

Cytokine production in peripheral blood cells of patients with differentiated thyroid cancer: elevated Th2/Th9 cytokine production before and reduced Th2 cytokine production after radioactive iodine therapy

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Abstract Cytokines play a key role in the regulation of cells of the immune system and also have been implicated in the pathogenesis of malignant diseases. The aim of this study was to evaluate cytokine profiles in patients with differentiated thyroid cancer (DTC) before and 7 days after radioactive iodine (¹³¹I) therapy. Cytokine levels were determined in supernatants obtained from phytohemagglutinin-stimulated whole blood cultures of 13 patients with DTC and 13 control subjects. The concentrations of selected cytokines: Th1—interferon gamma (IFN- γ), interleukin 2 (IL-2) and tumor necrosis factor alpha (TNF- α); Th2—interleukin 4 (IL-4), interleukin 5 (IL-5), interleukin 13 (IL-13) and interleukin 10 (IL-10); Th9—interleukin-9 (IL-9); and Th17—interleukin 17 (IL-17A) were measured using multiplex cytokine detection systems for Human Th1/Th2/Th9/Th17/Th22. We have shown that peripheral blood cells of DTC patients produce significantly higher concentrations of Th2/Th9 cytokines (IL-5, IL-13 and IL-9) than control subjects. The ¹³¹I therapy led to reduced secretion of Th2 cytokines (IL-4, IL-5 and IL-13). Despite this, the calculated cytokine ratios (Th1/Th2) in DTC patients before and 7 days after ¹³¹I therapy were not different

from those in healthy subjects. DTC patients have significantly higher concentrations of Th2/Th9 cytokines (IL-5, IL-13 and IL-9) than control subjects. There is no influence of hypothyroidism or stage of disease on cytokine production in DTC patients before ¹³¹I therapy. The radioactive ¹³¹I therapy leads to reduced secretion of Th2 cytokines (IL-4, IL-5 and IL-13). Additional studies are needed to determine the significance of these findings.

Keywords Cytokines · Differentiated thyroid cancer · Radioactive iodine therapy · Th1/Th2 ratio

Abbreviations

¹³¹ I	Iodine 131
DTC	Differentiated thyroid cancer
EANM	European Association of Nuclear Medicine
IFN- γ	Interferon gamma
IL-2	Interleukin 2
IL-4	Interleukin 4
IL-5	Interleukin 5
IL-9	Interleukin 9
IL-10	Interleukin 10
IL-13	Interleukin 13
IL-17A	Interleukin 17A
PHA	Phytohemagglutinin
SD	Standard deviation
Th1	T-helper-1
Th2	T-helper-2
Th9	T-helper-9
Th17	T-helper-17
TNF- α	Tumor necrosis factor alpha
TSH	Thyroid-stimulating hormone
TxNOM0	Patients without any proven metastases
TxNxM0	Patients with nodal metastases

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Introduction

Thyroid cancer is the most common malignancy of the endocrine system, with an increasing incidence during the past 20 years [1]. Among thyroid malignancies, differentiated thyroid cancers (DTCs) are the most frequent (more than 90 %) and include papillary and follicular histological types [2]. Similarly to the thyroid cells from which they derive, DTC cells have the ability to concentrate iodine. This property enables administration of radioactive iodine (^{131}I) for ablation of remnant thyroid tissue and for treatment of iodine-avid metastases [3]. According to the European Association of Nuclear Medicine (EANM) guidelines, after surgery, some patients with DTC need to receive radioactive iodine ^{131}I therapy [4].

Cytokines play a key role in the regulation of cells of the immune system and also have been implicated in the pathogenesis of malignant diseases [5, 6]. Although most studies argue that secretion of T-helper-1 (Th1) cytokines is protective, while T-helper-2 (Th2) cytokines contribute to progression of malignancy [7, 8], there are other findings indicating a protective role for Th2 cytokines in anti-tumor immunity [9]. The concentrations of selected cytokines in patients with different types of cancers have been measured [10–13], but seldom in DTC patients [14, 15]. In one study, a Th2 or mixed type of response in tumor infiltrating and peripheral blood lymphocytes of patients with papillary thyroid carcinoma was shown [15]. Data regarding cytokine secretion in DTC patients treated with radioactive iodine are still rare. In one study, a significant increase of IL-6 two months after radioactive iodine therapy was found, while TNF- α level did not change after therapy [14]. The effect of radioactive iodine therapy on cytokine production in patients with hepatocellular carcinoma and Graves' disease has been analyzed. In patients with hepatocellular carcinoma, iodine-125 implantation stimulated the anti-tumor immune response by promoting Th2/Th1 deviation [16]. Also, in patients with Graves' disease treated with radioactive ^{131}I , a transient increase of Th2 cytokines (IL-4, IL-6 and IL-10) was obtained [17]. The aim of this study was to evaluate the cytokine profiles in DTC patients before and 7 days after ^{131}I therapy. Cytokine concentrations in patient's sera do not only reflect the ability of the immune cells to produce cytokines, as the levels may be influenced by the presence of various inhibitors, such as soluble receptors, anti-cytokine antibodies and receptor antagonists [18], as well as products from cells outside the immune system [19], including thyroid cancer cells [20]. To assess the cytokine-producing ability of peripheral blood cells in DTC patients, as well as the possible influence of radioactive iodine therapy, cytokine concentrations were measured in phytohemagglutinin (PHA)-stimulated whole blood cultures *in vitro*. We analyzed the secretion of

selected Th1 cytokines: interferon gamma (IFN- γ), interleukin 2 (IL-2) and tumor necrosis factor alpha (TNF- α); and Th2 cytokines: interleukin 4 (IL-4), interleukin 5 (IL-5), interleukin 13 (IL-13) and interleukin 10 (IL-10); the Th9 cytokine: interleukin 9 (IL-9) and the Th17 cytokine interleukin 17 (IL-17A). We showed that peripheral blood cells of DTC patients produce significantly higher amounts of Th2/Th9 cytokines (IL-5, IL-13 and IL-9) than control subjects. There was no influence of hypothyroidism or stage of disease on cytokine production in DTC patients before ^{131}I therapy. The ^{131}I therapy led to reduced secretion of Th2 cytokines (IL-4, IL-5 and IL-13). Despite this, the calculated cytokine ratios (Th1/Th2) in DTC patients before and 7 days after ^{131}I therapy were not different from those in healthy subjects.

Patients and methods

The study population included thirteen well-DTC patients (11 females and 2 males) of mean age 51.23 ± 14.9 years. Among them, ten (76.9 %) had papillary carcinomas, while three (23.1 %) had follicular carcinomas. All DTC patients included in this study underwent total thyroidectomy. Four to six weeks after surgery, and 10 days after a low-iodine diet, the patients were treated at the Nuclear Medicine Department of the Clinical Center, Kragujevac, according to the EANM guidelines [4], with fixed nominal activities of 3.7 GBq (100 mCi) of sodium (^{131}I) iodide administered orally. At the time of ^{131}I administration, all patients were hypothyroid after thyroid hormone withdrawal [concentration of thyroid-stimulating hormone (TSH) > 30 mIU/l]. None of the patients had acute infections, chronic inflammatory or autoimmune diseases or other conditions that could affect the tested parameters. The control group was composed of 13 healthy subjects (11 females and 2 males) of mean age 45.75 ± 12.89 years. The control subjects had no acute infectious, chronic inflammatory or autoimmune diseases. All control subjects were evaluated for thyroid function and thyroid antibodies. The mean concentration of TSH was 1.46 ± 0.72 mIU/l (range 0.4–3.5 mIU/l), and thyroid antibodies were negative. After venipuncture, blood samples (5 ml) from patients and control subjects were collected in tubes (vacutainer). Blood was taken from control subjects only once, while samples from DTC patients were obtained both before and 7 days after ^{131}I treatment.

The study was planned according to ethical guidelines following the Declaration of Helsinki. The institutional review committee approved our study protocol (number 01-5868) according to local biomedical research regulations. All patients and control subjects gave informed consent prior to enrollment in the study.

Whole blood culture

Whole blood (0.5 ml per subject) was added to 5 ml of RPMI-based complete medium containing fetal bovine serum, L-glutamine and PHA (GIBCO™ PB-MAX™ karyotyping medium, Invitrogen, CA, USA) and incubated at 37 °C for 72 h. The supernatant was harvested by centrifugation (1,400g for 12 min) and then stored at –20 °C until required.

Cytokine measurements

Cytokines were determined in supernatants obtained from whole blood cultures of DTC patients and controls. The supernatant samples were thawed and analyzed with the multiplex cytokine detection systems for Human Th1/Th2/Th9/Th17/Th22 13plex, FlowCytomix Multiplex (ebioscience Cat. no. BMS817FF) according to the manufacturer's instructions. All samples were acquired and analyzed on a FC500 Beckman Coulter flow cytometer. Collected data were analyzed using FlowCytomix™ Pro Software.

Statistical analysis

The data were expressed as range (minimum–maximum), mean \pm SD, and median values. The commercial SPSS version 10.0 for Windows was used for statistical analysis. Student's *t* test was employed for comparison of paired samples. For nonparametric variables, differences between two independent groups were determined by the Mann–Whitney *U* test, while the Wilcoxon test was used for dependent groups. The observed variables were compared by the bivariate correlation test and determination of Pearson/Spearman coefficient. *p* values <0.05 were considered to be statistically significant.

Results

We analyzed the cytokine-producing ability of peripheral blood cells from thirteen control subjects and thirteen DTC patients before and 7 days after 131-I therapy. Cytokine concentrations were measured in the supernatants of 72-h PHA-stimulated whole blood cultures in vitro.

The mean leukocyte count in healthy controls was $6.44 \pm 1.85 \times 10^9/l$ (range $3.19\text{--}8.34 \times 10^9/l$); in DTC patients before 131-I therapy, it was $6.77 \pm 1.02 \times 10^9/l$ (range $5.6\text{--}8.2 \times 10^9/l$), and in DTC patients after 131-I therapy $5.72 \pm 1.02 \times 10^9/l$ (range $4.6\text{--}7.0 \times 10^9/l$). There was no significant difference in mean leukocyte counts between healthy controls and DTC patients before therapy, while the difference between DTC patients before and

7 days after therapy was significant ($p < 0.001$). Since the measured values of cytokines were corrected to 1,000 stimulated cells, whereby dilution was taken into account, the cytokine concentrations are expressed as pg/ml per 1,000 stimulated leukocytes. In this way, the influence of differences in the number of PHA-stimulated cells on cytokine concentrations was excluded.

Cytokine production in PHA-stimulated whole blood cultures of control subjects and DTC patients before and after 131-I therapy

The cytokine concentrations in supernatants from PHA-stimulated whole blood cultures of control subjects and DTC patients before and after 131-I therapy are shown in Table 1.

There were no statistically significant differences in the production of the Th1 cytokines, IFN γ , IL-2 and TNF α between 72-h PHA-stimulated whole blood cultures from DTC patients and controls. On the other hand, PHA-stimulated whole blood cultures from DTC patients had significantly higher concentrations of the Th2 cytokines: IL-5 and IL-13, than control subjects. Also, PHA-stimulated whole blood cultures of DTC patients produced significantly more IL-9. There were no significant differences in production of IL-4, IL-10 and IL-17A between cell cultures of DTC patients and controls.

The mean concentration of TSH in our DTC patients before 131-I therapy was 108.99 ± 81.13 mIU/l. There were no significant correlations between the serum concentrations of TSH and the following cytokines in whole blood culture: IL-13 (Pearson $r = 0.209$, $p = 0.620$), IL-5 (Pearson $r = 0.242$, $p = 0.563$) and IL-9 (Pearson $r = 0.517$, $p = 0.190$). Also, there were no statistically significant differences in concentrations of IL-13 ($p = 0.737$), IL-5 ($p = 0.349$) and IL-9 ($p = 0.381$) between the subgroup of patients with TSH values below 100 mIU/l (56.38 ± 23.80 mIU/l) and that with TSH values above 100 mIU/l (196.67 ± 58.80 mIU/l).

To examine the impact of stage of disease on the production of cytokines, DTC patients were divided into two subgroups: patients with nodal metastases (TxNxM0) and patients without any proven metastases (TxN0M0). The results showed that no significant differences in concentrations of IFN- γ ($p = 0.924$), IL-2 ($p = 0.126$), TNF- α ($p = 0.528$), IL-4 ($p = 0.108$), IL-5 ($p = 0.120$), IL-13 ($p = 0.412$), IL-10 ($p = 0.307$), IL-9 ($p = 0.130$) and IL-17 ($p = 0.317$) between the patients without and with metastases before 131-I therapy.

Cytokine concentrations in PHA-stimulated whole blood cultures of DTC patients 7 days after 131-I therapy were significantly less than those before therapy for IL-13, IL-4 and IL-5 (Table 1).

The concentrations of four cytokines for which statistically significant differences were found are shown in Fig. 1.

Table 1 Cytokine concentrations in PHA-stimulated whole blood cultures of control subjects, DTC patients before and 7 days after 131-I therapy (*p* values indicate the significance of differences)

Cytokine	Control subjects			DTC patients before 131-I therapy			DTC patients 7 days after 131-I therapy		
	Range (minimum–maximum) (pg/ml)	X ± SD (pg/ml)	Median (pg/ml)	Range (minimum–maximum) (pg/ml)	X ± SD (pg/ml)	Median (pg/ml)	Range (minimum–maximum) (pg/ml)	X ± SD (pg/ml)	Median (pg/ml)
IFN- γ	430–58,040	22,940 ± 19,182	21,774	4,468–48,490	27,343 ± 15,691	28,821	4,386–54,190	30,150 ± 16,660	28,146
IL-2	0.0–8,124	2,141 ± 3,014	694	447–10,893	4,322 ± 3,768	2,998	472–2,423	1,419 ± 656	1,308
TNF- α	0.0–849	336 ± 291	291	0.0–8,071	1,330 ± 2,732	448	194–734	360 ± 169	306
IL-4	0.0–823	286 ± 293	288	181–1,134	494 ± 312 ^d	396	0.0–393	193 ± 170	240
IL-5	0.0–2,412	869 ± 842 ^a	740	518–4,763	2,232 ± 1608 ^e	1,396	0.0–2,769	826 ± 1,135	350
IL-13	0.0–1,300	460 ± 475 ^b	419	317–2,730	1,602 ± 954 ^f	1,783	450–1,666	709 ± 417	500
IL-10	0.0–1,415	702 ± 513	738	144–5,390	1,319 ± 1,744	643	304–2,580	770 ± 778	492
IL-9	0.0–142	42.4 ± 60.7 ^c	0	0.0–293	125.8 ± 87.3	122	0.0–226	89.1 ± 87.9	65
IL-17	1,101–14,970	4,891 ± 4,365	4,075	976–14,983	7,743 ± 4,561	8,252	1,041–25,465	7,262 ± 8,259	3,596

^a Statistical significant difference between DTC patients before therapy and control subjects, *p* = 0.046^b Statistical significant difference between DTC patients before therapy and control subjects, *p* = 0.012^c Statistical significant difference between DTC patients before therapy and control subjects, *p* = 0.038^d Statistical significant difference between DTC patients before and 7 days after therapy, *p* = 0.031^e Statistical significant difference between DTC patients before and 7 days after therapy, *p* = 0.038^f Statistical significant difference between DTC patients before and 7 days after therapy, *p* = 0.037

Fig. 1 The concentrations of IL-4, IL-5, IL-13 and IL-9 in control subjects and DTC patients before and 7 days after 131-I therapy

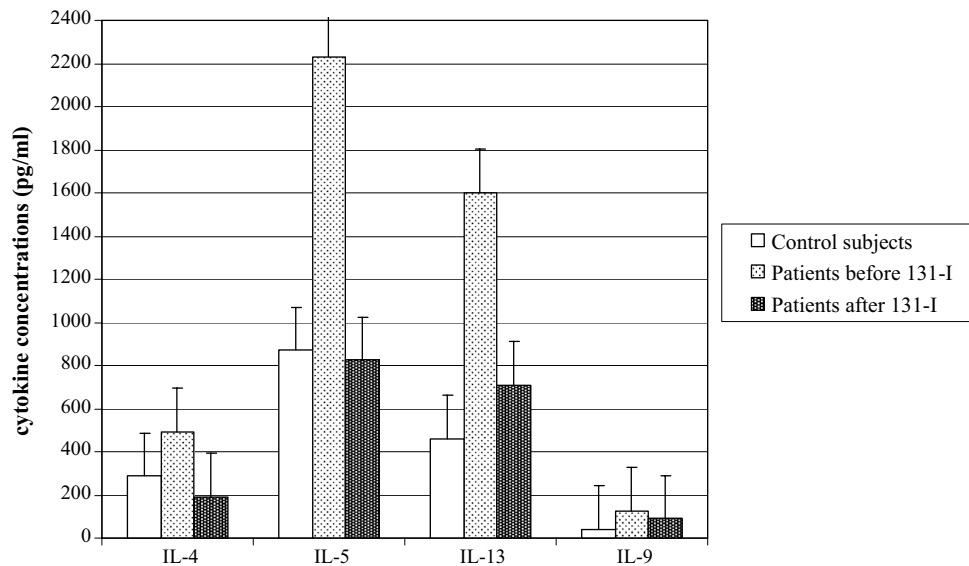


Table 2 Mean values ($X \pm SD$) of Th1/Th2 cytokine ratios for control subjects, DTC patient before and 7 days after 131-I therapy

Th1/Th2	Control subjects $X \pm SD$	DTC patients before 131-I therapy $X \pm SD$	DTC patients 7 days after 131-I therapy $X \pm SD$
IFN- γ /IL-4	46.2 \pm 46.4	66.6 \pm 60.4	47.9 \pm 53.6
IFN- γ /IL-5	24.0 \pm 21.9	17.9 \pm 14.0	18.2 \pm 21.3
IFN- γ /IL-10	29.4 \pm 22.8	43.4 \pm 31.3	59.5 \pm 39.2
TNF- α /IL-4	0.67 \pm 0.63	3.98 \pm 8.66	0.84 \pm 0.79
TNF- α /IL-5	0.34 \pm 0.27	0.45 \pm 0.52	0.56 \pm 1.01
TNF- α /IL-10	0.48 \pm 0.50	0.77 \pm 0.54	0.64 \pm 0.29

Th1/Th2 cytokine ratios in PHA-stimulated whole blood cultures of control subjects and DTC patients before and 7 days after 131-I therapy

Table 2 summarizes the mean values of Th1/Th2 cytokine ratios (IFN- γ or TNF- α vs. IL-4, IL-5 or IL-10) calculated for each DTC patient (before and after 131-I therapy) and control subjects individually.

There were no significant differences between DTC patients before therapy and healthy controls for Th1/Th2 cytokine ratios (IFN- γ /IL-4, IFN- γ /IL-5, IFN- γ /IL-10, TNF- α /IL-4, TNF- α /IL-5 and TNF- α /IL-10). Also, there were no statistically significant changes in Th1/Th2 cytokine ratios (IFN- γ /IL-4, IFN- γ /IL-5, IFN- γ /IL-10, TNF- α /IL-4, TNF- α /IL-5 and TNF- α /IL-10) after treatment in any patient.

Discussion

The aim of this study was to evaluate the cytokine-producing ability of peripheral blood cells in DTC patients

before and 7 days after 131-I therapy. Cytokine concentrations were measured in the supernatants of PHA-stimulated whole blood cultures in vitro. We showed that DTC patients produce significantly higher amounts of Th2/Th9 cytokines (IL-5, IL-13 and IL-9) than control subjects. Radioactive 131-I therapy led to reduced secretion of Th2 cytokines (IL-4, IL-5 and IL-13). Despite this, the Th1/Th2 ratios in DTC patients before and 7 days after 131-I therapy were not significantly different from those in healthy subjects.

It is generally accepted that Th1-type cytokines are critical for the induction and maintenance of anti-tumor cytotoxic T lymphocyte responses in vivo, while Th2-type cytokines may downregulate cell-mediated immunity, providing a microenvironment conducive to malignant disease progression [7, 8]. Cytokine concentrations, including the balance of Th1 and Th2 types, have been frequently studied in cancer patients [10–13, 21]. Cytokines were measured in patients' sera [13], samples of tumor tissue [10, 11, 22], cells isolated from peritoneal washings [12] and blood cells stimulated in vitro [23].

Imbalances between Th1 and Th2 immune responses have been attributed to immune dysregulation in patients with malignancies [24, 25]. A Th2 bias was described in both metastatic melanoma and renal cell carcinoma [13, 26]. Patients with renal cell carcinoma with significantly increased Th2 type cytokine production do not develop an effective anti-tumor immune response, despite significant infiltration by lymphocytes [27]. These data suggest that the malignancy may play an active role in “reprogramming” systemic immunity toward Th2 dominance that may be permissive for tumor progression/metastases. In this study, we detected an increase of Th2 cytokines (IL-5 and IL-13) in PHA-stimulated whole blood cultures from DTC patients compared to those from healthy controls. In addition, there was also an increase in IL-9 concentration. This indicates that, in response to a nonspecific stimulus, peripheral blood cells of DTC patients are directed to produce more Th2/Th9 cytokines than controls. On the basis of our results, we cannot consider the cause or the potential significance of these findings. However, autocrine production of IL-4 and IL-10 by thyroid cancer cells has been shown earlier [20]. Both cytokines are known as inducers of a Th2 type response.

Another possibility is that the Th2 type of immune response in DTC patients before ¹³¹I was induced as a part of the anti-tumor defense. While it is generally accepted that Th2 cytokines contribute to disease progression [7, 8], there are studies indicating a protective role for them in anti-tumor immunity [9]. Thus, particular components of the Th2-mediated immune response, such as IL-4 and eosinophils, can decrease tumor growth and initiate anti-tumor activity [28]. The basic biological activity of IL-5 is stimulation of eosinophil production in the bone marrow, as well as regulation of survival and activation of eosinophils [29]. Eosinophils have been found in peritumoral infiltrate from a number of tumors (colon carcinoma, planocellular carcinoma, lung adenocarcinoma and urinary bladder carcinoma) and are usually associated with a favorable prognosis (reviewed in references [28, 30]). In experimental animal models, it was shown that eosinophils activated by IL-5 suppress the growth of hepatocellular carcinoma *in vivo* and *in vitro* [31], and they can directly kill cells of genetically modified murine fibrosarcoma *in vitro* [32]. Furthermore, it was shown that tumor-specific CD4⁺ T lymphocytes with a Th2 cytokine profile can remove an established metastasis of melanoma in lungs, whereby eosinophil degranulation was induced in the tumor tissue [33].

Since two Th2 type cytokines, IL-13 and IL-4, have common and different receptors, their respective roles in tumor immunity are incompletely understood [9]. Recent findings suggest a negative influence of IL-4 on tumor growth [34–36], while data on the role of IL-13 are

contradictory [37–39]. According to findings indicating an anti-tumor effect of IL-13, the effect was probably mediated by accumulation of neutrophils and macrophages [37, 39], although action of IL-13 through eosinophils could not be excluded [9].

IL-9 was first described as a Th2 cytokine, and many of its effects are similar to those of IL-4 [40]. In the presence of IL-4 and TGFβ, both Th0 and Th2 cells can differentiate into TH9 lymphocytes, which secrete IL-9 and IL-10. Besides TH9 lymphocytes, other subpopulations of T lymphocytes may secrete IL-9 under certain conditions. IL-9 exerts pleiotropic effects on CD4⁺ T cell subsets and influences the development and maintenance of effectors' cells (reviewed in reference [40]). Thus, IL-9 regulates the development of inflammation and hyper-responsiveness in experimental autoimmune encephalomyelitis and rheumatoid arthritis [40]. Moreover, IL-9 may have a protective role in tumor immunity. In an experimental study, Purwar et al. [41] showed that abundant production of IL-9 suppressed the growth of melanoma cells in mice, while IL-9-blocking antibodies enhanced tumor growth.

Although numerous studies with animals [42, 43] and human subjects [44] have shown an influence of hypothyroidism on immune function, our results do not confirm that. Thus, statistical analysis excluded the association of any cytokine concentration with TSH values. This could be explained by differences in study design. Namely, we analyzed the selected cytokines in culture medium after stimulation with PHA *in vitro*. It is possible that strong mitogen stimulation *in vitro* prevents or significantly reduces the effect of hypothyroidism on cytokine production in blood cells. Given that our severely hypothyroid DTC patients produce more Th2/Th9 cytokines than controls, while production of cytokines Th1/Th17 is similar in both groups, we believe that the differences in cytokine profile were not caused by hypothyroidism.

Data regarding the cytokine secretion in DTC patients treated with radioactive iodine are very scarce. In a slightly different study design, Barsegian et al. [45] analyzed the production of pro-inflammatory cytokine IFN-γ and anti-inflammatory cytokine IL-10 in DTC patients treated with radioactive ¹³¹I. Upon stimulation with microbial antigen at the single cell level (using ELISPOT assay), there was an increase of IFN-γ production in DTC patients 1 day after ¹³¹I therapy with return to pretreatment values 7 days after therapy. Our results are in agreement with this. Namely, in both studies, the production of IFN-γ and IL-10 at day 7 after radioactive ¹³¹I therapy was similar to the values before therapy.

In this study, we obtained a statistically significant decrease of Th2 cytokine production (IL-4, IL-5 and IL-13) in DTC patients 7 days after ¹³¹I therapy. In patients with hepatocellular carcinoma, it was earlier shown that

iodine-125 implantation stimulates the anti-tumor immune response by promoting Th2/Th1 deviation [16]. Also, in patients with Graves' disease treated with radioactive 131-I, a transient increase of Th2 cytokines (IL-4, IL-6 and IL-10) with longer term increases in IFN- γ production was obtained [17]. In that study, the cytokine-producing cells were evaluated by ELISPOT assay. We believe that the results obtained in patients with Graves' disease might be different from ours with DTC patients, due to differences in the inflammatory cells included in thyroid cancer and autoimmune thyroid diseases. The reduction of Th2 cytokine in DTC patients after 131-I therapy could be beneficial if it allows the development of a stronger Th1 type of response to the tumor.

Analysis of the Th1/Th2 cytokine ratios showed no statistically significant difference between DTC patients before 131-I therapy and controls. Moreover, in our study, no statistically significant difference of the Th1/Th2 cytokine ratios in DTC patients before and after 131-I therapy was detected. These results indicate that, in spite of induction of the Th2 type of response and cytokine secretion in DTC patients before 131-I therapy, there was no marked shift from the Th1 toward the Th2 type immune response. Moreover, radioactive iodine therapy did not change the Th1/Th2 cytokine ratio in DTC patients.

The advantages of this study are: a simple assay for detection of the cytokine-producing potential of blood cells, no cytokines secreted from other cells (e.g., keratinocytes, osteoblasts, endothelial cells, adipose tissue cells, smooth muscle cells and skeletal muscle cells) and no interference by inhibitory molecules present in the patient's sera (soluble receptors, anti-cytokine antibodies, receptor antagonists). Despite the limitations of our investigation (small sample size, large individual variations of secreted cytokines, mitogen stimulation of blood cells in vitro), increased production of Th2/Th9 cytokines in patients with DTC before radioactive iodine therapy and reduced capacity of secretion of Th2 cytokines 7 days after 131-I therapy was demonstrated for the first time. As the role of the Th2/Th9 immune response in patients with tumors is still insufficiently clarified, new studies are necessary for evaluation of the role of IL-4, IL-5, IL-13 and IL-9 in tumor immune surveillance and tumor immunity during progression of malignancy.

In conclusion, DTC patients produce significantly higher concentrations of Th2/Th9 cytokines than control subjects, while Th1/Th17 cytokine levels did not differ from those in control subjects. There is no influence of hypothyroidism or stage of disease on cytokine production in DTC patients before 131-I therapy. Radioactive 131-I therapy leads to reduced secretion of Th2 cytokines. Additional studies are needed to determine the significance of these findings.

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Conflict of interest The authors declare that they have no conflict of interest.

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