Human chorionic gonadotropin (hCG) β -core fragment is produced by degradation of hCG or free hCG β in gestational trophoblastic tumors: a possible marker for early detection of persistent postmolar gestational trophoblastic disease

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Abstract

The present study was undertaken to investigate whether human chorionic gonadotropin (hCG) β -core fragment (hCG β cf) was directly produced by gestational trophoblastic tumors. Immunoreactivity of hCG β cf was demonstrated in the extracts as well as in the culture media of hydatidiform mole tissues. It was also present in the extracts of choriocarcinoma tissues, and its molar concentration exceeded that of intact hCG. The presence of hCG β cf was then confirmed by gel chromatography and Western blot analysis. Immunohistochemistry showed localization of hCG β cf immunoreactivity to the syncytiotrophoblasts and scattered cells in the stroma of mole tissue, and to syncytiotrophoblastic cells in choriocarcinoma. Immunoreactivity of hCG β cf was also detected in the sera of the patients with gestational trophoblastic disease,

although the hCG β cf/hCG ratio was less than one hundredth of that in the tissue extracts. Serial measurement of serum hCG β cf levels after mole evacuation showed that they declined much more rapidly than those of hCG and became undetectable in the patients with subsequent spontaneous resolution, while hCG β cf remained or became detectable before the rise of hCG was observed in the patients with subsequent persistent trophoblastic disease. Taken together, these results suggest that hCG β cf is directly produced by gestational trophoblastic tumors, and monitoring of hCG β cf in the serum after mole evacuation may be useful for early prediction of subsequent development of postmolar persistent trophoblastic disease.

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Introduction

Gestational trophoblastic disease (GTD) refers to a wide spectrum of diseases ranging from benign hydatidiform mole to highly malignant choriocarcinoma and placental site trophoblastic tumor. Hydatidiform mole is a specific form of abnormal pregnancy that is characterized by the presence of hydropic swelling of the chorionic villi and proliferation of the trophoblasts. It is associated with a substantial risk (5–10%) of subsequent persistent GTD and may eventually develop into choriocarcinoma (Lurain *et al.* 1983). Except for the serial measurement of human chorionic gonadotropin (hCG), there is no reliable predictor for the early detection of persistent GTD.

The hCG β -subunit core fragment (hCG β cf) is a form of hCG β composed of segments of hCG β residues 6–40 disulfide-linked to residues 55–92 and lacking the hCG β carboxy-terminal immunological determinant; it has a molecular mass of approximately 12 000–17 000 (Birken

et al. 1988, Blithe *et al.* 1988). hCG β cf is quantitatively the major form of immunoreactive hCG molecules in pregnancy urine, the concentration of which is 2 to 10 times higher than that of intact hCG on a molar basis (Kato & Braunstein 1988, Wehmann *et al.* 1990, de Medeiros *et al.* 1992), and it has been assumed that hCG β cf originates from the renal metabolism of intact hCG or free hCG β (Wehmann & Nisula 1980, Lefort *et al.* 1984, 1986). However, in a study involving the intravenous injection of recombinant hCG into healthy, non-pregnant women, the hCG β cf measured in urine was only 12.2% of the amount of hCG excreted in urine, and it was suggested that there may be more than one pathway responsible for the production of hCG β cf in pregnancy (Norman *et al.* 2000). In fact, placental tissue has been associated with the production of hCG β cf (Cole & Birken 1988, Udagawa *et al.* 1998). Moreover, we recently demonstrated that hCG β cf was directly produced by cancer cells *in vitro*, including choriocarcinoma cell lines (Okamoto *et al.*

2001). Also in that report, we detected hCGβcf immunoreactivity in the serum of the mice transplanted with choriocarcinoma cells that produced hCGβcf *in vitro*. In view of these observations, the present study was undertaken to investigate the presence of hCGβcf in the tissue specimens of hydatidiform mole and choriocarcinoma. Serum levels of hCGβcf in the patients with GTD were also determined.

Materials and Methods

Tissue and blood sample collection

Tissue samples were obtained from 10 patients with complete hydatidiform mole (9 to 14 weeks of gestation) and 5 patients with gestational choriocarcinoma; all were histologically confirmed and none of them had been treated with chemotherapy. They were collected immediately after delivery or at the time of surgery, snap frozen in liquid nitrogen and stored at -80°C . Fresh molar tissue was also used for organ culture. Some of the samples were fixed by immersion in 10% neutral buffered formalin for 12–24 h and processed for paraffin embedding by the standard methods.

Serum samples were obtained from 14 patients with complete hydatidiform mole, 4 of whom developed persistent GTD, and 4 patients with gestational choriocarcinoma. The 4 patients who developed postmolar persistent GTD were diagnosed as ‘clinical invasive mole’ on the basis that in weekly measurements of serum hCG levels there were two consecutive rising levels or levels reached a plateau for three or more consecutive weeks, combined with the ‘choriocarcinoma diagnostic score’, which is routinely used in Japan (Okamoto *et al.* 1998). The samples were stored at -80°C until use.

Written informed consent for the collection of tissue and serum samples was obtained from all patients, and the study was conducted in accordance with the institutional guidelines.

Organ culture of molar tissue

Organ culture of molar tissue was performed by the method described previously (Udagawa *et al.* 1998). Briefly, pieces of molar vesicles were rinsed in phosphate-buffered saline (PBS) and cultured in a six-well Transwell-COL chamber (Coster, Cambridge, MA, USA). Incubation was carried out in the culture medium (Dulbecco’s Minimal Essential Medium supplemented with 10% fetal calf serum) at 37°C in humidified 5% CO_2 . The culture media were harvested 0.5, 1, 1.5, 2, 4, or 24 h later for the measurement of intact hCG, free hCGβ and hCGβcf. Each experiment was performed in triplicate.

Tumor formation of choriocarcinoma cells injected into the nude mouse

The NaUCC-3 choriocarcinoma cell line, which was established and maintained in our department (Sugiura

et al. 1988), was cultured in RPMI 1640 supplemented with 10% fetal calf serum in a humidified atmosphere of 5% CO_2 at 37°C . Six-week-old female BALB/c nu/nu mice (obtained from Clea Japan Animals Inc, Tokyo, Japan) were injected with 1×10^6 NaUCC-3 cells subcutaneously in the flank. After tumor formation, the mice were killed, and the tumors were excised and snap frozen. The tumors were histologically proven to be choriocarcinomas (Okamoto *et al.* 2001).

Preparation of tissue extracts

The frozen tissue samples were homogenized with a motor-driven Teflon pestle for 10 min on ice in 1 ml extraction buffer (150 mM NaCl, 20 mM Tris (pH 7.5), 5 mM EDTA, 1% Nonidet P-40 containing 1 mM phenylmethylsulfonyl fluoride and 2 μg/ml aprotinin) per 100 mg tissue wet weight, and the tissue extracts were obtained after centrifugation at 15 000 r.p.m. for 20 min at 4°C .

Determination of intact hCG, free hCGβ, and hCGβcf

Levels of intact hCG, free hCGβ, and hCGβcf were determined by the enzyme immunoassays (EIAs) described elsewhere (Udagawa *et al.* 1998). The EIA for hCGβcf gave negligible cross-reactivity with intact hCG (0.034%) or free hCGβ (0.12%), but the results for hCGβcf were always corrected for cross-reaction with these molecules. Occasionally, the concentration of each molecule was calculated and compared on a molar basis, assuming that the molecular mass of hCGβcf is 15 000 (Birken *et al.* 1988).

Chromatography of the tissue extract

Tissue extracts of a mole and a choriocarcinoma were analyzed by chromatography on Superdex 75 (Pharmacia Biotech, Uppsala, Sweden). Aliquots (200 μl) of each sample were applied to a 1×30 cm column bed equilibrated with 50 mM sodium phosphate buffer containing 0.15 M NaCl and 0.02% sodium azide (pH 7.0). The flow rate of the column was adjusted to 0.5 ml/min, and 0.5 ml fractions were collected by gravity at 4°C . Each fraction was measured for intact hCG, free hCGβ or hCGβcf by the respective EIAs, and the data were calibrated on molecular size.

Western blot analysis

SDS-PAGE for immunoblotting was performed in 10% acrylamide slab gels under non-reducing conditions. Protein molecular weight standards (Kaleidoscope Prestained Standards; Bio-Rad Laboratories, Hercules, CA, USA) were used to calibrate the gels. Proteins were transferred to nitrocellulose membranes (Amersham, Aylesbury,

Table 1 Amounts of hCG, free hCGβ, and hCGβcf in the culture media of molar tissue. All experiments were performed in triplicate, and values are expressed as the mean $\pm$ s.d. (pmol/g tissue/4 h)

Sample	hCG	Freeβ	hCGβcf	Freeβ/hCG (%)	hCGβcf/hCG (%)
1	3720 $\pm$ 470	195 $\pm$ 33	177 $\pm$ 5.8	5.2	4.8
2	22 200 $\pm$ 3950	377 $\pm$ 31	586 $\pm$ 10.4	1.7	2.6
3	10 200 $\pm$ 2570	244 $\pm$ 44	1370 $\pm$ 360	2.4	13.4
4	3840 $\pm$ 524	343 $\pm$ 39	1347 $\pm$ 387	8.9	35.1
5	2980 $\pm$ 151	280 $\pm$ 84	1360 $\pm$ 163	9.4	45.6
6	4570 $\pm$ 672	275 $\pm$ 40	1640 $\pm$ 169	6.0	35.9
7	3220 $\pm$ 495	321 $\pm$ 59	1210 $\pm$ 410	10.0	37.6
8	2930 $\pm$ 392	282 $\pm$ 36	820 $\pm$ 240	9.6	28.0
9	950 $\pm$ 120	54 $\pm$ 20	99 $\pm$ 7.7	5.7	10.4
10	814 $\pm$ 148	140 $\pm$ 33	334 $\pm$ 67	17.2	41.0

Bucks, UK) and blocked overnight at 4 °C in 10% skimmed milk in TBST buffer (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, and 0.1% Tween 20). The membranes were incubated with B210 monoclonal antibody, which is highly specific to hCGβcf (Krichevsky *et al.* 1991) at a concentration of 50 µg/ml for 2 h at room temperature. After washing with TBST, biotinylated goat anti-mouse immunoglobulin G (IgG) (DAKO, Glostrup, Denmark) was added. Positive bands were visualized by an enhanced chemiluminescence detection system (ECL; Amersham) and exposure to Hyperfilm (Amersham).

Immunohistochemical study

Formalin-fixed, paraffin-embedded tissue sections were deparaffinized in xylene, and hydrated through a descending series of ethanol. They were blocked with normal goat serum, incubated with B210 monoclonal antibody, and then further incubated with biotinylated goat anti-mouse IgG (Vector, Peterborough, UK). Avidin-biotin-peroxidase complex (Vector) was applied, and visualization was carried out with a peroxidase solution containing 0.02% hydrogen peroxide and 0.1% 3,3'-diaminobenzidine-tetrahydrochloride (Merck, Darmstadt, Germany) in PBS. Sections were counterstained with hematoxylin. Control mouse IgG1 obtained from a non-immunized mouse was used, instead of the primary antibody, as a negative control. Also, specificity of the primary antibody was verified by the absence of staining when reacted with the primary antibody preabsorbed with an excess of hCGβcf.

Statistical analysis

Statistical analysis was performed using Wilcoxon signed-ranks test or Mann-Whitney *U* test. Significance was set at $P < 0.05$.

Results

Presence of hCGβcf immunoreactivity in the culture media and tissue extracts

Levels of intact hCG, free hCGβ, and hCGβcf were examined in the culture media of hydatidiform mole tissues. First, a time course study was carried out, and a representative result is presented in Fig. 1. Immunoreactivity of each molecule appeared as early as 30 min after the start of incubation, and increased until 4 h, after which it reached a plateau. Interestingly, a rise in hCGβcf appeared later than that of intact hCG. Then, the amounts of each molecule in the culture media (4-h incubation) were determined using 10 hydatidiform mole samples (Table 1). Immunoreactivity of hCGβcf was present in the culture media at levels from 2.6% to 45.6% (mean $\pm$ s.d.,

Figure 1 Presence of hCGβcf immunoreactivity in the culture media of molar tissue. Molar tissue (sample no. 4 in Table 1) was cultured for 0.5, 1, 1.5, 2, 4, or 24 h in triplicate. Values represent the means $\pm$ s.d.

Table 2 Amounts of hCG, free hCGβ, and hCGβcf in the extracts of mole and choriocarcinoma tissues. The sample numbers of mole tissue correspond to those in Table 1. All experiments were performed in triplicate, and values are expressed as the mean ± s.d. (pmol/g tissue)

Sample	hCG	Freeβ	hCGβcf	Freeβ/hCG (%)	hCGβcf/hCG (%)
Mole					
1	3757 ± 570	122 ± 35	1127 ± 158	3.2	30.0
2	12 230 ± 1395	192 ± 21	1433 ± 104	1.6	11.7
3	5251 ± 557	332 ± 74	640 ± 86	6.3	12.2
4	3136 ± 552	230 ± 40	1727 ± 398	7.3	55.1
5	1218 ± 251	108 ± 34	1820 ± 263	8.9	149
6	1485 ± 372	225 ± 40	1600 ± 200	15.2	108
7	3529 ± 395	311 ± 70	2060 ± 390	8.8	58.4
8	1858 ± 390	461 ± 86	1160 ± 230	24.8	62.4
9	1371 ± 220	187 ± 25	406 ± 76	13.6	29.6
10	1703 ± 248	247 ± 63	1560 ± 267	14.5	91.6
Choriocarcinoma					
1	32.7 ± 7.3	45.0 ± 9.2	93.3 ± 10.6	138	285
2	19.3 ± 2.3	20.2 ± 3.3	106 ± 11.3	105	549
3	4.8 ± 0.5	2.5 ± 0.3	13.3 ± 1.3	52	277
4	128 ± 18.9	623 ± 85.6	1762 ± 213	487	1380
5	362 ± 43.6	226 ± 33.5	884 ± 77.3	62	244
6*	399 ± 44.3	76.7 ± 7.7	1260 ± 187	19	316

*Tumor specimen formed by the inoculation of NaUCC-3 choriocarcinoma cells into a nude mouse.

25.4% ± 16.1%) of that of intact hCG, and it exceeded that of free hCGβ except for one sample.

The amounts of immunoreactivity of intact hCG, free hCGβ, and hCGβcf in the extracts of hydatidiform mole and choriocarcinoma tissues are presented in Table 2. In the molar tissues, immunoreactivity of hCGβcf was 11.7% to 149% (mean ± s.d., 60.8% ± 44.3%) of that of intact hCG, which was significantly higher than that in the culture media of molar tissues (Wilcoxon signed-ranks test, $P=0.0069$). On the other hand, no significant difference in the ratio of free hCGβ to intact hCG was observed between the culture media (1.7%–17.2%, 7.6% ± 4.5%) and tissue extracts (1.6%–24.8%, 10.4% ± 6.8%) of molar tissues ($P=0.0925$). In the choriocarcinoma tissues, the hCGβcf/hCG ratio was significantly elevated (mean ± s.d., 509% ± 441%) as compared with that in the molar tissues (Mann–Whitney U test, $P=0.0022$). Likewise, the free β/hCG ratio was significantly higher in the choriocarcinoma tissues (143% ± 173%) than in the molar tissues (10.4% ± 6.8%) ($P=0.0022$).

Chromatography of the tissue extracts

Tissue extracts of a mole and a choriocarcinoma were gel filtrated, and the eluent fractions were measured by the EIAs. The results are shown in Fig. 2. Intact hCG, free hCGβ, and hCGβcf were detected in the expected order of each molecule. The elution profiles indicated that the

tissue extracts of GTD contained hCGβcf, and its quantity was greater than that of intact hCG on a molar basis.

Western blot analysis

To confirm the presence of hCGβcf in the tissue extracts, immunoblot analysis was performed under non-reducing conditions using a tumor formed by the inoculation of NaUCC-3 choriocarcinoma cells into a nude mouse, in addition to tissue from a mole and a choriocarcinoma. An immunoreactive band with a molecular mass of approximately 15 kDa was seen, as was observed in the standard urinary hCGβcf (Fig. 3, arrow). Also, some faint bands with molecular masses of approximately 40–60 kDa were observed.

Immunohistochemical localization of hCGβcf in hydatidiform mole and choriocarcinoma

Intense immunoreactivity of hCGβcf was demonstrated in the syncytiotrophoblast of hydatidiform mole (Fig. 4A). Scattered cells in the villous stroma also showed positive immunoreactivity for hCGβcf. With respect to choriocarcinoma, the tissue showed the typical pathological appearance of choriocarcinoma comprising dimorphic populations of malignant cytotrophoblasts and syncytiotrophoblasts, the latter of which revealed positive immunoreactivity for hCGβcf (Fig. 4B, arrows). None of the

Figure 2 Elution profile of the tissue extracts by gel chromatography on Superdex 75. (A) Hydatidiform mole (sample no. 6 in Table 2). (B) Choriocarcinoma (sample no. 1 in Table 2). Elution fractions were assayed for intact hCG, free hCG β and hCG β cf. The elution positions of the standard intact hCG, free hCG β and hCG β cf are indicated by the arrows.

control sections treated either with hCG β cf–preincubated primary antibody (Fig. 4C and D) or with non-immune mouse IgG (data not shown) revealed any significant staining.

Detection of hCG β cf in the sera of patients with GTD

Levels of immunoreactivity of intact hCG, free hCG β , and hCG β cf in the sera of patients with GTD were measured. Not only intact hCG and free hCG β but also hCG β cf were present in their sera (Table 3). The hCG β cf/hCG ratio was 0.02%–0.39% (mean $\pm$ s.d., 0.19% $\pm$ 0.10%) in hydatidiform mole, 0.06%–0.70% (0.41% $\pm$ 0.28%) in invasive mole, and 0.28%–1.31% (0.84% $\pm$ 0.59%) in choriocarcinoma. Among these three groups, statistical significance was observed only between

Figure 3 Immunoblots of the tissue extracts stained with B210 antibody under non-reducing conditions. Lane 1, standard urinary hCG β cf (2 ng/ml; provided by Colombia University, NY, USA); lane 2, hydatidiform mole; lane 3, choriocarcinoma; lane 4, tumor formed by the inoculation of NaUCC-3 choriocarcinoma cells into a nude mouse. The arrow indicates the position of major immunoreactivity of hCG β cf.

hydatidiform mole and choriocarcinoma ($P=0.011$). In the four patients who developed persistent GTD after uterine evacuation, the hCG β cf/hCG ratio was higher, although not significantly so, than that prior to mole evacuation (patients 10–13 in Table 3, $P=0.327$).

Serum levels of hCG β cf were monitored in the patients with hydatidiform mole after uterine evacuation. In the cases with subsequent spontaneous remission, hCG β cf levels declined much more rapidly than those of hCG and became undetectable after evacuation (Fig. 5A). On the other hand, in the cases with subsequent persistent GTD, hCG β cf remained or became detectable before the rise of hCG levels was observed (Fig. 5B), and it rapidly became undetectable after the initiation of chemotherapy. In the patients with choriocarcinoma, serum hCG β cf levels were relatively low compared with those of the patients with hydatidiform mole, and they rapidly decreased and became undetectable after the initiation of chemotherapy (data not shown).

Discussion

hCG β cf is the principal form of hCG β subunit in pregnancy urine, and it has been generally assumed that it originates from the renal metabolism of intact hCG or free hCG β (Birken *et al.* 1988, Wehmann *et al.* 1989, 1990, de Medeiros *et al.* 1992). However, we recently reported that hCG β cf was directly produced not only by placental tissue (Udagawa *et al.* 1998) but also by cancer cells *in vitro*, including choriocarcinoma cell lines (Okamoto *et al.* 2001). As for trophoblastic disease, there are limited data describing hCG β cf immunoreactivity in the tissue (Kardana *et al.* 1988, Khan *et al.* 2000), and the present study was performed to examine the presence and

Figure 4 Immunohistochemical staining of hydatidiform mole and choriocarcinoma tissues with B210 antibody. (A) Hydatidiform mole. hCGβcf immunoreactivity is prominent in the syncytiotrophoblast. Stromal cells are also positive for hCGβcf. Bar=40 μm. (B) Choriocarcinoma. The tumor shows the typical dimorphic pattern of choriocarcinoma, and the syncytiotrophoblastic cells are positive for hCGβcf (arrows). Bar=40 μm. (C, D) Negative controls for A and B respectively in which the sections were treated with the primary antibody preincubated with an excess of hCGβcf.

production of hCGβcf in gestational trophoblastic tumors using clinical tissue specimens.

Immunoreactivity of hCGβcf was detected in the culture media of hydatidiform mole tissues. It was also detectable in the tissue extracts of mole and choriocarcinoma. Then gel chromatography of the tissue extracts and Western blot analysis were performed, which showed that the hCGβcf immunoreactive material was indistinguishable from hCGβcf derived from pregnancy urine. Thus, it could be concluded that hCGβcf is produced by mole and choriocarcinoma tissues, although there may exist some differences, such as that in carbohydrate moieties (de Medeiros *et al.* 1993), between hCGβcf in pregnancy urine and that in trophoblastic tumors.

In the culture media of molar tissue, hCGβcf had a molar concentration of approximately 25% of that of intact hCG, and its levels were significantly elevated (approximately 60%) in the tissue extracts of the same samples that were used for the organ culture. This may indicate that, besides direct release, hCGβcf could be retained in the tissue by local degradation of intact hCG or free hCGβ produced by the syncytiotrophoblast. The result from immunohistochemical staining of molar tissue, which showed that the stromal cells as well as the syncytiotrophoblasts were positive for hCGβcf, supports this hypothesis. With respect to choriocarcinoma, the immunohistochemistry showed that the syncytiotrophoblastic cells

were mainly positive for hCGβcf. In our previous report (Okamoto *et al.* 2001), the hCGβcf/hCG ratio in the culture medium of NaUCC-3 choriocarcinoma cells *in vitro* was 11%, while the present study showed that the ratio increased to 316% in the extract of the tumor formed by the inoculation of the same cells into a nude mouse, and similar levels were also observed in the extracts of the other clinical specimens of choriocarcinoma (Table 2). Thus, it seems likely that the presence of stromal tissue could promote production of hCGβcf through local degradation of hCG or free hCGβ in gestational trophoblastic tumors.

Previously, we reported that the major form of hCGβcf immunoreactivity secreted by cancer cells *in vitro* was larger than the standard hCGβcf, possibly a trimer of hCGβcf (Okamoto *et al.* 2001), which was compatible with the intrinsic characteristic of hCGβcf that polymerization could occur when it exists in high concentration in solution (Cole *et al.* 1999). On the other hand, the present study demonstrated that the size of the major immunoreactivity of hCGβcf in the tumor formed by NaUCC-3 choriocarcinoma cells as well as in a clinical specimen of choriocarcinoma was identical to that of the standard urinary hCGβcf (Fig. 3). This result suggests that, even though polymerization of the hCGβcf molecule may occur, aggregated forms of hCGβcf could be degraded into the monomer when cancer cells exist *in vivo*, possibly by local degradation.

Table 3 Serum levels of hCG, free hCG β , and hCG β cf in the patients with GTD

Patient	hCG (pmol/ml)	Free β (pmol/ml)	hCG β cf (pmol/ml)	Free β /hCG (%)	hCG β cf/hCG (%)
Mole					
1	250	5.3	0.30	2.1	0.12
2	219	8.9	0.43	4.1	0.20
3	201	12.1	0.37	6.0	0.18
4	441	38.7	1.7	8.8	0.39
5	47	0.73	0.07	1.6	0.15
6	163	12.3	0.45	7.5	0.28
7	520	28.4	0.99	5.5	0.19
8	872	20.9	1.1	2.4	0.13
9	45	0.93	0.09	2.1	0.20
10*	755	2.9	0.83	0.38	0.11
11*	2226	19.7	0.42	0.88	0.02
12*	3755	71.8	3.43	1.9	0.09
13*	371	8.5	1.23	2.3	0.33
Invasive Mole					
10*	2.2	0.05	0.014	2.3	0.64
11*	113	2.6	0.073	2.3	0.06
12*	120	5.4	0.24	4.5	0.20
13*	12.8	0.39	0.056	3.0	0.44
14	2.7	0.04	0.019	1.5	0.70
Choriocarcinoma					
15	0.38	0.03	0.005	7.9	1.31
16	2.17	0.18	0.006	8.3	0.28
17	1.04	0.44	0.013	42.3	1.25
18	1.07	0.41	0.004	38.3	0.37

*Patients 10–13 developed persistent GTD (clinical invasive mole) after evacuation of hydatidiform mole.

In contrast to accounting for most of the hCG immuno-reactivity in pregnancy urine, hCG β cf is present only in minute amounts in pregnancy serum (Alfthan & Stenman 1990, Wehmann *et al.* 1990, Udagawa *et al.* 1998). Little information is currently available regarding concentrations of hCG β cf in the serum of patients with GTD, and to the best of our knowledge, the present study is the first to demonstrate the presence of hCG β cf immunoreactivity in their serum. Khan *et al.* (2000) failed to detect hCG β cf in the serum of patients with hydatidiform mole. This discrepancy is likely to be due to the difference in the specificity of the antibody against hCG β cf. The antibody used in their study was not specific to hCG β cf, but it cross-reacted 100% with free hCG β (Yoshimura *et al.* 1994), resulting in reduced ability to discriminate the hCG β cf molecule from hCG β , especially in the serum, and it seems that their hCG β cf assay underestimated the actual hCG β cf levels. Also, it is not surprising that using the technique of immunohistochemistry they observed only weak staining for hCG β cf in the syncytiotrophoblasts of molar tissue.

We previously showed that the level of hCG β cf immunoreactivity was suppressed in pregnancy serum although hCG β cf was abundant in the placenta (Udagawa *et al.* 1998), which is probably due to its short half-life in the serum (less than 5 min) as compared with that of intact

hCG (39 h) or free hCG β (4 h) (Wehmann & Nisula 1979, Wehmann *et al.* 1989). The present study demonstrated that this is also the case in the serum of patients with GTD. The mean ratio of hCG β cf to hCG was 60.8% in the molar tissue while it was 0.19% in the serum of patients with mole. In choriocarcinoma, the mean hCG β cf/hCG ratio was 509% in the tissue while it was 0.84% in the serum. Thus, the hCG β cf/hCG ratio in the serum was reduced to less than one hundredth of that in the tissue. As compared with normal pregnancy, the hCG β cf/hCG ratio in the serum of the patients with mole was comparable to that in pregnancy serum (0.10%–0.19%) (Udagawa *et al.* 1998), and the ratio was elevated in choriocarcinoma. The increased hCG β synthesis in choriocarcinoma seems to contribute to this phenomenon; the ratio of free hCG β to intact hCG in the tissue was significantly higher in choriocarcinoma than in mole (Table 2), and that ratio in the serum was also elevated in choriocarcinoma (Table 3). Consistent with these data, Ozturk *et al.* (1988) reported that, during normal pregnancy, the serum free hCG β /hCG ratio remained constant at approximately 0.5% after 5 weeks of gestation, while serum free hCG β levels were elevated in the patients with GTD, reaching more than 5% of hCG in choriocarcinoma cases.

Figure 5 Serum hCG β cf levels in the patients with hydatidiform mole after mole evacuation. (A) A patient (no. 4 in Table 3) with subsequent spontaneous remission. (B) A patient (no. 11 in Table 3) with subsequent persistent GTD.

Serum hCG levels are usually extremely elevated, occasionally exceeding 1 000 000 mIU/ml (corresponding to approximately 3000 pmol/ml), in the patients with hydatidiform mole at the time of mole evacuation and, due to its long circulating half-life, its levels gradually decrease after mole evacuation. Therefore, a relatively small amount of hCG secreted by the persistent trophoblasts may be masked by a large amount of remaining, circulating hCG derived from the evacuated molar tissue, resulting in a time lag before the rise of hCG levels becomes clinically manifest. Follow-up with assessment of hCG levels alone is thus often lengthy, requiring weeks or months to rule out persistent postmolar GTD. The present study showed that,

due to its short half-life, serum hCG β cf levels rapidly declined and became undetectable after uterine evacuation in the patients with hydatidiform mole with subsequent spontaneous resolution, while they remained elevated or started to rise before persistent GTD was diagnosed based on the rise of hCG levels. This means that detection of hCG β cf in the serum after mole evacuation may reflect persistence of viable trophoblasts more sensitively than hCG. On the other hand, once the chemotherapy was initiated, hCG β cf became undetectable more rapidly than hCG because of its relatively low levels in the serum and rapid clearance from the circulation as compared with hCG. Therefore, it seems that hCG β cf measurement may not be suitable for the follow-up of the patients receiving chemotherapy. Several indicators of high risk for persistent postmolar GTD have been proposed such as markedly elevated hCG levels, uterine size larger than gestational age, the presence of large theca lutein cysts (Curry *et al.* 1975), or the use of radiological methods (Kohorn *et al.* 1998), but the detection of any of these risk factors does not ensure the diagnosis of persistent GTD. Considering that, at present, measurement of hCG remains the most reliable means of identifying persistent postmolar GTD, monitoring of hCG β cf in the management of patients with hydatidiform mole merits further investigation.

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