) mIU/l, *vs.* 0,62 (0.09–2.39) mIU/l $p < 0.02$ for TSH, and 15.5 (9.5–25.1) pmol/l *vs.* 11.5 (7.2–14.9) pmol/l, $p = 0.16$ for fT4 respectively (Fig. 2).

After ^{131}I treatment a significant increase in percentages of $CD3^+$, $CD4^+$ and $CD3^+HLA\text{-}DR^+$ lymphocyte subsets was ob-

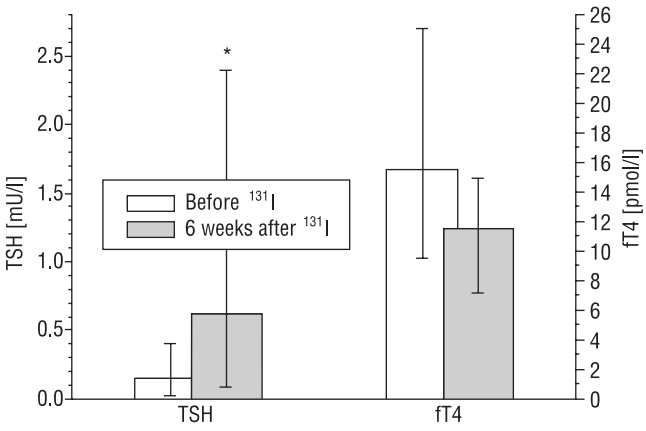

Figure 2. Changes in TSH and fT4 concentration before and 6 weeks after ^{131}I treatment. Box — median value, whiskers — lower and upper quartiles values; Wilcoxon's test, *$p < 0.02$.

served in total group (n = 30) in comparison with the initial evaluation: (median ± quartiles values) 69.8 (67.9–73.7)% *vs.* 74.2 (71.7–75.8)%, $p < 0.0001$ for $CD3^+$, 44.6 (38.7–47.4)% *vs.* 48.5 (44.6–51.6)%, $p < 0.00001$ for $CD4^+$ and 3.5 (1.9–5.9)% *vs.* 5.3 (3.5–7.2)%, $p < 0.005$ for $CD3^+HLA\text{-}DR^+$. Contrary, significant decrease in percentage of $CD19^+$ cells was noticed during the study: 13.3 (10.3–15.7)% *vs.* 9.3 (7.9–13.0)%, $p < 0.00001$.

No significant changes in the percentage of cytotoxic ($CD8^+$) and NK ($CD3^-CD16^+CD56^+$) cells were observed during this study in whole group (Fig. 3).

In patients with present ocular symptoms (n = 26), lymphocyte subsets were compared between groups with and without ocular signs improvement after RAI.

The patients with worsening or no regression of TAO (n = 7) had initial percentages of $CD3^+$ and $CD8^+$ lymphocytes significantly lower in comparison to the patients with subjective improvement (regression) of TAO (n = 19) after ^{131}I treatment (Fig. 4):

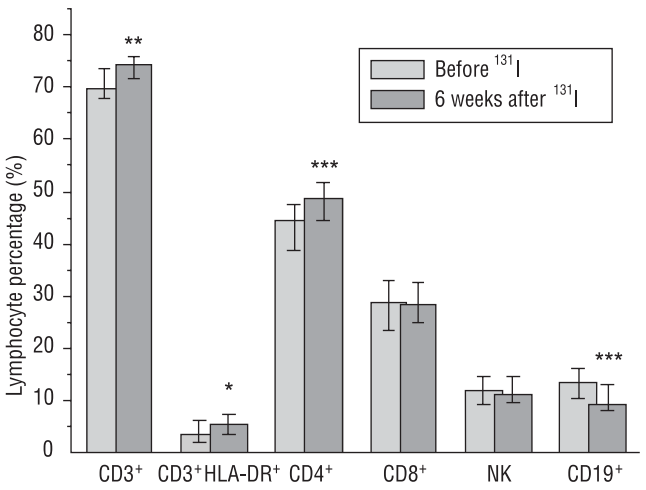

Figure 3. Changes in percent of peripheral blood lymphocyte subsets before and 6 weeks after ^{131}I treatment. Box — median, whiskers — lower and upper quartiles; Wilcoxon's test, *$p < 0.005$, **$p < 0.0001$, ***$p < 0,00001$.

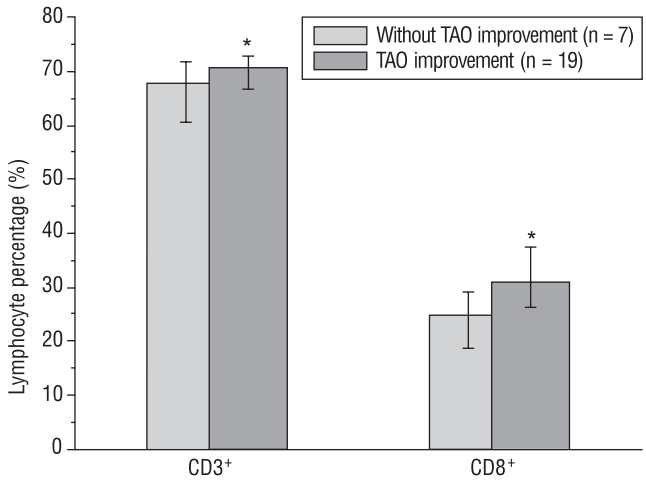

Figure 4. Differences in percent of $CD3^+$ and $CD8^+$ peripheral blood lymphocyte subsets before ^{131}I treatment in group of patients with thyroid-associated ophthalmopathy (TAO). Box — median value, whiskers — lower and upper quartile values; Mann-Whitney's test, *$p < 0.05$.

67.9 (60.6–71.9)% *vs.* 70.6 (66.6–72.9)%, $p < 0.05$ for $CD3^+$ and 24.8 (18.5–29.3)% *vs.* 30.9 (26.3–37.4)%, $p < 0.05$ for $CD8^+$ subsets respectively.

Discussion

Our study indicates distinct immunological response involvement after ^{131}I administration in patients with hyperthyroidism of Graves' disease.

The relative increase of $CD4^+$ (Th) population seems to be a dominant feature in the picture of immunological changes 6 weeks after ^{131}I application. It is associated with a relative increase in $CD3^+HLA\text{-}DR^+$ lymphocytes which correspond to activated T cells.

The parallel increase of both T cell subsets suggests that it would be the same lymphocyte population but further investigations are needed. These events were associated with an adequate decrease in $CD19^+$ (B cells) population, which is either a relative change or results from their redistribution beyond blood vessels to secondary lymphoid organs, like lymphonodules and spleen or the affected thyroid gland. Changes in PB lymphocyte subsets caused by ^{131}I treatment of Graves' disease observed in our study are similar to results of other investigators, showing the involvement of acquired cellular immunity after radiation damage of thyroid gland [4]. Lack of any response in $CD8^+$ cytotoxic lymphocytes and NK cells suggests that they do not participate directly in immunological disturbances after radioiodine therapy. It is likely that ^{131}I transiently activate Th2 cell-mediated processes with increase in IL-4 production and B cells stimulation next to non-specific inflammatory processes with increase in IL-6 and TNFα production [13].

Different retrospective and prospective studies have produced conflicting data on the effect of the radioiodine therapy on the development or exacerbation of TAO [14, 15]. Some investigators conclude that the appearance or worsening of ophthalmopathy occurs more often in radioiodine treated patients than in antithyroid drugs treated patients but this is often transient and can

be prevented by the administration of prednisone [16]. In our study we observed subjective ocular symptoms in 26 patients during the study. Improvement (progression) of TAO was noticed in 19 patients. Appearance or maintenance of TAO in 6th week after RAI treatment was found in 7 persons.

Analysis of orbital T cell infiltration indicates that $CD4^+$ lymphocytes dominate in the retrobulbar space of patients with TAO [5, 17]. Directly comparing those data with results obtained in peripheral blood could produce interpretation problems. We have not observed any differences in $CD4^+$ population which could reflect alterations in orbital infiltrates. But significantly lower percentages of $CD3^+$ and $CD8^+$ lymphocytes in the group of patients with persisted subjective ocular signs after RAI treatment which were observed before ^{131}I administration in comparison to the group with improvement of ophthalmopathy could express impairment mechanisms of immunological suppression exerted by some $CD8^+$ T cell clones [3]. According to this hypothesis, the decreased percentages of $CD3^+$ and $CD8^+$ lymphocytes could help make a prediction of ocular symptoms persisting after radioiodine treatment in patients with Graves' disease.

Conclusions

The changes in peripheral blood lymphocyte subsets caused by ^{131}I treatment of Graves' disease indicate involvement of acquired cellular immunity after radiation damage of thyroid gland.

The decreased initial percentage of $CD8^+$ and $CD3^+$ lymphocytes could help make a prediction of ocular symptoms persisting after radioiodine treatment in some patients with ophthalmopathy.

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