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Relationship of Carbohydrate Antigen 19-9 and Lewis Antigens in Pancreatic Cancer[1]

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ABSTRACT

Carbohydrate antigen (CA) 19-9 identified by a murine monoclonal antibody against a colorectal carcinoma antigen is thought to be a sialylated Lewis (Le)^a blood group antigen and occurs in high concentration in serum of patients with pancreatic carcinoma. This study was designed to identify the relationship between Lewis antigens and CA 19-9 in patients with pancreatic cancer. The following analyses were performed in 20 pancreatic cancer patients: Le^a and Le^b antigen phenotype in saliva (modified enzyme-linked immunosorbent assay) or on red cells (hemagglutination); CA 19-9 levels (radioimmunoassay) in serum; and CA 19-9 and Le^a and Le^b expression (immunoperoxidase assay) on tumor tissue. Le^{a-b-} patients based on salivary phenotype failed to express CA 19-9 in tumor tissue and had normal or low levels of CA 19-9 (<37 units/ml) in serum ($P = 0.0011$, *versus* Le^{a+b-} and Le^{a-b+} patients). Eighty-eight % of Le^{a+b-} and Le^{a-b+} patients had elevated serum CA 19-9 levels (>37 units/ml). All Le^{a+b-} and Le^{a-b+} patients expressed both Le^a and Le^b antigens in tumor tissue. These results support the view that Le^{a-b-} pancreatic cancer patients cannot manufacture CA 19-9. Surprisingly, Le^a-positive patients express Le^b antigen in tumor tissue; in this subgroup, Le^b antigen may be a tumor-specific biomarker.

INTRODUCTION

CA[3] 19-9 is a tumor-associated antigen which is now known to be a sialylated Le^a antigen (1). This antigen was originally defined by a monoclonal antibody produced by a hybridoma prepared from spleen cells of a mouse immunized with human colorectal carcinoma cell line SW1116 (2). It is now known that CA 19-9 is normally present in salivary mucus and in physiological exocrine pancreatic secretions (3, 4).

Elevated CA 19-9 levels (>37 units/ml) have been described in a variety of gastrointestinal malignancies (5), particularly in pancreatic adenocarcinoma (6–10). In various series, elevated CA 19-9 expression is found in 69 to 92% of pancreatic cancer patients. Koprowski *et al.* (11) have hypothesized that patients with a Le^{a-b-} phenotype should be unable to synthesize CA 19-9 since normal individuals with a Le^{a-b-} phenotype do not express CA 19-9 in their secretions (3). Confirmation of this relationship would better define the clinical utility of this biomarker in pancreatic cancer.

In this study, MoAbs recognizing CA 19-9 (MoAb 19-9) and Le antigens a and b (MoAb Le^a and Le^b) were used to determine Le antigen phenotype in saliva, serum CA 19-9 levels, and Le antigen and CA 19-9 expression in malignant tissue in patients with pancreatic adenocarcinoma.

PATIENTS AND METHODS

Tumor tissue (primary or metastatic) was obtained from patients with adenocarcinoma of the pancreas undergoing clinical evaluation at the University of Nebraska Medical Center. Sections of paraffin-embedded formalin- or Bouin-fixed tissue were prepared and stained using an immunoperoxidase assay as previously described (12) with murine MoAb 19-9 and CO-514 and CO-431 (MoAb Le^a and Le^b) with specificity for Le^a and Le^b antigens, respectively (13). Tissue was examined by light microscopy and graded according to the percentage of cells expressing surface antigen.

Serum CA 19-9 was quantitated with a radioimmunoassay kit (courtesy of Centocor, Malvern, PA) using the appropriate monoclonal antibody. Established normal values of 6 to 37 units/ml were used in interpreting results.

When possible, a fasting saliva specimen was obtained. Le antigens in saliva were qualitatively demonstrated using an enzyme-linked immunosorbent assay with MoAb Le^a or Le^b. In brief, heat-inactivated saliva (100 μl) diluted with carbonate-bicarbonate buffer (pH 9.6) was applied to microtiter plate wells and incubated at 4°C overnight. After washing in PBS, 100 μl of bovine serum albumin in PBS were added and incubated one h at room temperature. After further washings in PBS, 100 μl of 1:5 diluted culture supernatant containing MoAbs Le^a or Le^b followed by 100 μl of 1:100 diluted peroxidase-conjugated affinity-purified goat anti-mouse immunoglobulin and 100 μl of peroxidase substrate (4 mg of *o*-phenylenediamine:40 μl of 30% H_2O_2 in 10 ml of phosphate-citrate buffer, pH 5.0) were sequentially added to the wells. Brown staining of the wells indicated a positive reaction. In those patients who could not provide a saliva specimen, RBC-associated Le antigens were determined using a standard hemagglutination assay with commercially available polyclonal antiserum.

Data were analyzed by χ^2 analysis.

RESULTS

Table 1 outlines the immunohistological findings in 20 patients with the associated Lewis antigen phenotype in saliva or on RBC and the corresponding serum CA 19-9 levels. No Le^{a+b+} phenotypes occurred in this series; all Le-positive patients were either Le^{a+b-} or Le^{a-b+}. Histologically, all tumors were adenocarcinomas of ductal type.

CA 19-9 and Le antigens were absent in tissue from three patients with Le^{a-b-} salivary phenotypes. In these patients, serum CA 19-9 expression was ≤37 units/ml. In the remaining patients, normal or excessive levels of serum CA 19-9 were present. The failure of Le^{a-b-} patients to produce elevated CA 19-9 levels was highly significant ($P = 0.0011$). This relationship is detailed in Table 2.

Overall, 75% of the patients analyzed demonstrated a serum CA 19-9 level of >37 units/ml. In patients with Le^{a+b-} and Le^{a-b+} phenotypes, the sensitivity (true positive proportion) of elevated serum CA 19-9 levels was 88%.

In two cases, multiple sites of tumor involvement were available for immunohistology. Heterogeneity of Le antigen and CA 19-9 expression was apparent (Table 1).

All patients with Le^{a+b-} and Le^{a-b+} phenotype demonstrated inappropriate Le antigen expression (variation from salivary or RBC Le antigen phenotype) in malignant tissue. In most of these patients, a high percentage of malignant cells demonstrated both Le^a and Le^b expression.

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[3] The abbreviations used are: CA, carbohydrate antigen; Le, Lewis; MoAb, monoclonal antibody; PBS, phosphate-buffered saline.

Table 1 *Comparative studies of Lewis antigen and CA 19-9 expression in tumor tissue, saliva, and serum of pancreatic cancer patients*

Tumor immunohistology on 20 patients with pancreatic cancer paired with Lewis antigen phenotype and serum CA 19-9 levels are shown. In 2 patients (F. E. and B. R.) multiple sites of involvement were analyzed. Lewis phenotype represents a qualitative determination in saliva unless otherwise noted RBC.

	Tumor immunohistology[a]			Lewis phenotype[a]		
Patient	Le^a	Le^b	CA 19-9	Le^a	Le^b	Serum CA 19-9 level (normal range, 6–37 units/ml)
1 H. M.	++	++++	++++	+	−	54
2 J. K.	++++	++++	++++	+	− (RBC)	108,897
3 I. H.	++++	++++	++++	+	−	69,342
4 E. S.	++	+	−	+	−	318
5 F. E. 1	−	++++	+	−	+ (RBC)	314
F. E. 2	+	++++	++++			
6 B. R. 1	++	++++	++	−	+	545
B. R. 2	++++	+++	+			
B. R. 3	++++	++++	++++			
B. R. 4	++++	+	+			
7 C. K.	+	+	+	−	+	6,031
8 K. W.	++	++	++	−	+	74
9 J. G.	++++	++++	++++	−	+	67
10 M. E.	++++	++++	++++	−	+ (RBC)	1,587
11 C. H.	+++	+++	+++	−	+	22,220
12 F. E.	++++	++++	++++	−	+	7,700
13 A. B.	++++	++++	++++	−	+	79,156
14 J. C.	+++	++	++++	−	+	530
15 R. S.	+	+	+++	−	+ (RBC)	99
16 R. L.	++++	++++	−	−	+	15
17 V. D.	++++	++++	++++	−	+	24
18 G. B.	−	+	−	−	−	4
19 J. F.	−	−	−	−	−	14
20 G. F.	−	−	−	−	−	10

[a] Key for immunohistology: −, negative; +, 0–5% of cells positive; ++, 5–30% of cells positive; +++, 30–70% of cells positive; and ++++, 70–100% of cells positive.

Table 2 *Lewis phenotype and serum CA 19-9 antigen levels in pancreatic cancer ($P < 0.0011$ by χ^2 analysis)*

CA19-9 levels in serum by radioimmunoassay are compared with Lewis phenotype in saliva.

	CA 19-9 > 37 units/ml	CA 19-9 ≤ 37 units/ml
Le^{a+b-} and Le^{a-b+}	15	2
Le^{a-b-}	0	3

DISCUSSION

The results of this study confirm the previously reported high sensitivity of the serum CA 19-9 assay in patients with exocrine pancreatic adenocarcinoma. However, the clinical role of this biomarker remains to be defined. In the United States, approximately 24,000 cases of pancreatic adenocarcinoma occur annually (14). Since all patients with resectable pancreatic cancer do not appear to express an elevated CA 19-9 level (15), it is doubtful that this biomarker would be suitable for population-based screening with the goal of detecting early stage disease and improving the already dismal 5-yr survival rate of 2%.

CA 19-9 levels may, however, have an important role in the differential diagnosis of a pancreatic mass caused by tumor or from chronic fibrosing pancreatitis, a disease characterized by normal levels (<37 units/ml) of CA 19-9 (6, 8, 10). Serial CA 19-9 levels may also predict disease activity. In most patients with pancreatic adenocarcinoma (16) and in selected patients with colon cancer (17), rising serial CA 19-9 levels reliably predict disease progression.

Our results indicate that the clinical utility of CA 19-9 antigen is limited by the corresponding salivary Le antigen phenotype. CA 19-9 antigen cannot be used as a biomarker in patients with Le^{a-b-} phenotype. Approximately 5% of the population belong to this group (18). Furthermore, restricting the use of this antigen to patients with a positive Le antigen phenotype increases the sensitivity and certainly the reliability of this biomarker.

Perfect correlation of CA 19-9 expression in tumor tissue and in serum was not apparent in our analysis. One of 2 Le-positive patients who failed to determine CA 19-9 in tumor tissue paradoxically demonstrated high levels of CA 19-9 in serum. This is probably a further demonstration of heterogeneity in tumor antigen expression, since only a metastatic and not a primary site was analyzed for immunohistology in this particular case. Conversely, one patient with strong positivity for CA 19-9 at the primary site in the head of the pancreas failed to demonstrate elevated CA 19-9 levels in serum. This patient has been followed prospectively, and monthly CA 19-9 levels have remained within normal limits. Interestingly, the primary site of tumor involvement with established CA 19-9 expression has remained in remission following localized radiation therapy.

Apparent "inappropriate" expression of Le antigens (variation from Le antigen phenotype in saliva or on RBC) occurred in almost all Le^{a-b+} and Le^{a+b-} cases. This phenomenon has been previously described in patients with colon carcinoma, although it appears that less than half of the patients with colon carcinoma demonstrate "inappropriate" Le antigen expression (19). It is unclear whether Le antigen expression in pancreatic cancer tissue is truly "inappropriate," since the expression of Le antigens in normal pancreatic tissue compared with salivary Le antigen phenotype has not been studied. The normal pancreatic tissue of these patients was not available for analysis. In an independent study, Uchida *et al.* (12) found that Le^a was expressed in centroacinar and terminal ductular cells of all specimens examined. However, in that study, the pancreatic tissue was retrospectively obtained from cadavers and, thus, the salivary Lewis phenotype of the individuals was not known.

As is the case with CA 19-9, it is likely that increased levels

of Le^a and Le^b antigens could be detected in the sera of these patients. Currently, a commercial quantitative assay for these antigens is not available. However, serum has been banked from all of these reported cases so that quantitation of Le^a and Le^b in the serum can be assessed in the future.

The unexpected finding of Le^b antigen on tumor tissue of patients with salivary Le^{a+b-} phenotype has also been described in human colon tumors (20), including benign colon polyps (21). This observation suggests that activation or derepression of the secretor gene may occur in the tumors of nonsecretors (22). Thus, in these cases, Le^b antigen may truly represent a tumor-specific antigen suitable for radioimmunodetection or antibody-targeted therapy.

Since CA 19-9 is a sialylated Le^a antigen, change in the secretor status in tumor tissue might alter circulating levels of this antigen. In this small study, Le^{a+b-} pancreatic cancer patients did not appear to have higher levels of serum CA 19-9 compared to Le^{a-b+} patients. However, this relationship would be better evaluated in a larger group of patients stratified for extent of disease, since serum CA 19-9 levels are clearly related to tumor burden (16).

In summary, our results suggest that comparative analyses of Le antigen phenotype and CA 19-9 expression in patients with suspected or proven pancreatic malignancy can increase the sensitivity of this biomarker. The appearance of unexpected Le antigens in tumor tissue suggests that serological or immunohistochemical analysis for "inappropriate" Le antigen expression in patients with pancreatic carcinoma may also define other useful biomarkers in patients with pancreatic cancer.

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