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CANCER ANTIGEN 1251S LOCALLY PRODUCED IN THE PERITONEAL CAVITY DURING CONTINUOUS AMBULATORY PERITONEAL DIALYSIS

Ger C.M. Koomen,1,2 Michiel G.H. Betjes,3 Oésirée Zemel,2 Raymond T. Krediet,2 and Frans J. Hoek1

Departments of Clinical Chemistryl and Nephrology,2 Academic Medical Center, University of Amsterdam,. Department of Cell Biology,3 Free University, Amsterdam, The Netherlands

The local production of cancer antigen (CA) 125 in the peritoneal cavity of 14 continuous ambulatory peritoneal dialysis patients was studied. In addition, the relationship between the concentration of mesothelial cells and CA 125 in the peritoneal dialysate effluent was examined. The median results and ranges were as follows: plasma CA 125 14 U/mL (range 10 23), dialysate CA 125 18 U/mL (range 5.2 76), dialysate/plasma ratio 19 (range 0.61 -5.4), and number of mesothelial cells 400/mL (range 10 5000). Peritoneal concentrations of mesothelial cellsand CA 125 were positively correlated ($r = 0.50$, $p < 0.01$). Using a monoclonal antibody, CA 125-positive cells were found in the cytospin preparations of the cells of dialysis effluents. All these CA 125 positive cells were also positive for cytokeratin used as a mesothelial cell marker. *In vitro* experiments using mesothelial cells in monolayers showed a linear increase in CA 125 concentration both in time and in relation to the number of mesothelial cells. From these experiments a production rate of 24 U/hour/1 06 cells could be calculated.

It is therefore concluded that CA 125 is locally produced in the peritoneal cavity during CAPD and that the mesothelial cells are the major source of this CA 125.

KEY WORDS: Cancer antigen (CA) 125; mesothelial cells; peritoneum.

The development of the monoclonal antibody, OC125, directed against a glycoprotein produced by human epithelial ovarian cancer cells was reported by *Bastet al.* in 1981 (1). They showed that this antibody reacts with an antigenic determinant, termed cancer antigen (CA) 125, that is expressed on more than 80% of all nonmucinous epithelial ovarian tumors (1,2). However, it has become clear that CA 125 is not a tumor-specific antigen. Its presence has been demonstrated in fetal as well as adult normal tissues derived from coelomic epithelium. For example, CA 125 is present in breast ducts, bronchial epithelium and glands, in the luminal surfaces of the gall bladder, and in mesothelial cells lining the pleura and peritoneal cavity (3,4). CA 125 could also be detected in normal peritoneal fluid obtained in women undergoing laparoscopyfor evaluation ofinfertility(5).

Mesothelial cells line the entire peritoneal cavity. For this reason these cells are the most important intraperitoneal cells quantitatively. The mesothelial cell has the capability of secreting different types of substances. Both lubricants (phospholipids and glucosaminoglycans) and agents which suppress adhesion (prostacyclin and tissue plasminogen activators) are produced (6). Also, increased production of cytokines and prostanoids by cultured mesothelial cells has been shown after stimulation with supernatants derived from peritoneal macrophages (7). It may therefore be of importance to investigate the *in viva* role of the peritoneal mesothelial cells during peritoneal dialysis.

In this study we provide evidence that the mesothelial cell produces CA 125 during continuous ambulatory peritoneal dialysis (CAPD). To determine the origin of the CA 125 in the dialysate, we did two types ofinvestigations. First, we studied dialysate/ plasma ratios ofCA 125 in CAPD patients to find out whether its concentration in the effluent was the result of transperitoneal transport from the circulation or of local production in the peritoneal cavity. Second, we analyzed whether the observed local production could be attributed to mesothelial cells.

MATERIALS AND METHODS

SOURCE OF PATIENT MATERIAL

Twenty-four overnight peritoneal dialysate samples of 14 CAPD patients (10 male, 4 female) were

Correspondence to: G.C.M. Koomen, SLIC FO-116, Department of Clinical Chemistry , Academic Medical Center , Meibergdreef9, 1105 AZ Amsterdam, the Netherlands.

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collected. The dialysate samples of 4 patients were collected twice and of 3 patients three times within one month. From each patient one plasma sample was obtained at the time of the peritoneal night exchange. The mean age of the patients was 48 years (range 23 69 years). Duration of time on CAPD prior to the study ranged from 4 107 months (mean 26 months). The duration of the night exchanges and the drained volumes were measured.

METHOD

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All samples were stored at -20°C until analysis. The concentration of CA 125 was measured with a commercial microparticle enzyme immunoassay based on the monoclonal antibody against CA 125 (Abbott IMx, North Chicago, U.S.A.). The method was validated for dialysate by comparing dilution ranges of plasma and dialysate for parallelism. The agreement with the standard curves was good; therefore, the measurements were performed without any pretreatment of the samples, except centrifugation at *1500g* for 10 minutes. The tota] number of cells was counted using a Bürker counting chamber. A differential cell count was performed on cy to spin preparations (centrifugation for 5 minutes, *50g,* 23°C) after J enner-Giemsa staining by examining 400 cells as described previously (8).

Human mesothelial cells were obtained by enzymatic digestion of pieces of human omentum as described by Stylianou *et al.* (9), and cultured as described by *Betjeset al.* (10). The CA 125 production by mesothelial cells *in vitra* was estimated by culturing various numbers of these mesothelial cells for different periods of time. Two types of experiments were done. In the first one, 50000 cells were cultured for various periods ranging from 1 -8 days. In the other, various numbers of cells ranging from 50000 800000 were cultured for 48 hours. The supernatant of 1 mL was taken at the end of the incubation period, and CA 125 was determined as described above.

To further investigate whether the CA 125 was of mainly mesothelial origin, cells of drained dialysates of 5 patients were concentrated by centrifuging 50 mL at *1500g* for 5 minutes. From this concentrated cell suspension cytospin preparations were made as described above. The cell precipitates of these drained dialysates and the cells of human omentum were incubated with the monoclonal CAM 5.2 of the IgG2a subclass directed against cytokeratin (DAKO-CK1, Dakopatts, Copenhagen, Denmark), a protein predominantly occurring in mesothelial cells, and with the monoclonal OC125, of the IgGla subclass against CA 125 (International CIS, Gif-sur-Yvette, France). After a wash step, this was followed by incubation with peroxidase and alkaline phosphatase-labeled antibodies directed against the respective IgG subclasses. *After* a second wash step, the two color reactions were performed.

STATISTIC

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A first analysis showed that the distribution of CA 125 values in the dialysates was not normal. Consequently, we adopted nonparametric tests. The Wilcoxon test for paired observations was used to compare plasma and dialysate CA 125 concentrations. The Spearman rank test was used to test the correlation between the number of mesothelial cells per milliliter and the concentration of CA 125 in the draineddialysates.

RESULTS

The results of the CA 125 concentrations in dialysate and plasma are given in Table 1 as well as the number of mesothelial cells per milliliter found in the dialysate. The CA 125 value in dialysate appeared to be significantly higher than the paired plasma value ($p < 0.05$, $n = 14$). This finding is also reflected in a median dialysate/plasma ratio of 1.9. In the 7 patients in which the dialysate CA 125 measurement was repeated within one month, a mean variation in CA 125 concentration of 23% (range 5% -43%) was calculated. The observed range of plasma values of 10 23 U/mLis within the normal range, which is up to 35 U/mL. A good correlation was found between the number of mesothelial cells per milliliter and the CA 125 concentration in the dialysate, as shown in Figure 1. The Spearman rank test gave a correlation coefficient of 0.50 ($n = 24$, $p < 0.01$). The double labeling with CAM 5.2 and OC125 showed that the cells of the effluents that were positive for CA 125 were also positive for cytokeratin. The amount of cytokeratin positive cells, also positive for CA 125 was between 50% and 80%. In addition, it appeared that about 80% of the mesothelial cells obtained from omentum contained antigens which reacted with OC125. A preparation in which all cell nuclei were

TABLE 1
The plasma and dialysate concentrations and the D/P ratio of CA 125. In addition, the number of mesothelial cells per milliliter effluent is given.

	Median Value	Range
Plasma CA 125 (U/mL) (N = 14)	14	10–23
Dialysate CA 125(U/mL) (N = 24)	18	5.2–76
Dialysate/plasma ratio	1.9	0.61–5.4
Mesothelial cells	400	10–5000

stained in addition to the CA 125 labeling is shown in Figure 2. Most of the mesothelial cells contained CA 125 antigens, although there was a variability in the intensity of the staining between the cells. The predominant localization of the antigen appeared to be on the membrane of the cells.

Data on *in vitra* production of CA 125 were obtained in three ways. First, for 50000 mesothelial cells, obtained from omentum, the incubation time in the medium was varied from 1 to 8 days and the CA 125 increase was measured (Figure 3, left panel). A linear increase over the tested time range was observed. Second, the number of mesothelial cells per milliliter of incubation medium was varied from 50000 to 800000, while the incubation time was fixed at 48 hours. Again, a linear increase in the CA 125 production was observed (Figure 3, right panel). The

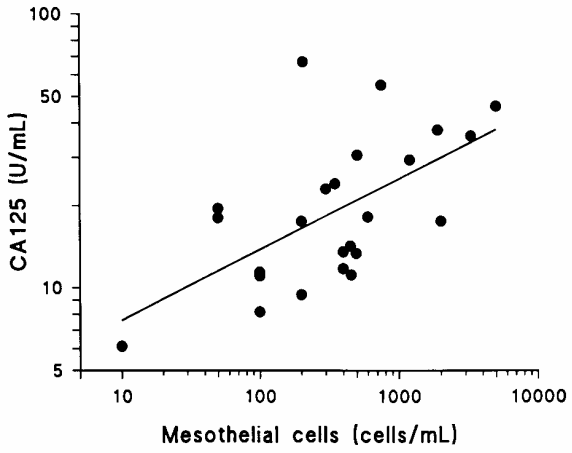

Figure 1 — The relationship between the number of mesothelial cells per milliliter and the concentration of CA 125 in peritoneal dialysate.

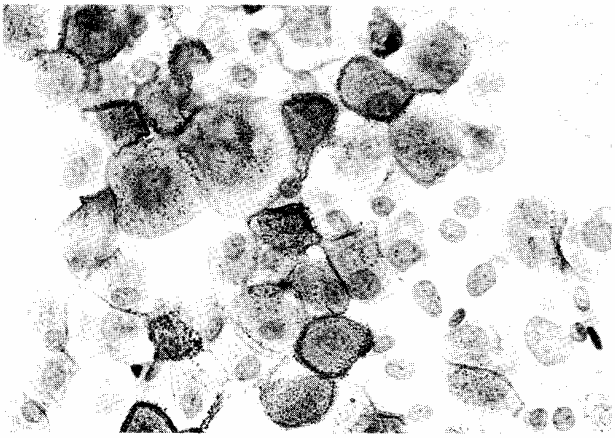

Figure 2 — Mesothelial cells isolated from human omentum. The CA 125 positive cells are stained. All cell nuclei are stained with hematoxylin.

mean production rate calculated for these experiments was 24 U/hour/106 mesothelial cells. Third, from the night exchange dialysate of 2 CAPD patients all cells were concentrated as described in the Methods section and 500 000 of these cells were incubated with 1 mL of medium for 24 hours. The CA 125 production in this period was 15 U. For both dialysates 3% of the cells were mesothelial cells according to the differential cell counts. Therefore, the incubations were performed with 15000 mesothelial cells, resulting in a CA 125 production rate of 40 U/hour/106 mesothelial cells. This production rate, although somewhat higher, is still in the same range as the production rate calculated for mesothelial cells obtained from omentum.

DISCUSSION

In the present study evidence was obtained that CA 125 in drained dialysate of CAPD patients is produced by the mesothelium. *Anin vitra* production rate of24 U/hour/106 mesothelial cells could be calculated.

The mesothelial cells lining the peritoneum are able to secrete a large number of substances, such as phosphatidylcholine, proteoglycans, prostacyclin, and tissue plasminogen activator (6). Therefore, the mesothelial cell is likely to be important for the function of the peritoneal membrane during peritoneal dialysis. Cultured mesothelial cells also appeared to be able to synthesize the neutrophil chemoattractant IL-8, after stimulation with either Il-lβ orTNFα(11). Muijsken *et al.* showed *in vitra* that the surface of peritoneal mesothelial cells provides favorable conditions for the elimination of staphylococci (12). Therefore, the peritoneal mesothelium is probably also important in host defense mechanisms against bacteria. This is possibly the explanation for the inverse relationship between the number of mesothelial cells in the effluent ofCAPD patients and their peritonitis incidence (13,14). All these findings underline the potential importance of the peritoneal mesothelial cell during peritoneal dialysis. Therefore, a readily measurable parameter that is a reflection of the mesothelial mass or activity will be of great help for the *in viva* investigations of the role of the mesothelium.

Our *in viva* results show that CA 125 in the dialysate must have been produced locally. The mean plasma concentration was significantly lower than the dialysate concentration, so diffusion from plasma cannot be the main source of the dialysate CA 125. Even the lowest estimation of the molecular weight of the CA 125 glycoprotein of approximately 200 kilodalton (15) makes it evident that diffusion cannot contribute more than 1% or 2% to the total CA 125

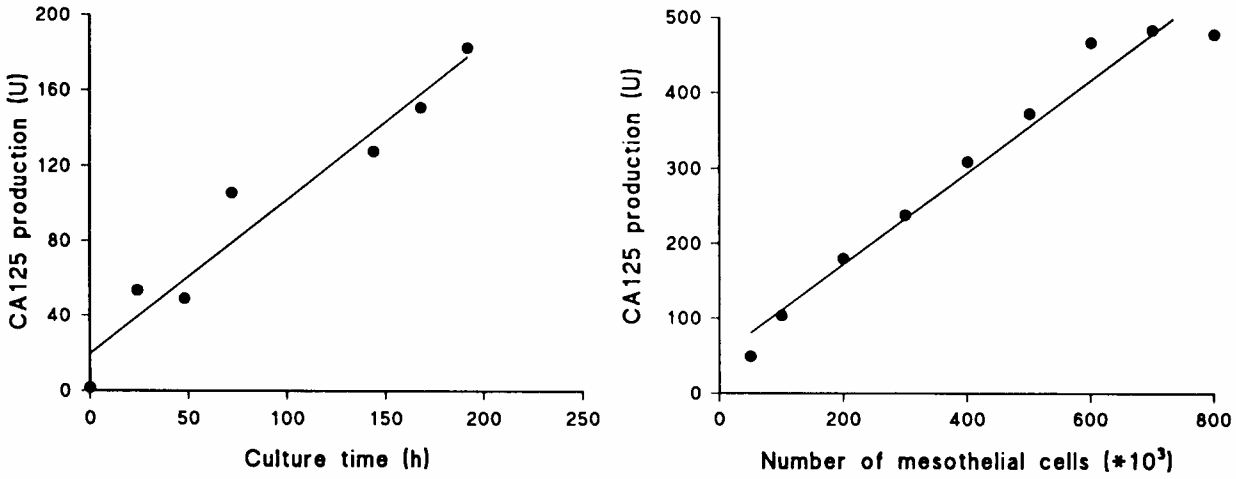

Figure 3 — The CA 125 production by mesothelial cells. The production in time by 50 000 cells is given in the left panel ($r = 0.97$, $p < 0.01$). The 48-hour production by various numbers of mesothelial cells is shown in the right panel ($r = 0.98$, $p < 0.01$).

concentration found in the dialysate. This percentage is calculated with the assumption that CA 125 diffuses maximally with the same rate across the peritoneal membrane as the smaller IgG protein (Mw = 155 kilodalton, clearance 0.05 mL/minute) (16). During a night exchange of10 hours with2 L and a CA 125 plasma concentration of14 U/mL, diffusion would result in a dialysate concentration ofo.2 U/mL. The *in vitra* experiments show that the peritoneal mesothelial cells can account for this local production of CA 125. The cells of both the omentum and the effluents were positive for cytokeratin, as well as for CA 125. Because cytokeratin can be considered to be a specific identification marker for mesothelial cells in these cases (7), it can be concluded that the CA 125 positive cells are also mesothelial cells.

Production of CA 125 by peritoneal mesothelial cells is not found exclusively during CAPD. Kabawat *et al.* were able to show the CA 125 positive determi nant on mesothelial cells lining the human peritoneum especially in areas of inflammation and adhesion (3). Elevated concentrations ofCA 125 have also been found in ascites in patients with malignant, but also with nonmalignant ascites, resulting from liver disease (17,18). In these cases the plasma levels were well above lOO U/mL. Interestingly, paracentesis of ascites was followed by a marked decrease in plasma CA 125 levels (19), suggesting that the increased plasma levels ofCA 125 during ascites are the result ofCA 125 production in the peritoneal cavity.

No mechanism by which elevated CA 125 levels in ascites can lead to increased plasma concentrations was given in the above-mentioned studies. During CAPD, a continuous convective flow of fluid and intraperitoneally administered solutes out of the peritoneal cavity is present (20,21). The magnitude of this lymphatic absorption rate ranges from 0.1 3.0 mL/minute, with a median of 1.0 mL/minute (22). Assuming a mean peritoneal dialysate CA 125 concentration of9 U/mL over the day, which is half the median night exchange value, and a lymphatic flow of 1 mL/minute, a flow of 9 U CA 125/minute into the plasma can be calculated. The reported half-life time of CA 125 is 4.8 days in humans (23). When we assume that the distribution volume of CA 125 is equal to that of albumin, which is 6.7 L for a CAPD patient of 70 kg (24), it is possible to calculate the elimination rate for CA 125. This gives a value ofo.67 mL/minute usingfirst-order kinetics. With the elimination rate of 0.67 mL/minute and the influx of 9 U/ minute a plasma equilibrium concentration of9/0.67 = 13 U/mL can be calculated. The measured median plasma concentration in our patients was 14 U/mL. This implies that the measured plasma levels in CAPD patients can roughly be explained by this mechanism.

The good correlation between the concentration of CA 125 and mesothelial cells in the dialysate might suggest that the CA 125 concentration in the dialysate in a given patient was produced by the drained mesothelial cells only. However, the data obtained *in vitra* on the production rate ofCA 125 by the drained mesothelial cells make it unlikely that more than 1 % of the CA 125 in the dialysate is produced by the mesothelial cells in the effluent. The calculated *in vitra* production rate of 24 U/hour/106 mesothelial cells means that with a median value of 400 mesothelial cells per milliliter in a night exchange of 10 hours, only 0.1 U/mL CA 125 is to be expected. We measured a median concentration of18 U/mL. There

fore, we assume that the mesothelial cells lining the peritoneal cavity are the major source of CA 125 during CAPD. The *in vitra* results show a linear relation between the number of mesothelial cells and the amount ofCA 125 secreted. Therefore, it is tempting to speculate that the amount ofCA 125 drained is a reflection of the mesothelial mass in the peritoneal cavity. However, further investigations are needed to analyze whether this holds true in the *in viva* situation. **If** so, CA 125 can be useful in the investigation of the role of the peritoneal mesothelium during CAPD.

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