Tumoricidal Cytokines Enhance Radioiodine Uptake in Cultured Thyroid Cancer Cells

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We explored whether the stimulation of iodine uptake by interferons seen in a rat thyroid cell line is reproducible in human thyroid cancer and thus applicable to enhance the efficacy of radioiodine therapy. **Methods:** Surgical specimens from 12 papillary and 2 follicular adenocarcinomas were minced and seeded in culture trays. After 14–16 days in a medium supplemented with 5% calf serum, we measured cellular uptake of ^{125}I during a 40-min incubation period. **Results:** In 8 of 12 papillary and all 2 follicular carcinomas, interferon-gamma significantly stimulated iodine incorporation. The four nonresponder tumors had lower basal iodine uptake and relatively less differentiation of histologic features. The effect was dose dependent (0–100 U/ml), and the average maximum increase in responding cases was 35.1% over basal values. Tumor necrosis factor-alpha alone did not alter uptake, but at 300 U/ml it further enhanced the effect of interferon-gamma in the two follicular tumors. In addition to the pure cytokines, supernatant from lymphocyte culture conditioned with a bacterial immunostimulator also boosted radioiodine trapping in thyroid cancer cells. **Conclusion:** These in vitro results warrant animal experiments to test potential usefulness of tumoricidal cytokines in radioiodine therapy.

Key Words: thyroid cancer; radioiodine therapy; cytokines

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Radioiodine treatment has been the first choice for remote metastases from differentiated thyroid carcinoma (*1,2*). Among the many factors that affect the outcome of this therapy, intensity of accumulation in metastatic lesions is one of the most important (*3,4*). Any enhancement in tumor uptake could lead to improved therapeutic efficacy. We previously reported a more than twofold increase of iodine uptake by interferons in the rat thyroid cell line FRTL5 (*5*). To assess whether this phenomenon is applicable to radioiodine therapy, we studied the effect of interferons on iodine trapping in a primary monolayer culture of human thyroid cancer cells.

In contrast, bacterial immunostimulators and other biologic response modifiers have recently drawn much attention as adjunctive therapy for various malignant diseases (*6*). Of these, OK-432, a streptococcal preparation, has been shown to induce a multitude of cytokines, including interferon (IFN)-gamma and tumor necrosis factor (TNF)-alpha (*7,8*). Therefore, in addition to pure cytokines, we also examined effect of OK-432-conditioned supernatant from human lymphocyte culture on iodine uptake by thyroid cancer cells.

MATERIALS AND METHODS

We used commercially available reagents and cultures: immunoradiometric assay kits for thyroglobulin (Tg), collagenase type V-S, Coon's modification of Ham's F-12 medium, hydroxyethyl piperazine ethanesulfonic acid (HEPES), D-glucose and sodium iodide, human recombinant IFN-gamma; mouse recombinant TNF-alpha, RPMI 1640 medium, calf and fetal calf sera, lymphocyte separation medium, carrier-free Na ^{125}I and OK-432, an attenuated streptococcal preparation.

Thyroid Cell Culture

Thyroid cancer tissues were obtained at surgery from 14 female patients whose profiles are summarized in Table 1. The tumors were processed aseptically, as described previously for thyroid adenomas (*9*), with some modifications. Briefly, tumor blocks were freed of connective tissues, minced finely with scissors and digested with 0.1% collagenase in phosphate-buffered saline (PBS), pH 7.2, for 10 min at 37°C. After 1 min of sedimentation, the first supernatant was discarded, and the sediment was further digested in fresh collagenase solution for 30 min. Released cells were collected and filtered through cheesecloth. The process was repeated three times, and the pooled filtrate was washed once with RPMI 1640 supplemented with 5% calf serum, penicillin and streptomycin (washing medium). After hemolysis with Tris-buffered ammonium chloride, the cells were washed three times with the washing medium and seeded in 24-well culture trays at 2×10^5/well in 1 ml of Coon's modified Ham's F-12 supplemented with 5% calf serum, bovine thyroid-stimulating hormone (TSH), insulin, transferrin, somatostatin, hydrocortisone and glycyl-L-histidyl-L-lysine acetate and the antibiotics. Seven to 10 days later, the cells were washed twice with PBS and further cultured in the similar medium but devoid of TSH for 5–7 days. During these culture periods, cells were fed with fresh medium every 3–4 days.

Separation and Stimulation of Human Peripheral Lymphocytes

Heparinized venous blood from healthy volunteers was processed by discontinuous density gradient centrifugation at $1400 \times g$ for 30 min on the lymphocyte separation medium. Cells in the interface were collected, washed three times with the washing medium and resuspended in RPMI-1640 supplemented with 10% fetal calf serum and the antibiotics, with or without 0.01 mg/ml of OK-432. After 2 days of incubation, the mixture was centrifuged, and the supernatant was collected and stored at −70°C until use.

Iodine Uptake Assay

Iodine uptake assay was performed according to Weiss et al. (*10*). Briefly, cells were rinsed once with Hank's balanced salt solution containing HEPES and glucose (HBSS), incubated with 3.7 kBq Na ^{125}I and 10 μM NaI in HBSS for 40 min at 37°C, washed twice with ice-cold HBSS, extracted with ethanol and counted in a gamma counter.

Statistical Analysis

All comparisons of numerical data were carried out with analysis of variance of triplicate samples.

RESULTS

As shown in Table 2, cultured thyroid cancer cells trapped approximately 0.5%–1% of the total radioiodine added (roughly 20,000 cpm/well), and IFN-gamma significantly increased uptake in 10 of 14 cases. The effect was dose dependent, as illustrated in Figure 1 for a representative case. The mean

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TABLE 1
Patient Characteristics

Patient no.	Age (yr)	Histologic findings	Serum Tg (ng/ml)
1	73	PAC	321
2	44	PAC	218
3	70	PAC	104
4	62	PAC	127
5	67	PAC	<1.5
6	73	FAC	12.2
7	63	PAC	<1.5
8	57	PAC	<1.5
9	22	PAC	118
10	37	PAC	596
11	40	PAC	40.7
12	56	FAC	35.5
13	53	PAC	2.8
14	69	PAC	244

PAC = papillary adenocarcinoma; FAC = follicular adenocarcinoma; Tg = thyroglobulin.

maximal increase over basal uptake was 35.1% in the 10 responsive cases. The four nonresponsive cases tended to have lower basal uptake than did the responsive cases and low or undetectable levels of serum Tg measured within 2 wk before surgical resection (Table 1). Tumor necrosis factor-alpha alone did not significantly change radioiodine trapping (data not shown). At 300 U/ml, however, it enhanced the effect of IFN-gamma in cases 6 and 12 and, although not significantly, nearly 20% in case 14 (Table 2).

Unlike FRTL5 cells, human cancer cells did not respond to TSH stimulation (up to 10 mU/ml) in the iodine uptake assay (data not shown).

The effect of the supernatant from lymphocyte culture was studied in cases 6, 9 and 11. As shown in Figure 2, OK-432-conditioned preparation stimulated iodine uptake as strongly as did the combination of IFN-gamma and TNF-alpha, whereas the control supernatant had only a slight, nonsignificant effect

TABLE 2
Enhancement of Iodine Uptake in Thyroid Cancer Cells by Cytokines (300 U/ml IFN-gamma with or without 300 U/ml TNF-alpha)

Patient no.	Basal value (cpm)	With IFN-gamma (% of basal value)	With IFN-gamma and TNF-alpha (% of basal value)
1	1464 ± 69	148.0 ± 12.1*	Not done
2	1448 ± 80	128.5 ± 7.3†	Not done
3	1270 ± 39	119.1 ± 7.2†	125.0 ± 5.8†
4	1010 ± 36	128.4 ± 6.1†	138.1 ± 8.6†
5	930 ± 98	120.1 ± 9.9	121.2 ± 8.9
6	1508 ± 85	134.0 ± 11.3†	148.4 ± 10.2*‡
7	1266 ± 121	114.1 ± 9.2	118.2 ± 10.4
8	792 ± 69	109.3 ± 11.5	112.6 ± 9.2
9	2457 ± 198	139.6 ± 8.8*	147.9 ± 11.8*
10	1279 ± 93	124.3 ± 6.5†	124.8 ± 6.9†
11	714 ± 53	124.1 ± 5.4†	132.4 ± 6.7†
12	2095 ± 189	147.8 ± 7.8*	162.2 ± 11.4*‡
13	879 ± 124	117.4 ± 8.9	110.4 ± 6.6
14	1099 ± 196	156.9 ± 14.8*	176.0 ± 15.7*

*$p < 0.01$, †$p < 0.05$, significantly different from basal values.
‡$p < 0.05$, significantly different from IFN-gamma alone.
Data are expressed as mean ± s.d. of triplicate samples.

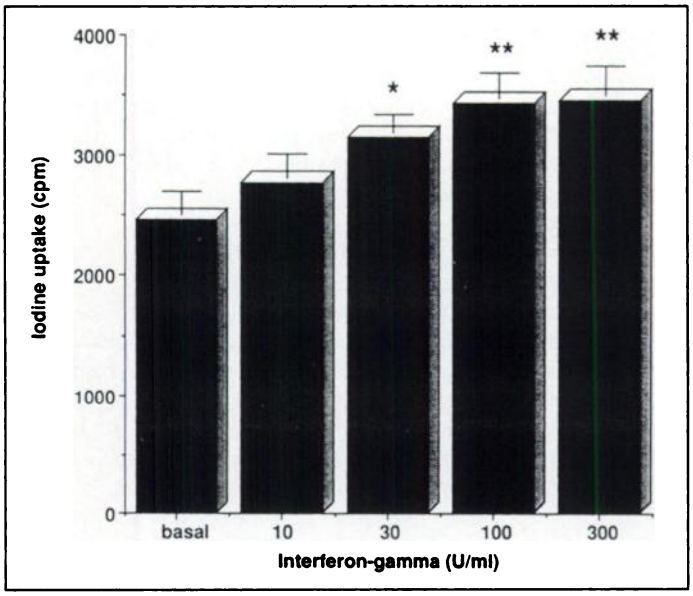

FIGURE 1. Dose-dependent enhancement of iodine uptake by IFN-gamma in a representative primary culture of thyroid cancer (Patient 9). The assay was performed in triplicate. Mean values (columns) and standard deviations (thin vertical lines) are shown. *$p < 0.05$, **$p < 0.01$, significantly different from basal levels.

in case 6. Similar results were seen in the other two cases; iodine uptake in the presence of OK-432-conditioned supernatant (10% in volume) was 142.2% of basal levels in case 9 and 145.4% in case 11.

In no instance did either IFN-gamma alone or the combination of IFN-gamma and TNF-alpha cause any apparent morphological changes.

DISCUSSION

The present study demonstrated that IFN-gamma boosts iodine uptake not only in the FRTL5 cell line, but also in most cases of human thyroid cancer, although to a lesser extent

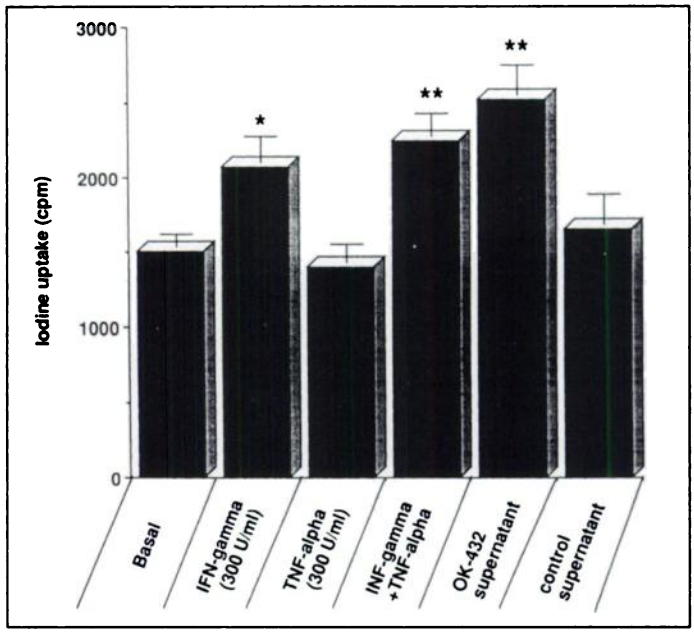

FIGURE 2. Enhancing effect of the OK-432-treated lymphocyte supernatant (diluted 1:10) on iodine uptake in thyroid cancer cells in primary culture (Patient 6). The test was performed in triplicate. Mean values (columns) and standard deviations (thin vertical lines) are shown. *$p < 0.05$, **$p < 0.01$, significantly different from basal levels.

(approximately 120%–150% increase in the former versus 20%–60% in the latter). As expected from their low basal iodine uptake and low serum Tg levels, the four nonresponding tumors were generally less differentiated, although not entirely undifferentiated, on histopathological examination. Therefore, the enhancing effect of IFN-gamma seems to be greater on more differentiated cancers, which retain the characteristics of normal thyrocytes.

The mechanism for this stimulatory effect is largely elusive, like many other biologic actions of interferons (*11*). We could at least preclude an increase in intracellular cyclic adenosine monophosphate (cAMP) as a candidate because IFN-gamma inhibits other cAMP-mediated functions in thyroid cells (*5,12,13*) and does not alter the content of this substance in FRTL5 (*5*) or in thyroid cancer cells (*unpublished data*, 1995) Given the results of others for benign thyrocytes (*13,14*), discordant from ours for cancer cells, exploration of the underlying mechanism is all the more important, although the methods used in the three works are slightly different from one another.

Tumor necrosis factor-alpha has been shown to increase binding of IFN-gamma to its receptor (*15*), and this appeared to be the most likely explanation for the supportive role of TNF-alpha seen in the iodine uptake.

In our experimental setting, human thyroid cancer cells did not respond to TSH, despite the reported preservation of a functional adenylate cyclase pathway (*16*). A possible explanation for this unresponsiveness is a failure in the link between cAMP generation and differentiated functions, such as iodine trapping in malignant thyrocytes.

As expected from previous reports (*7,8*), OK-432 stimulated lymphocytes to produce factors, presumably including IFN-gamma and TNF-alpha, which can stimulate iodine uptake in thyroid cancer cells. In our previous study (*17*), intratumoral injection of this bacterial preparation induced TNF-alpha secretion in situ and also increased expression of HLA-DR antigen, probably by stimulating local production of IFN-gamma (*5,12,13*). Hence, in future in vivo studies, we could substitute local application of OK-432 for expensive pure cytokine preparations, which may cause severe adverse effects when administered systemically.

Before considering any clinical application of the observed effects of IFN-gamma and TNF-alpha, animal studies are needed to see whether similar events take place in vivo and to determine optimal timing, combination and route of administration for the two cytokines or their inducers. If reproduced in animals, our present in vitro results could lead to the combined use of cytokines and ^{131}I therapy with improved tumor uptake. The well-established tumoricidal ability of interferons and tumor necrosis factor may provide further merit in eliminating malignant thyrocytes.

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