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CA125 production by the peritoneum: in-vitro and in-vivo studies

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The source of CA125 synthesis is still debated. Endometrial, peritoneal, ovarian and amniotic cells have been demonstrated to produce and secrete CA125. Different studies show that the peritoneum is a source of CA125. The present study aimed at investigating *in vivo* and *in vitro* the peritoneal contribution to circulating CA125. Cultures of uterine peritoneum, abdominal peritoneum and myometrium explants were performed and CA125 measured in the culture medium. To modulate the potential production of CA125, the explants were cultured with or without cycloheximide, bacterial lipopolysaccharide (LPS) or ascitic fluid. In a prospective study, we compared a group of patients after abdominal surgery (n = 19; nine men, 10 women) with a group after extra-abdominal surgery (n = 21; 11 men, 10 women), in order to detect a postoperative increase of serum CA125. De-novo synthesis of CA125 could not be demonstrated in the cultures of uterine and abdominal peritoneum and in myometrium, but CA125 concentrations were detectable in the culture medium without being modulated by cycloheximide, LPS or ascitic fluid. After peritoneal surgery, the proportion of patients with increased serum CA125 was significantly higher ($P < 0.03$) after abdominal surgery as compared with extra-abdominal surgery. This is considered as indirect evidence for in-vivo production of CA125 by the peritoneum.

Key words: CA125/cell culture/myometrium/ovarian cancer/peritoneum

Introduction

Cancer antigen 125 (CA125) is an antigenic determinant recognized by a murine monoclonal antibody OC125 (Bast *et al.*, 1981). It has been used as a tumour marker for ovarian cancer since the description of high concentrations of CA125 in 80% of patients with epithelial ovarian cancer (Bast *et al.*, 1983). However, this elevation is not specific and has also been observed in many physiological and pathological conditions: during menses (Lehtovirta *et al.*, 1990; Bon *et al.*, 1999), endometriosis (Barbieri *et al.*, 1986; Pittaway and Fayez, 1987; Takahashi *et al.*, 1990; Garzetti *et al.*, 1994; Abrao *et al.*, 1997; Ho *et al.*, 1997), early pregnancy, pelvic inflammatory disease (Halila *et al.*, 1986; Paavonen *et al.*, 1989; Takahashi *et al.*, 1990), uterine fibroids (Bischof *et al.*, 1992), endometrial cancer (Duk *et al.*, 1988), gastro-intestinal cancer (Haga *et al.*, 1986; Bergmann *et al.*, 1987), breast cancer and other cancers. Monitoring CA125 concentrations is of limited clinical value in the screening and diagnosis of ovarian cancer, but CA125 concentration is a good marker for the follow-up of this disease.

CA125 is immunolocalized in the amnion and derivatives of fetal coelomic epithelia, decidua and placenta (Kabawat *et al.*, 1983). In adult tissues it is found in the female genital tract epithelium except on the surface of the ovary (Kabawat *et al.*, 1983; de Bruijn *et al.*, 1986). The epithelium of the pancreas, colon, gall bladder, stomach, lung, kidney, breast (Dietel *et al.*, 1986; Nouwen *et al.*, 1987), and endometriotic lesions is also positive for CA125 (Fedele *et al.*, 1988). Many body fluids such as milk, amniotic fluid, peritoneal or pleural fluid, cervical mucus and seminal fluid contain CA125 (de Bruijn *et al.*, 1986; O'Brien *et al.*, 1986; Bergmann *et al.*, 1987; Jacobs *et al.*, 1988; Schwartz *et al.*, 1989; Meisser *et al.*, 1996). Four sites of CA125 synthesis have been studied so far. Endometrial, decidual, amniotic and peritoneal cells have been shown to produce CA125 *in vitro* (Bischof *et al.*, 1986; Barbati *et al.*, 1990; Weintraub *et al.*, 1990; Zeimet *et al.*, 1997, 1998). The normal ovary does not seem to be an important source of CA125 production, since after ovarian stimulation CA125 concentrations do not increase and there is no CA125 concentration gradient from the ovaries to the peripheral circulation (Bischof, unpublished data). However, primary cultures of human ovarian surface epithelial cells secrete detectable but modest concentrations of CA125 (Zeimet *et al.*, 1998). Different studies suggest that the peritoneum is an important source of CA125. The antigen can be detected immunohistochemically in the mesothelial cells of peritoneum, pleura and pericardium (Kabawat *et al.*, 1983). Two studies (Zeillemaker *et al.*, 1994; Zeimet *et al.*, 1998) demonstrated CA125 secretion by monolayers of mesothelial cells in culture. The role of peritoneal inflammation in the increase of the marker is also suggested by pathological conditions such as ruptured ectopic pregnancy (Bischof *et al.*, 1989), pelvic inflammatory disease and endometriosis (Johansson *et al.*, 1998). Direct in-vitro evidence demonstrating the effects of inflammatory cytokines such as interleukin-1β (IL-1β) or tumour necrosis factor-α (TNF-α) on human peritoneal cells is controversial (Zeillemaker *et al.*, 1994; Zeimet *et al.*, 1998). Furthermore, elevated CA125 concentrations have been found after abdominal surgery: Redman *et al.* (Redman *et al.*, 1988) observed an elevation in peritoneal fluid but not in the sera,

Talbot *et al.* (Talbot *et al.*, 1989) found a postoperative CA125 increase after laparotomy in the sera of patients with normal preoperative concentration, while a third study (Yedema *et al.*, 1993) showed a significant CA125 increase in the serum after laparotomy for benign diseases and non-ovarian malignancies.

The purpose of the present study was to investigate *in vivo* and *in vitro* if the peritoneum is a source of CA125 and if it contributes to circulating CA125. In practical terms, we wanted to see if the increase in CA125 was higher after a peritoneal surgery and if the proportion of patients having an increased CA125 concentration was higher in patients having undergone a peritoneal surgery compared with non-peritoneal surgery.

Materials and methods

Tissue cultures

Uterine peritoneum and myometrium were obtained from patients undergoing hysterectomy for reasons other than malignancy. Abdominal peritoneum was obtained from women undergoing elective laparotomy.

After removal, the tissue pieces were minced with scissors, washed and placed in HBSS (Hanks' balanced salt solution, Sigma, St Louis, MO, USA) containing 200 IU/ml penicillin (Hoechst, Darmstadt, Germany), 200 µg/ml streptomycin (Hoechst) and 2.5 µg/ml fungizone (Gibco, Basle, Switzerland).

Explants of uterine and abdominal peritoneum, and myometrium were cultured in RPMI 1640 (Merck, Darmstaadt, Germany) containing 10% FCS (fetal calf serum) (Animed, Basel, Switzerland), 100 ng/ml streptomycine, 0.1 IU/ml penicillin and 2.5 ng/ml fungizone (referred to as complete RPMI hereafter). To mimic bacterial infection, complete RPMI was supplemented in some experiments by 100 µg/ml lipopolysaccharides (LPS; Sigma, Buchs, Switzerland). In other experiments, ascitic fluid (30% v/v) was added to complete RPMI to mimic cancer. Finally, complete RPMI was also supplemented sometimes with cycloheximide (10 µg/ml, Sigma) to inhibit protein synthesis.

Media were collected on days 2, 5 and 8 and CA125 concentration determined in duplicate in the supernatants by an immunoradiometric assay kit (CIS Biointernational, Saclay, France) according to the instructions of the manufacturer. Total proteins were measured in each supernatant with the protein Biorad kit (Biorad, Munchen, Germany) using bovine serum albumin as the standard. The concentration of CA125 in media supplemented with ascitic fluid but in absence of tissue was subtracted from the CA125 concentrations found in the culture supernatants supplemented with ascitic fluid and in presence of tissue.

Results were expressed as units of CA125 per mg total protein. Statistical analyses were performed on log transformed values by analysis of variance (ANOVA) and paired *t*-test (when appropriate) using the Statview program (Abacus, Berkeley, CA, USA).

Prospective study

In a prospective study we analysed a population of 40 patients admitted for surgery under general anaesthesia. The first group included nine men and 10 women undergoing abdominal surgery with peritoneum opening. Cases with endometriosis or ovarian cancer were excluded. The second group included 11 men and 10 women undergoing surgical intervention elsewhere. Data concerning age, sex, preoperative diagnosis, type of surgery, anatomicopathological diagnosis, length of the anaesthesia and the operation were recorded. A 5 ml blood specimen on heparin was obtained 24–48 h preoperatively and 48 h postoperatively from each patient. After centrifugation (10 min at 1500 *g*), plasma samples were stored at –20°C until assayed. CA125 concentration was determined in duplicate by the same immunoradiometric assay as described above. The change between pre- and postoperative CA125 concentrations was calculated and log transformed. Statistical analyses were performed by Student's *t* and χ^2 tests.

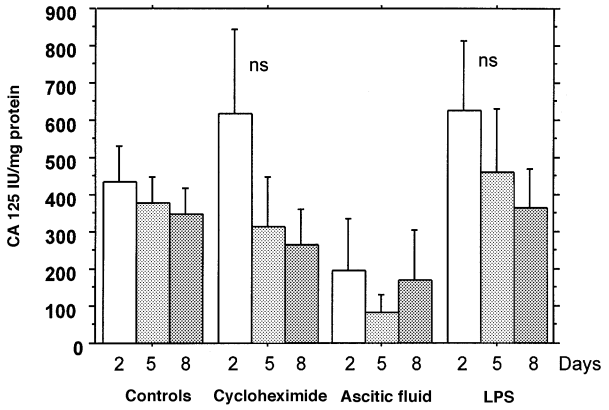

Figure 1. CA125 concentrations in uterine peritoneal explant cultures ($n = 12$) according to the duration of incubation. Controls were cultured in complete RPMI (see Materials and methods). Explants were also cultured in RPMI supplemented with 10 µg/ml cycloheximide ($n = 12$) with 30% v/v ascitic fluid ($n = 9$) and with 100 µg/ml lipopolysaccharides (LPS; $n = 4$).

Results

Tissue cultures

In the uterine peritoneal explant cultures ($n = 12$), we observed CA125 concentrations varying between 83.9 ± 135.1 and 627.8 ± 367.9 IU/mg protein (mean ± SD) according to the days of culture and the treatments. Irrespective of the culture conditions, CA125 concentrations gradually decreased with the time of incubation. However, no viability test was done to exclude the possibility that this decrease was due to cell death. The release of CA125 in cultures of uterine peritoneal explants was not significantly changed by the presence of cycloheximide (a protein synthesis inhibitor) ($n = 12$; Figure 1). Ascitic fluid ($n = 9$) or lipopolysaccharides ($n = 4$) did not change significantly the CA125 concentrations as compared to complete RPMI alone (Figure 1).

Cultures of abdominal peritoneal explants ($n = 11$), uterine peritoneal explants ($n = 12$) and myometrial explants ($n = 9$) released similar CA125 concentrations. The concentrations of CA125 were not significantly different between the tissues for the same incubation time (Figure 2). Similarly to uterine peritoneal explants, the presence of cycloheximide did not change significantly the CA125 concentrations released by myometrial explant cultures ($n = 9$, Figure 3).

Prospective study

The prospective study included two groups of patients: the first group (nine men and 10 women) having abdominal surgery with opening of the peritoneum and the second (11 men and 10 women) with extraperitoneal surgery. Details of the patients and the corresponding CA125 concentrations are given in

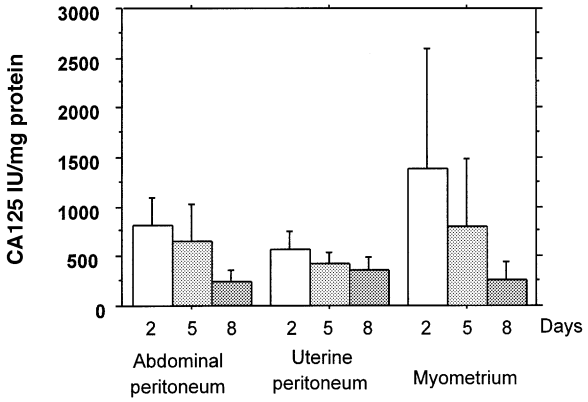

Figure 2. CA125 concentrations in cultures of abdominal ($n = 4$) and uterine ($n = 12$) peritoneal explants and myometrial explants ($n = 9$), according to the duration of incubation.

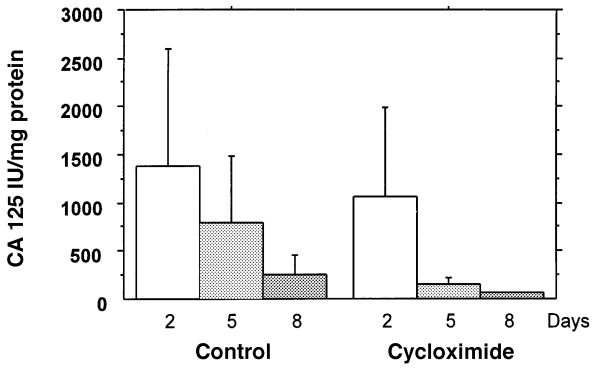

Figure 3. CA125 concentration in cultures of myometrial explants ($n = 9$) supplemented or not with 10 μg/ml cycloheximide.

Table I. Of the 40 patients, seven (three men and four women) had a preoperative CA125 concentration above 35 IU/ml and were excluded from further analysis as discussed below.

Table II shows the characteristics of the remaining patients with a preoperative CA125 <35 IU/ml. The groups are comparable in age. The mean postoperative value of CA125 in the group 'peritoneal surgery – men' was high (55.8 IU/ml ± 63.2) with a high standard deviation. This is due to one case of gastrojejunal anastomosis cancer with a postoperative CA125 concentration of 167.11 IU/ml. The postoperative CA125 values were not significantly different from the preoperative ones when considering the different groups (peritoneal surgery or extraperitoneal surgery in men and/or women). Since the CA125 assay had an interassay coefficient of variation of 5.5%, we counted the number of patients who had a postoperative CA125 concentration above the preoperative concentration +5.5% and considered this increase as significant. With this approach, 53.3% of patients had an increased postoperative CA125 in the group of peritoneal surgery whereas only 16.7% of the patients had such an increase in the group of extraperitoneal surgery. This was statistically significant (χ^2 test, $P < 0.03$). Analysing the female subgroup for the same parameters, 33% of women with peritoneal surgery had a higher postoperative CA125 concentration against 0% in the extraperitoneal group ($P < 0.03$). In the male group, we found 83.3 against 27.3% ($P < 0.05$).

Discussion

We could not demonstrate in-vitro de-novo synthesis of CA125 under our experimental conditions since an inhibitor of protein synthesis such as cycloheximide did not change the concentrations of CA125 found in the incubation medium. Addition of cycloheximide has been shown to decrease significantly the concentrations of CA125 secreted in the medium by explants of decidua (Bischof *et al.*, 1986). Our results suggest that uterine and abdominal peritoneum, and myometrium are not a source of CA125 production. In the explant cultures we noticed that the CA125 concentrations decreased progressively with the time of incubation. This can be explained either by inhibition of de-novo production of the antigen in our culture conditions or by a release of stored CA125. It must be added, however, that the interpretation of these data is somewhat difficult because the half-life of CA125 *in vitro* is unknown and the viability of the explants in culture could not be assessed.

Our observations seem to exclude the possibility that these tissues actively produce CA125 *in vitro*. Different inflammatory situations such as pelvic inflammatory disease, endometriosis (especially with adhesions), malignant pathologies with ascites and peritonitis are known to induce high circulating concentrations of CA125. In order to mimic cancer or an inflammation, peritoneal explant cultures were performed in medium supplemented with ascitic fluid or LPS, speculating that these two substances could stimulate potential CA125 production. Even under these conditions, we do not find a significant effect on the CA125 release. This observation is in contrast with previous results (Zeillemaker *et al.*, 1994). These last authors cultured mesothelial cells for 6 h and observed that the secretion of CA125 by cells grown in medium with LPS, IL-1β or TNF-α was statistically higher than in unstimulated cells. However, the kinetics of the two studies are different: we measured CA125 after 2–8 days. This could perhaps explain the different results. The difference in the culture medium and the use of antibiotics certainly does not explain our negative results.

Among the 40 patients included in the prospective study, seven had a preoperative CA125 >35 IU/ml associated with malignant (pancreas, lymphoma, gastric and breast) and benign (uterine fibroid, thyroid) conditions. This underlines the lack of specificity of this marker for ovarian cancer. We excluded these cases from further analysis in order to avoid a possible decrease in postoperative CA125 due to tumour removal, as shown previously (Yedema *et al.*, 1993). Although our sample size was limited, we observed a significant difference in the proportion of patients who had a postoperative increase of CA125 when patients with abdominal and extra-abdominal surgery were compared. This suggests that, *in vivo*, the peritoneum might contribute to circulating CA125 concentrations. These results confirm earlier studies (Talbot *et al.*, 1989; Yedema *et al.*, 1993) in which a postoperative increase of CA125 after laparotomy was observed. In both studies, CA125 measurements were performed between 4 and 28 days after surgery and the maximal rise in CA125 was detected 2–4 weeks postoperatively (Talbot *et al.*, 1989). Our postoperative

Table I. Diagnosis, preoperative and postoperative CA125 concentrations in 40 men and women undergoing peritoneal or extraperitoneal surgery

Men			Women		
Diagnosis	CA125 preop (IU/ml)	CA125 postop (IU/ml)	Diagnosis	CA125 preop (IU/ml)	Ca125 postop (IU/ml)
Peritoneal surgery			**Peritoneal surgery**		
Pancreatic cancer	333.4	208	Myoma	117.6	43.6
Malignant lymphoma	99.1	84.7	Serous ovary cystadenoma	31.6	27.5
Gastric sarcoma	66.3	43.1	Colic invagination	30.5	43.8
Gastrojejunal anastomosis carcinoma	27.4	167.1	Endometrial carcinoma	30	19.2
Colon carcinoma	16.2	22.4	Adenomyosis	24	19.8
Sigmoiditis	15	97.3	Adenomyosis	18	20.8
Peritoneal pseudomyxoma	14.8	12	Colon carcinoma	13.6	25.2
Rupture of abdominal scar	11.4	17.8	Serous ovary cyst	11	9.5
Abdominal arterial bypass	6	18.4	Stomach carcinoma	9.8	6
			Myoma	9.8	8.2
Extraperitoneal surgery			**Extraperitoneal surgery**		
Prostatic hyperplasia	23.6	21.4	Thyroid hyperplasia nodular	69.5	56.1
Carotid stenosis	23.2	19.1	Breast cancer	43.8	31.4
Dysphagia	15.8	16.9	Breast cancer	42.6	33.9
Occlusive arterial disease	13.6	10	Femur and wrist fracture	30.8	27.7
Spinal stenosis	11.6	18.2	Multinodulary goitre	14.2	13
Spinal stenosis	10.4	6.9	Papillary thyroid cancer	12.2	11.6
Acromial syndrome	8	6.5	Shoulder synovitis	9.6	7
Occlusive arterial disease	7.9	7.3	Breast cancer	8.3	8.2
Revision of sternum	7.2	6.8	Occlusive arterial disease	8	8.4
Spinal stenosis	5.2	11.9	Breast cancer	7.9	6.4
Varicose veins	4.9	3.8			

Preop = preoperatively; postop = postoperatively.

Table II. Characteristics of patients with preoperative CA125 concentration <35 IU/ml

	Peritoneal surgery		Extraperitoneal surgery	
	Men (n = 6)	Women (n = 9)	Men (n = 11)	Women (n = 7)
Mean age (years, SD)	63 ± 16	57.1 ± 12	59.6 ± 11	59.9 ± 16
Length of surgery (mean, SD)	179 ± 150	118 ± 43	165 ± 136	130 ± 9.1
Length of anaesthesia (mean, SD)	216 ± 154	149 ± 40	205 ± 150	172 ± 89
Preoperative CA125 (IU/ml, SD)	15.1 ± 7	19.8 ± 9.3	12 ± 6.5	13 ± 8.2
Postoperative CA125 (IU/ml, SD)	55.8 ± 63.2	20 ± 11.6	11.7 ± 6.4	11.8 ± 7.4
Postoperative–preoperative CA125 (IU/ml, SD)	40.8 ± 57.6	0.2 ± 7.8	–0.27 ± 3.7	–1.2 ± 1.3

measurements were done after 48 h and an increased CA125 concentration, although not statistically significant, could already be observed. This marginal increase is taken as indirect evidence of the peritoneal contribution to circulating CA125 concentration. It must be admitted, however, that in the five cases with abdominal tumours, the contribution of tumour manipulation to increased postoperative CA125 concentrations cannot be excluded.

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