peroxidase was performed. Staining was carried out with carbazole, a precipitating substrate of peroxidase. The same blotting method was used in PAG gradient gels for determination of molecular weight.

Results: The minimum concentration of Tg in serum to detect MH patterns by immunoblotting was about 1,000 ng/ml. This method revealed an identical MH pattern of isolated thyroidal Tg as compared to the direct staining procedure. By immunoblotting serum Tg of two patients with Graves' disease (Tg levels 1,234 and 1,523 ng/ml) exhibited the same MH pattern as thyroidal TG (6 bands, pH range 4.2 to 4.6). In the serum of a patient with a colloid cyst we found an MH pattern of low iodinated and desialylated Tg (pH range 4.4 to 4.8) in agreement with earlier findings in the colloid fluid of thyroid cysts.

In the sera of three patients with metastatic carcinoma, however, a different MH pattern of Tg was found in the range of sialylated and desialylated Tg (pH 4.4 to 4.8). After complete desialylation of the whole sera an additional band, not seen before in benign thyroid diseases, appeared at pH 5.0.

The molecular weight of serum Tg, as determined by PAG gradient gel electrophoresis in patients with follicular carcinoma and Graves' disease, was in the same order of magnitude as normal thyroidal Tg (300,000 Daltons).

Conclusion: Our investigation demonstrates an identical MH pattern of isolated thyroidal Tg and serum Tg in Graves' disease, as well as an identical pattern in thyroid colloid cyst fluid and Tg in the sera of these patients. This identity of the thyroidal and serum MH pattern may be suggestive of a passive efflux of Tg, at least in these benign thyroid diseases. It may be postulated that active secretion, on the other hand, would lead to modifications of Tg, i.e. deiodination and/or degradation resulting in a different MH pattern.

An abnormal MH pattern of serum Tg was found in three patients with metastatic follicular carcinoma, both before and after in vitro desialylation. Therefore this characteristic MH pattern is more likely to result from a different peptide structure than from a different carbohydrate chain.

Supported by the Deutsche Forschungsgemeinschaft

64. Thyroglobulin as tumor marker and 201-thallium scan in the follow-up of differentiated thyroid cancer


Thyroglobulin (TG), a thyroid-specific glycoprotein, can be detected by radioimmunoassay (RIA) in the majority of normal individuals. In patients who have had a total thyroidectomy for differentiated thyroid carcinoma no circulating TG should be detected unless metastases are present. 201-thallium-chloride (201-Tl) is a radioisotope with a biological and physiological behaviour similar to potassium. It is known that 201-Tl is concentrated in primary lesions of thyroid cancer, and also in their metastases. The aim of the present prospective study was to determine the usefulness of serial measurements of TG concentration in combination with whole-body 201-Tl scintigraphy in patients with differentiated thyroid carcinoma.

Methods: 80 patients with histologically proven differentiated follicular or papillary thyroid carcinoma were examined. All had undergone total thyroidectomy followed by 131-I-ablation for remaining tissue. Each patient stopped T 3-medication for two weeks. Blood was drawn for measurement of TSH and TG on the last day before stopping T 3 and on the 7th and 14th day after T 3-withdrawal. On the 7th day 3 mCi 131-I was given orally, and scintigraphy was performed one week later. On the 14th day the whole-body scan after i.v. administration of 2 mCi 201-Tl was registered.

Serum TG was determined by a double antibody radioimmunoassay; TSH, T 4 and T 3 measurements were performed by RIA.

Results: In 74 patients without residual thyroid tissue and a negative whole-body 201-Tl and/or 131-I scan, the TG concentration in plasma was undetectable. In 5 of the remaining 6 patients, TG was measurable while the patients were receiving T 3 therapy, and undetectable in one. After one and two weeks off T 3, TG increased in all patients in parallel with TSH (Fig. 1).
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3. In 65.
2. It is approximately 100.
1. It is demonstrated that defined peptide regions of the isolated subunits, which are readily accessible for different proteolytic enzymes, are protected in the native hCG molecule and vice versa. These investigations give a hint at those regions of the molecule which may be readily attacked by proteases as well as at specificity of the enzyme.

Figure 1: TSH and TG values before (day 0) and on the 7th and 14th day after T3 withdrawal in 6 patients with metastatic thyroid cancer.

Of 5 patients with abnormal 201-Tl scintigraphy, 2 had residual local tissue, and 3 distant metastases. Of these cases the 131-I-scan detected metastases in only one and local tissue in two patients.

Conclusions:
1. TG is TSH-dependent and increases after withdrawal of T3 replacement therapy.
2. TG measurements under T3 replacement therapy are less sensitive than measurements after withdrawal of T3.
3. 201-Tl scintigraphy can replace 131-I scanning in follow-up controls.
4. The combination of serum TG and 201-Tl scintigraphy seems to be superior to either one alone and can be performed while the patient is on replacement therapy.

65. Proteolytic degradation of native hCG: possible implications for hormone structure and tumor diagnostics

W. E. MERZ, U. MERKEL, Institut für Biochemie II, Universität Heidelberg

In the present study it is shown that the hCG specific COOH-terminal peptide region β-115–145 may be cleaved off enzymatically from native hCG. This finding is of interest in respect to the role of hCG as a tumor marker: a large number of tumors secrete or activate proteolytic enzymes which may give rise to hCG fragments and therefore to an impairment of immunological monitoring. Furthermore it is demonstrated that defined peptide regions of the isolated subunits, which are readily accessible for different proteolytic enzymes, are protected in the native hCG molecule and vice versa. These investigations give a hint at those regions of the molecule which may be readily attacked by proteases as well as at specificity of the enzyme.

Digestion of highly purified native hCG (13,000 IU/mg) by trypsin (soluble or immobilized enzyme; hCG: trypsin = 200: 1, 0.05 M NH₄HCO₃ pH 8.6) results in a rapid cleavage of peptide bonds of the β-subunit portion of hCG, whereas the α-subunit portion seems to remain unimpaired approximately up to 1 h. In contrast, the isolated α-subunit is readily cleaved at 8 out of 9 possible positions. This indicates that at least some of the tryptic cleavage sites of the α-subunit, especially those in the region α-35–52, seem to be hidden in the hCG molecule. Receptor binding activity of hCG decreases within 10 min of digestion to 3,180 IU/mg (95% c.I. 3,070–3,290) and reaches 2,120 IU/mg (2,130–2,310) after 30 min of treatment. Biological activity in vivo (rat prostate assay) after 10 min of diges-