Characterization of CA 19-9 Bearing Mucins as Physiological Exocrine Pancreatic Secretion Products

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ABSTRACT

The monoclonal antibody CA 19-9 reacts with a carbohydrate epitope (sialylated lacto-N-fucopentaose II), which was shown to be a part of a ganglioside extracted from a colon carcinoma cell line as well as of a mucin isolated from gastrointestinal tract tumor patients' sera.

Recently, when we compared CA 19-9 levels in pancreatic juices and corresponding serum samples from a large group of patients, we showed the high serum values to be indicative solely for a malignant disease. In contrast, the overall high CA 19-9 content in pancreatic juices from all diagnostic groups raised the question about the antigenic moieties in these samples.

By means of thin layer chromatography of glycolipids with subsequent antibody overlay, gel chromatography, and density gradient analysis, we found only the mucin form in all sources investigated. Thus we conclude that the discrimination potential of the CA 19-9 assay in serum is based on an altered secretion or distribution in pancreatic tumors.

INTRODUCTION

It is well established that carbohydrate residues, recognized by monoclonal antibodies, can be found on different "carriers," i.e., glycoproteins and glycolipids (1–3). One recent example is given by the gastrointestinal cancer associated antigen known as CA 19-9 (4, 5). The epitope recognized by mab 1116 NS 19 (6) was defined as sialylated lacto-N-fucopentaose II, which was initially found to be a component of a ganglioside extracted from a human colon carcinoma cell line (7, 8) and later to be linked to glycoproteins (9) and mucins as well. The latter antigenic form was proven to be the cancer-associated antigen in the sera of gastrointestinal tract tumor patients (10), which by means of commercially available radioimmunoassays has been widely used in the diagnosis and monitoring of gastrointestinal tract malignancies.

Previously, we compared the CA 19-9 content in pancreatic juice and serum samples from different groups of patients. In contrast to specific high CA 19-9 levels in the serum samples of the pancreatic cancer group, pancreatic secretion analyses revealed high CA 19-9 contents in pancreatic cancer patients, as well as in chronic pancreatitis patients and in controls (11, 12). These findings raised the question of whether all antigenic moieties can be traced in pancreatic secretions of all diagnostic groups or whether the secretion of the glycolipid or mucin is characteristic for the malignant state. To answer these questions we took an equivalent approach as described for serum samples (10) to study pancreatic juice samples from different sources all containing high levels of CA 19-9.

MATERIALS AND METHODS

Pancreas secretion- and serum-samples were obtained from patients of three diagnostic groups formed on the basis of standard diagnostic procedures (12):

Group I—cancer of the exocrine pancreas
Group II—chronic pancreatitis
Group III—control group consisting of patients in whom a pancreatic or other gastrointestinal tract disorder was excluded.

Two sera of group I and three pancreatic juices of the respective diagnostic groups were mixed each and diluted in CA 19-9 negative pooled serum of healthy donors to a final CA 19-9 concentration of 2000 units/ml.

125I-anti-CA 19-9 monoclonal antibody (maximal radioactivity, 0.35 μCi/ml) was provided by Centocor, Malvern, Pa. 125I-sheep anti-mouse IgG antibodies from Amersham/Buchler, Braunschweig, Federal Republic of Germany, were subjected to Bio-Gel P4 column chromatography for further purification.

Total Lipid Extraction and Thin Layer Chromatography. Total lipid extracts from pancreatic juices and sera were prepared as described previously (13), using samples of 2.0 ml, which were mixed with 5.0 ml of methanol and 2.5 ml of chloroform. Reextraction was done after resuspending the pellet in 1.0 ml H2O and 7.5 ml of chloroform-methanol (1:2 by volume). The finally yielded material was dissolved in 2.5 ml of chloroform, methanol, and water (60:30:4.5 by volume). Total lipid extract from colon carcinoma SW 1116 cells was a generous gift from V. Ginsburg (NIH, Bethesda, MD). Reference gangliosides and glycolipids (globoside, monosialoganglioside, disialoganglioside, trisialoganglioside, and ceramide trihexoside) were from Supelco, Inc., Bellefonte, PA. Samples of 10 μl were applied to thin layer chromatography with subsequent antibody overlay as described (14).

Gel Chromatography. Sephacryl S-400 (Pharmacia, Upsala, Sweden) column preparation and fractionation of serum and pancreatic juice samples were done according to the method of Magnani et al. (10). Fractions (3 ml) were collected and assayed for CA 19-9 by solid phase bioimmunoassay as well as by DDIA.1

Density Gradient Ultracentrifugation. Ultracentrifugation of the CA 19-9 positive pooled fractions obtained by gel chromatography was done in 5 ml CsCl isopycnic density gradients in phosphate-buffered saline, pH 7.4, containing 4 μg guanidine-HCl (10). Gradients were formed by centrifugation in a Beckmann SW 65 Ti rotor at 49,000 rpm for 68 h at 10°C. Fractions of approximately 0.27 ml were collected and 30-μl volumes were assayed for CA 19-9 by solid phase radioimmunoassay.

Radioimmunoassay. In solid phase radioimmunoassay (10) 125I-anti-CA 19-9 monoclonal antibody was applied as a direct detection system. DDIA (15) was handled according to the manufacturer's recommendations (Centocor, Malvern, PA) to determine CA 19-9 activity in pancreatic juice and serum samples, as well as in fractions collected from gel chromatography.

RESULTS

After total lipid extraction of the CA 19-9 positive colon carcinoma cell line SW 1116 and pancreatic juice samples from three diagnostic groups (group I, pancreatic cancer; group II, chronic pancreatitis; group III, control), each sample containing CA 19-9 (2000 units/ml) as determined by DDIA, thin layer chromatography with 125I-CA 19-9 antibody overlay revealed no similarities in the autoradiographic staining between glycolipids extracted from colon carcinoma cells SW 1116 and the

1 The abbreviation used is: DDIA, double determinant immunoassay.

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2 To whom requests for reprints should be addressed.

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three pancreatic secretion samples (Fig. 1). The same chromatogram using highly purified 125I-sheep anti-mouse IgG antibody overlay as control showed an identical staining pattern in lanes 2–5, thereby showing that the material running in the position of GM₁ (Fig. 1, lane 1) is exclusively specific for CA 19-9 antibody.

In an additional experiment using the same samples as in the thin layer chromatography in an indirect solid phase immunoassay replacing the iodinated by an unlabeled CA 19-9 antibody in combination with an enzyme labeled anti-mouse-IgG antibody, we obtained a strong signal (extinction 1.8) with the same amount of SW 1116 total lipid extract as in thin layer chromatography, whereas even a 10-fold amount of the three pancreatic juice total lipid extracts was completely negative.

To determine the molecular size(s) of the CA 19-9 antigen(s) in pancreatic juices, we subjected samples from group I, II, and III patients and serum from a pancreatic tumor patient serving as a reference sample to Sephacryl S-400 column chromatography. As shown in Fig. 2, the CA 19-9 positive fractions as determined by solid phase radioimmunoassay eluted in the void volume of the column, indicating a molecular weight of at least $2 \times 10^6$. The same result was obtained using the DDIA (data not shown).

In order to rule out aggregation of mono-sialo-gangliosides eventually mimicking high molecular weight antigens with repetitive determinants (mucins) we performed CsCl-density gra-

dient centrifugation in the presence of 4 M guanidine-HCl, using the pooled peak fractions from the Sephacryl S-400 column. All three pancreatic juice samples contained CA 19-9 positive antigen with a specific density of >1.5 g/ml, indicating carbohydrate rich mucins (16, 17) and excluding aggregation of the antigenic material (Fig. 3, top). To be certain that the absence of the CA 19-9 positive ganglioside in the top fraction of the gradient was not due to losses incurred in the foregoing gel chromatography, we subjected one unprocessed sample of pancreatic juice and serum each containing high levels of CA 19-9 to the same procedure (Fig. 3, bottom). These controls showed the same density as the chromatographed material with a slightly reduced maximum of 1.47 g/ml and a somewhat broader antigen distribution in the gradient.

DISCUSSION

When we first reported our preliminary data on the biochemical characterization of CA 19-9 antigen in pancreatic secretions (11) we were interested in elucidating our surprising finding, that this tumor marker did not discriminate between benign and malignant pancreatic diseases in more than 200 pancreatic juice samples in contrast to the respective serum samples (12). This raised the questions of (a) which CA 19-9 bearing moieties are found in pancreatic secretions and (b) whether the release of one of these, i.e., the glycolipid or the mucin, is in particular associated with pancreatic tumors.

The results presented here show that the CA 19-9 activity in pancreatic secretions is bound to high molecular weight antigens with a molecular weight of more than $2 \times 10^6$, which in CsCl density gradient centrifugation exhibit mucin properties, whereas no CA 19-9 positive ganglioside was detectable, either in thin layer chromatography or in solid phase immunoassay.

These data were obtained with all samples regardless of their diagnostic group and independently of their processing. This is in agreement with recent reports showing a wide, almost ubiquitous distribution of these mucins, which have been detected in the coelomic fluid of sea urchins and also with high titers in

**Fig. 1.** Thin layer chromatography of total lipid extract from various pancreas secretions and of colon carcinoma cell line SW-1116 overlaid with 125I-anti-CA 19-9 monoclonal antibody (1 x 10^6 cpm/ml). Lane I, SW 1116 extracts containing the monosialoganglioside antigen; lane 2, CA 19-9 negative, pancreatic juice; lanes 3–5, pancreatic juice samples of 3 diagnostic groups: lane 3, group I; lane 4, group II; lane 5, group III. Reference gangliosides and glycolipids: Cer, ceramide trihexoside; GL₄, globoside; GM₁, monosialoganglioside; GD₁, disialoganglioside; GT₁, trisialoganglioside.

**Fig. 2.** Gel chromatography of various pancreas secretion samples (●, group I; △, group II; ◆, group III) and serum (□). Fractions were monitored for CA 19-9 by solid phase radioimmunoassay. Marker substances: Dextran Blue 2000 (DB); IgG; and dinitrophenyl-alanin (DNP-Alanin).

**Fig. 3.** CsCl isopycnic density gradients containing 4 M guanidine-HCl; density (○) is in g/ml. Top, CA 19-9 positive material obtained from gel chromatography in various pancreatic secretions (●, group I; △, group II; ◆, group III); bottom, untreated serum (○) and juice (●) of pancreas cancer patients. Fractions were monitored for CA 19-9 by solid phase radioimmunoassay.
a variety of human secretions, including milk, saliva, and seminal plasma (9, 10, 18).

Although our investigations do not exclude the existence of the CA 19-9 ganglioside in the membrane of pancreatic cells as has been reported for the colon carcinoma cell line SW 1116 (7), they strongly indicate that the mucin form is the only CA 19-9 bearing antigen released into pancreatic secretions.

Since the detection of this antigen solely in human serum is highly indicative for a malignant state, the present study strongly suggests that an altered secretion or distribution in pancreatic tumors leads to the differential levels of this antigen in serum.

The detection of CA 19-9 antigen in sera of nude mice carrying pancreatic tumor transplants (19) suggests a direct involvement of the tumor instead of eventual secondary effects triggered by the tumor. The hypothesis of an altered secretion has recently been supported by electron microscopic studies (20) showing transformed pancreatic duct cells with loss of polarity and with mucin granules secreted into the interstitial space.

Thus, as in tumor-induced ectopic hormone production, a physiological product acquires tumor marker properties. In the case of CA 19-9 bearing mucins the mechanism by which this physiological exocrine pancreatic secretion product accumulates in the blood of pancreatic cancer patients is not yet understood. Whether spill-over effects due to tumor necrosis, pancreatic duct obstructions, or undirected secretions subsequent to altered polarity of malignant transformed pancreatic epithelials account alone or in concert for the appearance of CA 19-9 bearing mucins in the sera of pancreatic tumor patients requires further elucidation.

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