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Cancer-associated isoenzyme of serum galactosyltransferase

(polyacrylamide gel electrophoresis)

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ABSTRACT **Galactosyltransferase activity was assayed in sera from 58 patients with various types of cancer. On discontinuous polyacrylamide gel electrophoresis a slow-moving peak of galactosyltransferase activity (isoenzyme II) was found to be present in the serum of 43 of these patients in addition to the major isoenzyme I. Isoenzyme II was found in only 2 of 39 patients with various nonmalignant disorders and was not detected in the serum of 22 normal control subjects. There was no correlation between the presence of this electrophoretically distinct isoenzyme and total serum galactosyltransferase activity, alkaline phosphatase, levels of carcinoembryonic antigen, or blood type. However, patients with widespread metastases had significantly higher isoenzyme II levels than those with no metastases or with limited local spread. Further studies will be necessary to evaluate the clinical usefulness of this serum galactosyltransferase isoenzyme in the diagnosis and monitoring of patients with neoplastic disease.**

Galactosyltransferase activity has been identified with intracellular membranes, primarily the Golgi membrane fraction (1). Recent reports have also demonstrated the presence of galactosyltransferase activity on the cell surface membrane of various cell types which include rat intestinal crypt cells (2), human fetal cells (2), and chemically induced rat tumor cells (3). Bosmann and Hall (4) reported elevated levels of sialyltransferases in homogenates of tumor cells from human breast and colon. Kessel and Allen reported elevated sialyltransferase activity in the plasma of cancer patients (5). Galactosyltransferase activity has also been detected in rat and human serum as a soluble activity (6, 7), but the total activity was not previously found to be elevated in cancer patients unless liver disease was also present (8). However, by means of discontinuous polyacrylamide gel electrophoresis Podolsky and Weiser were able to detect in cancer patients a slower moving peak of activity that separated well from the major, more anodally directed peak of activity found in all patients (9). The present report describes the cancer patients who were tested for serum galactosyltransferase isoenzymes, compares them with disease and normal controls, and examines possible clinical and laboratory correlations with serum levels of these isoenzymes.

MATERIALS AND METHODS

Biochemical Assays. Galactosyltransferase activity in whole serum was measured as previously described (9). The glycoprotein acceptor used for this enzyme assay was fetuin minus its terminal sialic acid and penultimate galactose residues (10).

Polyacrylamide gel electrophoresis of sera and detection of galactosyltransferase activity from slices of polyacrylamide gels were performed by the method of Podolsky and Weiser (9). It was observed that the cancer-associated cathodal peak of activity (isoenzyme II) could not be detected unless the gel reagents were first re-crystallized. *N*-acetylglucosamine (1 mM) was added to the gel eluting solution to stabilize the enzyme; it did not affect galactosyltransferase activity with fetuin acceptor. Polyacrylamide electrophoresis appeared to separate two peaks of galactosyltransferase (fetuin) activity which entered the gel. No galactosyltransferase activity with endogenous acceptor was detected. The major peak, which was found in all sera, was the more anodal one, while the smaller peak (isoenzyme II) was near the origin but separated from the activity remaining at the origin (9). These isoenzymes have been separated and partially purified and shown to have their original R_F values on re-electrophoresis (unpublished observations).

Serum. Serum samples were obtained primarily from patients hospitalized on the surgical service of the Massachusetts General Hospital. Serum was stored at 0–4° and although as much as 40% of the total serum galactosyltransferase activity could be lost after 3 days' storage, further loss over 20 days' storage was not detected. However, storage did not seem to affect the levels of isoenzyme II. Nevertheless, all data reported were determined from fresh (<48 hr old) sera.

RESULTS

Measurement of total serum galactosyltransferase activity from patients with carcinoma demonstrated a mean level of activity higher than in normal control subjects or patients with nonmalignant disorders, but the increase was not statistically significant (Fig. 1). After polyacrylamide electrophoresis the major normal peak, isoenzyme I, was also found in greater amounts in sera from patients with carcinoma (Fig. 2) but this, again, was not statistically significant. However, the presence of isoenzyme II was only detected in sera of patients with cancer (Fig. 2 and Table 1) with two exceptions to date (Table 2). One of these exceptions was an 87-year-old woman with profound anemia who demonstrated an "apple-core" radiological sign in the sigmoid colon on barium enema that did not change its appearance after glucagon administration. Laparotomy apparently only showed diverticulae, and no tissue was removed for pathologic examination. The other exception was a patient with active nontropical sprue, a condition known to be associated with increased intestinal epithelial cell turnover (see below).

Clinical data of patients whose sera demonstrated isoenzyme II are presented in Table 1. Most tumors were adenocarcinomas of entodermally derived tissue, although isoenzyme II was also found in several patients with squamous cell carcinoma. One patient with isoenzyme II had a colonic adenocarcinoma which extended into the colonic fat but with negative nodes. In this patient, isoenzyme II was demonstrated in two separate sera preoperatively but was not detectable after removal of the tumor.

To date we have observed no correlation with blood type

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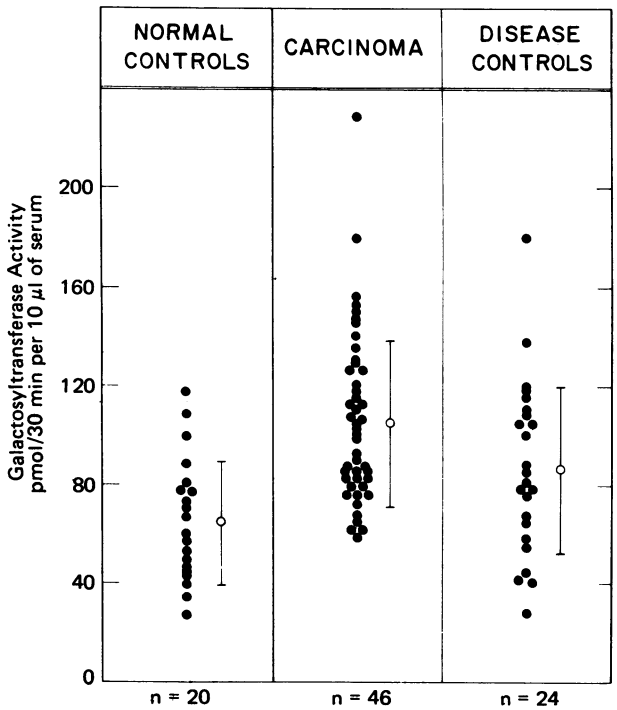

FIG. 1. Total human serum galactosyltransferase activity in 10 μl samples of fresh serum (<1 day old). Normal controls = 65.3 ± 18.6 (mean ± SD), carcinoma = 105.3 ± 33.2, disease controls = 86.6 ± 33.2; $P > 0.05$.

or with the levels of serum alkaline phosphatase, transaminase, or bilirubin. However, the level of galactosyltransferase isoenzyme II activity appeared to correlate with widespread metastases, as shown in Fig. 3 ($P < 0.001$).

Of 17 patients with no detectable isoenzyme II, eleven also had tumors of entodermally derived organs (Table 1). Sera from 22 normal, non-age matched controls (laboratory personnel) were evaluated for presence of galactosyltransferase isoenzymes I and II. While all contained the normal isoenzyme I, we were unable to detect the slow moving isoenzyme II in these control sera.

As with normal controls, none of the disease controls demonstrated isoenzyme II (Table 2) with the two exceptions mentioned above.† These disease controls included two patients (first two listed in Table 2) with prior histologically proven carcinoma who at the time of this study had no evidence of tumor. The other disease controls included patients with various forms of liver disease, pancreatitis, cholecystitis, inflammatory bowel disease, and peptic ulcer. Three patients with uremia did demonstrate increased total galactosyltransferase activity but no evidence of isoenzyme II.

In six patients levels of carcinoembryonic antigen were determined at the same time as the galactosyltransferase isoenzymes were assayed. Four had "normal" levels of this antigen but had evidence of galactosyltransferase isoenzyme II activity; in the two others, isoenzyme II was not detectable but their serum revealed high carcinoembryonic antigen levels. Thus, to date there has been no consistent correlation between these two assays, suggesting that carcinoembryonic antigen and galactosyltransferase isoenzyme II represent different chemical entities.

† **Note Added in Proof.** Six additional patients with gluten-sensitive enteropathy have been tested and their sera demonstrated the presence of isoenzyme II. Three other patients with severe alcoholic hepatitis but with no evidence of malignant disease also demonstrated isoenzyme II in their sera.

Table 1. Galactosyltransferase isoenzyme II in malignant disorders

Diagnoses	No. tested	No. demonstrating isoenzyme II
Bronchogenic carcinoma	8	7
Breast adenocarcinoma	6	4
Esophageal cancer (squamous cell)	4	3
Stomach adenocarcinoma	7	6
Pancreas adenocarcinoma	7	6
Colon-rectal adenocarcinoma	17	13
Gallbladder adenocarcinoma	1	1
Chronic lymphocytic leukemia	1	1
Polycythemia rubra vera	1	1
Lymphomas	2	0
Undifferentiated (source unknown) carcinoma	2	1
Ovarian and testicular cancer	2	0
Total	58	43 (74.1%)

DISCUSSION

The enzymes known generically as glycosyltransferases participate in the biosynthesis of complex carbohydrates. They are commonly found as membrane-bound enzymes within the interior of the cell; the Golgi membrane may, in fact, be identified biochemically by the presence of these glycosyltransferases (1). The function of adding sugars to proteins is not clear, although the nature of the terminal sugar appears to be important in the control of secretion and in the clearance of circulating glycoproteins (11). A significant portion of serum proteins are glycoproteins and the biosynthesis of ABO blood group substances also requires sugar additions through the action of glycosyltransferases (12). Although some glycosyltransferases appear to be membrane-associated enzymes when prepared from tissue homogenates, these transferases have also been detected as soluble enzymes in various body fluids, including rat and human serum (6, 7).

Human serum galactosyltransferase has been previously studied by Kim *et al.* (7), who demonstrated increased activity of total serum galactosyltransferase in patients with liver disease. They did not find any increased levels in patients with cancer unless the liver was involved; however, their report included only five patients with cancer (8). We also found increased total galactosyltransferase activity in three patients with renal insufficiency, as has been reported by Kirschbaum (13), but did not detect isoenzyme II in these patients. The data presented in this study are compatible with the findings of Kim *et al.* (7) in regard to total serum galactosyltransferase activity (Fig. 1), but differ in that an isoenzyme of serum galactosyltransferase previously reported from this laboratory (9) was present in patients with malignancy. The data indicate that this galactosyltransferase isoenzyme, which moved more slowly on polyacrylamide electrophoresis, was demonstrable in patients with cancer of the lung, breast, stomach, colon, and pancreas (Table 1). It was also detected in a patient with chronic lymphocytic leukemia and one patient with polycythemia rubra vera; to date it has not been observed in patients with plasma cell myeloma or histiocytic lymphoma.

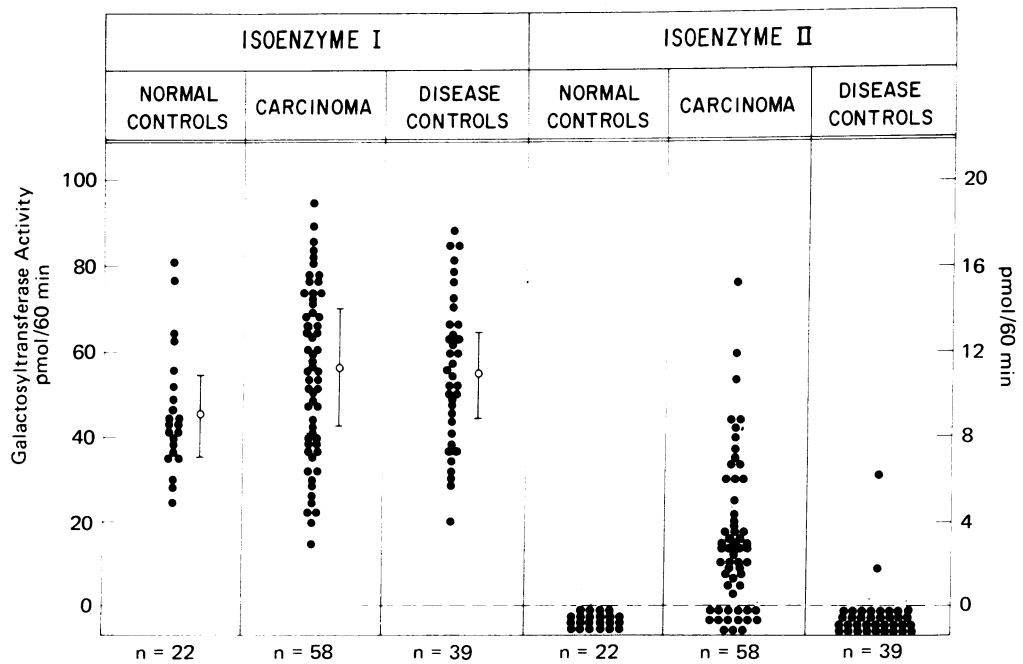

FIG. 2. Human serum galactosyltransferase isoenzyme levels. Data on patients analyzed by these procedures are presented in Tables 1 and 2. Serum (25 μl aliquot) was electrophoresed on polyacrylamide gel and slices of the gel were assayed for galactosyltransferase activity. Mean isoenzyme I levels ± SD (anodal peak) were: normal control, 45.3 ± 9.3; carcinoma, 56.6 ± 13.3; disease controls, 55.3 ± 10.0; $P > 0.05$ Isoenzyme II (cathodal peak) was found only in patients with carcinoma with the exception of two disease controls (see *text*).

In the cases studied, the incidence of false negatives appeared to be approximately 30% and only two cases of apparent false positives were found. As described above, one of the latter group had a constricting lesion of the sigmoid colon seen by x-ray but which was not found on laparotomy; however, tissue was not obtained to exclude carcinoma. The other patient had untreated active nontropical sprue (gluten-induced enteropathy), a condition known to be associated with increased mitotic activity of intestinal mucosal cells. The latter observation suggests that the presence of the isoenzyme II in serum may be a reflection of an increase in the number of cells undergoing cell division. It remains to be determined if isoenzyme II in celiac disease reflects increased cell division or is intrinsic to the disease.

The appearance of this unusual galactosyltransferase isoenzyme was not correlated with serum levels of alkaline phosphatase or with blood type. Kim *et al.* (14) had previously shown that serum *N*-acetyl-D-galactosaminyltransferase activity was found in individuals with blood groups A and AB but not in those with blood groups B or O. However, they could not show any correlation of serum galactosyltransferase activity with blood type (2). We also observed no correlation of blood type with either total serum galactosyltransferase activity or with galactosyltransferase isoenzymes.

The levels of isoenzyme II appeared to be greater in patients with widespread metastases (Fig. 3); the levels did not correlate with serum carcinoembryonic antigen titers. However, while there was an increase in the level of isoenzyme II in patients with metastases, this was not necessarily reflected by an increase in the total serum galactosyltransferase activity. In fact, when total serum galactosyltransferase activity was found to be increased, this usually appeared to be associated with an increase in isoenzyme I rather than II.

The tissue source of this apparently cancer-associated isoenzyme has not yet been determined. On the basis of clinical data, Kim *et al.* have suggested that most of the serum galactosyltransferase activity is derived from normal liver tissue (8). However, it should be noted that human intestinal fetal cells, rat intestinal crypt cells, and chemically induced rat intestinal adenocarcinomas have surface galactosyltrans-

Table 2. Galactosyltransferase isoenzyme II in nonmalignant disorders

Diagnoses	No. tested	No. demonstrating isoenzyme II
Post-operative radical mastectomies with no evidence of metastases	2	0
Gastrointestinal diseases (sigmoid polyps, rectal adenoma, esophageal stricture, peptic ulcer disease, nonspecific gastroenteritis, ulcerative colitis, regional enteritis, Wilson's disease, cholecystitis, pancreatitis, cirrhosis)	24	0
Other illnesses (peripheral vascular disease, aortic aneurysm, appendicitis with abscess, uremia, uterine fibroids, inguinal hernia)	11	0
Diverticulosis, ? sigmoid colon cancer	1	1
Celiac disease	1	1
Total	39	2 (5.1%)

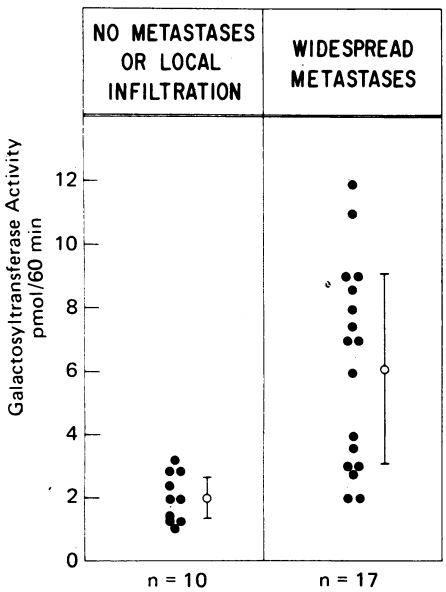

FIG. 3. Human serum galactosyltransferase isoenzyme II: comparison of patients with localized versus metastatic carcinoma. Polyacrylamide gel electrophoresis and galactosyltransferase activity were performed as in Fig. 2. Localized carcinoma, mean activity ± SD: 2.0 ± 0.5; widespread metastatic carcinoma: 6.1 ± 3.0. $P < 0.001$.

ferase activities which are not detectable in normal intestinal epithelial cells (2, 3). High levels of galactosyltransferase activity have also been shown in amniotic fluid (15) and in the fluid bathing the chick embryo brain (16). Therefore, it is possible that galactosyltransferase isoenzyme II may represent a carcinoembryonic cell membrane factor which has gained access to the circulation. We have preliminary evidence that isoenzyme II can be "shed" from the surface of neoplastic cells (unpublished observations).

At present the assay for serum galactosyltransferase isoenzyme II is not a simple laboratory procedure. However, it may be possible to increase the sensitivity and simplicity of the assay with increased knowledge of the biochemical properties of isoenzyme II. The determination of the clinical usefulness of this test requires more extensive studies, including investigations to demonstrate the mechanism whereby a small malignant lesion leads to a positive serum test and to determine whether the assay for isoenzyme II will be of value in monitoring patients following surgical resection or treatment of their malignancy.

It will also be important to determine the biological significance of the appearance of isoenzyme II in serum. Roth *et al.* demonstrated the presence of cell surface glycosyltransferases on embryonic chick neural retinal cells (17). Bosmann *et al.* observed these enzymes in tissue culture cells and showed that the enzyme activity increased after viral transformation (18). Keenan and Morré (19) argued against a cell surface membrane localization for glycosyltransferases but Shur and Roth (20) have recently reviewed the data, and strongly support the concept of a cell surface membrane location. Roseman has suggested the possibility that these cell surface glycosyltransferases may play a role in cell adhesion, contact inhibition of movement, and control of cell division—features which appear to be important in the social behavior of tumor cells (21). It is possible that further studies of galactosyltransferase isoenzyme II in patients with malignancy as well as in tissue culture systems may be able to test the validity of this hypothesis.

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