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Evaluation of Serum Fucosyltransferases in Malignancy*

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Abstract—*Incorporation of [^{14}C]-fucose into the endogenous acceptor of serum and into the exogenous acceptors desialofetuin (α_2-fucosyltransferase) and desialodegalactofetuin (α_3-fucosyltransferase) was determined in controls and in patients with various malignant diseases. Seventy-five percent of untreated cancer patients showed increased incorporation rates into the endogenous acceptor of serum. Increased rates of α_2-fucosyltransferase activity were observed in 67% and of α_3-fucosyltransferase activity in 49% of patients respectively. After successful chemotherapy a fall of fucosyltransferase activities could be observed and in non-responding patients an increase of fucosyltransferase activities during and after therapy was found. Determination of fucosyltransferase activities might provide an additional biological marker in monitoring cancer patients.*

INTRODUCTION

FUCOSE has been found to be incorporated in membrane glycoproteins of mammalian cells [1, 2], blood group substances [3, 4] and plasma glycoproteins [5, 6], where it occupies a terminal position in the carbohydrate units. Fucosyltransferases transfer L-fucose from GDP-L-fucose into specific glycoproteins, into α-1-2-linkage to galactosyl residues (α_2-fucosyltransferase), into α-1-4-linkage to *N*-acetylglucosaminyl residues (α_4-fucosyltransferase) and into α-1-3-linkage to D-glucosyl or *N*-acetylglucosaminyl residues (α_3-fucosyltransferase).

In human serum α_2- and α_3-fucosyltransferases occur, whereas α_4-fucosyltransferases cannot be detected [6]. In tumor tissue of animals with experimental neoplasms [7, 8] and in serum of cancer patients [9–13] elevated fucosyltransferase activities have been reported, the enzymes being secreted by the malignant cells or released during cell degradation [14].

After successful chemotherapy or surgical removal of the tumor a decrease of fucosyltransferase levels was observed [15].

The purpose of the present study was to evaluate the determination of fucosyltransferase activities in various malignant diseases and to correlate the enzyme levels to the success of chemotherapy.

MATERIALS AND METHODS

GDP-L-[U-^{14}C]-fucose, sp. act.118 mCi/mmol, was purchased from the Radiochemical Centre, Amersham, U.K. Fetuin from fetal calf serum came from Sigma Chemical Co., St. Louis, MO, U.S.A. All other reagents were of the highest purity commercially available.

Serum fucosyltransferase activities were determined according to the method of Munro and Schachter [5] in the modification described by Bauer *et al.* [15]. Incorporation of [^{14}C]-fucose into the endogenous acceptors of serum and into the exogenous acceptors desialofetuin (α_2-fucosyltransferase) and desialodegalactofetuin (α_3-fucosyltransferase) was measured. *N*-Acetylneuraminic acid and galactose were removed from fetal calf serum as described by Spiro [16].

In the test a 120 μl-system, pH 5.5, containing 30 μl serum, 0.5 nmol GDP-L-[U-^{14}C]-fucose, 4 μmol morpholinoethane sulfonic acid, 2 μmol $MgCl_2$ and 0.4 μmol sodium azide was incubated for 21 hr at 37°C. To determine α_2- and α_3-fucosyltransferase activities either 0.5 mg lyophilized desialofetuin or desialodegalactofetuin were added to the incubation mixture. Protein-bound activity was determined subjecting the samples to a washing procedure with trichloroacetic acid, ether and ethanol [17].

α_2- and α_3-fucosyltransferase activities were

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calculated by the difference, correcting for endogenous levels of other acceptors. Results are reported in terms of dpm/min/ml of serum, each value representing the mean of duplicate determinations.

Enzyme kinetic studies are shown in Fig. 1. Variation of substrate concentrations between 0.5 and 2 nmol [^{14}C]-fucose and addition of 0.5–1.5 mg of sugar acceptors to the test system had no effect on the amount of product formed in the assay.

Blood samples were taken in the fasting state and serum was separated by centrifugation for 10 min at 4000 *g*.

Serum fucosyltransferase activities were measured in 94 age-matched normal controls (male and female subjects aged 18–65 yr, with no evidence of cancer or any other disease).

One hundred and fifty-three untreated patients suffering from various malignant diseases were examined. In all cases malignancy was histologically verified: 28 patients with disseminated testicular cancer (5 seminoma and 23 non-seminomatous germ cell tumors), 19 patients with Hodgkin's disease, 9 non-Hodgkin's lymphoma patients, 17 patients with leukemia (8 acute myeloic leukemia, 2 chronic myeloic leukemia and 7 chronic lymphatic leukemia), 35 patients with lung cancer, 15 patients with breast cancer and a group of 30 patients suffering from various other tumors (5 gastrointestinal cancer, 7 prostatic cancer and 18 other carcinoma patients) were investigated. In addition, fucosyltransferase activities were measured in 21 patients after successful removal of the primary testicular tumor and no evidence of metastases in a careful clinical staging and in 8 patients with Hodgkin's disease in drug-induced complete remission.

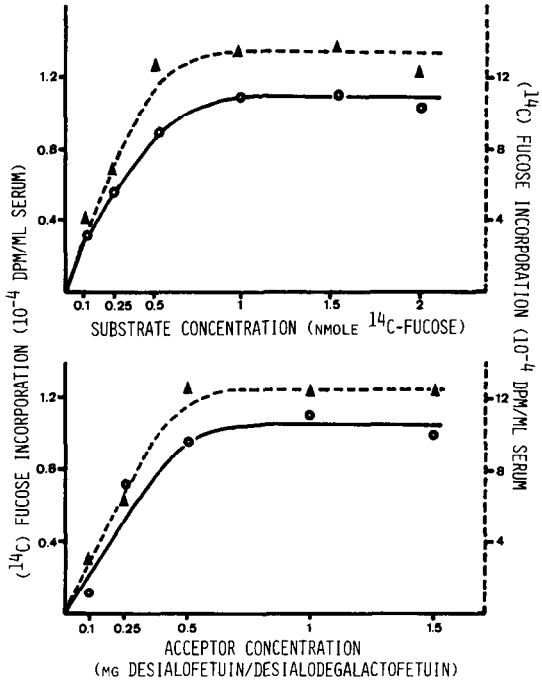

Fig. 1. The effect of varying the concentration of [^{14}C]*-fucose (upper part of the figure) and exogenous acceptors (lower part of the figure) on serum* α_2*-fucosyltransferase* (O——O) *and* α_3*-fycosyltransferase* (▲– – – –▲) *activities. The serum sample was provided by a normal donor. Incubation mixtures were the same as described in the text, except that varied amounts of* [^{14}C]*-fucose* (0.1–2 nmol) *and lyophilized desialofetuin or desialodegalactofetuin* (0.1–1.5 mg) *were added.*

RESULTS

Mean incorporation rates into the endogenous acceptor of serum from controls were 31,000 dpm/ml serum with a standard deviation of 10,800, into the exogenous acceptor desialofetuin 9800 ± 5100 dpm/ml serum and into desialodegalactofetuin 71,000 ± 29,000 dpm/ml serum. The upper limit of the normal range ($\bar{x}+2$ S.D.) was taken as 53,000 dpm/ml serum for the endogenous acceptor and 20,000 and 130,000 dpm/ml serum for α_2- and α_3-fucosyltransferase respectively (dotted line in Figs 2–6).

Seventy-five percent of untreated cancer patients showed increased incorporation rates into the endogenous acceptor of serum (Fig. 2). In patients with breast carcinoma and lung cancer increased incorporation rates into the endogenous acceptor of serum were detected in 87 and 83% respectively. α_2-Fucosyltransferase activities were above the normal range in 67% of patients (Fig. 3), this enzyme activity being most elevated in leukemia (89% of patients) and breast cancer (86% of patients). Increased α_3-fucosyltransferase activities were measured in 49% of cancer patients (Fig. 4).

Elevated enzyme activities were found in patients with metastatic disease but also in an early stage of the malignant disease (Fig. 5). Increased incorporation rates into the endogenous acceptor of serum were measured in 86% of patients with lung cancer without metastases and in 80% with metastatic disease respectively. Elevated α_2-fucosyltransferase activities occurred in 45% of patients without and in 61% with metastases; and elevated α_3-fucosyltransferase activities were detected in 33% of lung cancer patients without and in 53% with metastases respectively.

In disseminated testicular cancer [^{14}C]-fucose incorporation into the endogenous acceptor of serum was increased in 61% of patients, α_2-fucosyltransferase activities were above the normal range in 65% and occurrence of metastases was indicated by high α_3-fucosyltransferase activities in only 19% of cases. In patients with metastatic seminoma increased incorporation

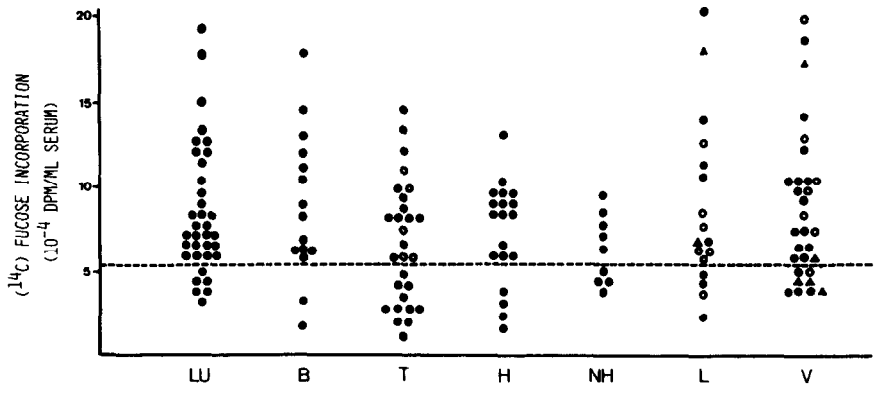

Fig. 2. [^{14}C]-*Fucose incorporation into the endogenous acceptor of serum from patients with various malignant diseases.* _____ *Upper limit of the normal range* ($\bar{x}$ + 2 *S.D.*) [*LU: lung cancer; B: breast cancer; T: testicular tumors* (○ *seminoma patients,* ● *non-seminomatous germ cell tumors*); *H: Hodgkin's disease; NH: non-Hodgkin's lymphoma; L: leukemia* (● *acute myeloic leukemia,* ▲ *chronic myeloic leukemia,* ○ *chronic lymphatic leukemia*); *V: various tumors* (○ *prostatic cancer,* ▲ *gastrointestinal tumors,* ● *other cancer patients*)].

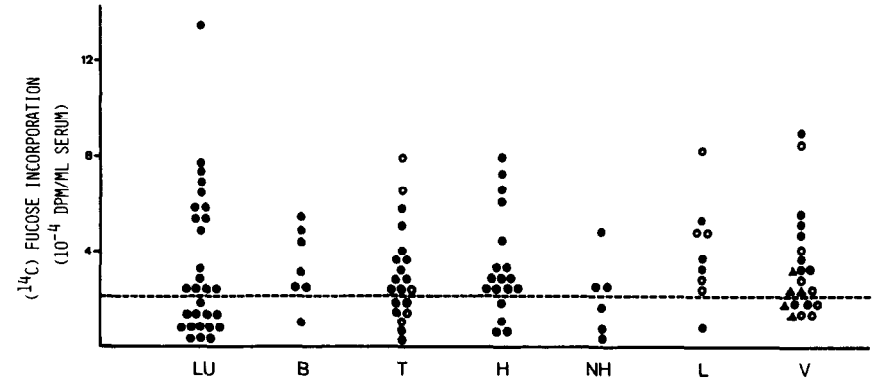

Fig. 3. α_2-*Fucosyltransferase activities in various malignant diseases.* _____ *Upper limit of the normal range* ($\bar{x}$ + 2 *S.D.*) [*LU: lung cancer; B: breast cancer; T: testicular tumors* (○ *seminoma patients,* ● *non-seminomatous germ cell tumors*); *H: Hodgkin's disease; NH: non-Hodgkin's lymphoma; L: leukemia* (● *acute myeloic leukemia,* ○ *chronic lymphatic leukemia*); *V: various tumors* (○ *prostatic cancer,* ▲ *gastrointestinal tumors,* ● *other cancer patients*)].

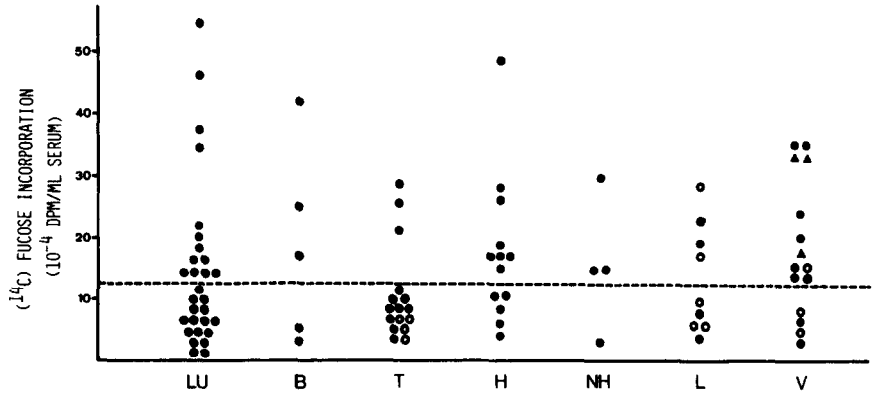

Fig. 4. α_3-*Fucosyltransferase activities in various malignant diseases.* _____ *Upper limit of the normal range* ($\bar{x}$ + 2 *S.D.*) [*LU: lung cancer; B: breast cancer; T: testicular tumors* (○ *seminoma patients,* ● *non-seminomatous germ cell tumors*); *H: Hodgkin's disease; NH: non-Hodgkin's lymphoma; L: leukemia* (● *acute myeloic leukemia,* ○ *chronic lymphatic leukemia*); *V: various tumors* (○ *prostatic cancer,* ▲ *gastrointestinal tumors,* ● *other cancer patients*)].

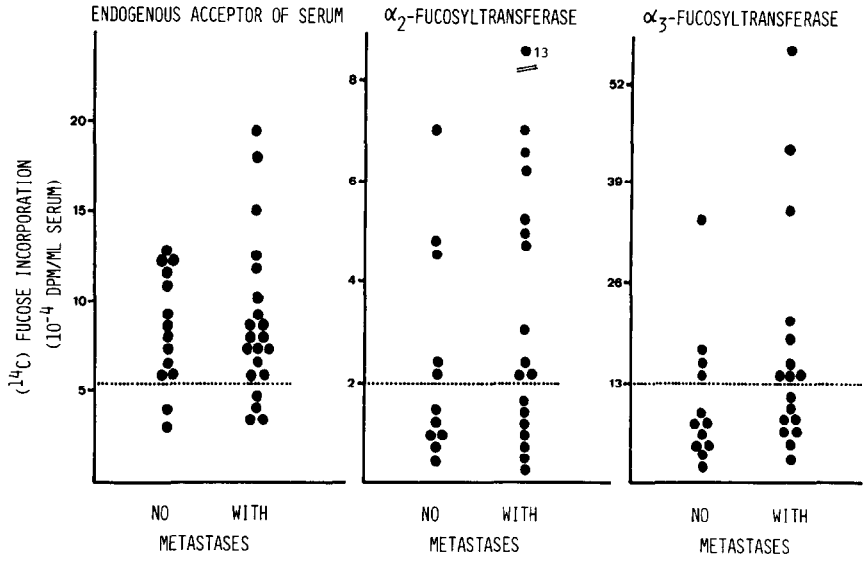

Fig. 5. Fucosyltransferase activities in patients with lung cancer with no evidence of metastases and with metastatic disease. (Endogenous acceptor of serum, α_2- and α_3-fucosyltransferase.) ..... Upper limit of the normal range ($\bar{x}$ + 2 S.D.)

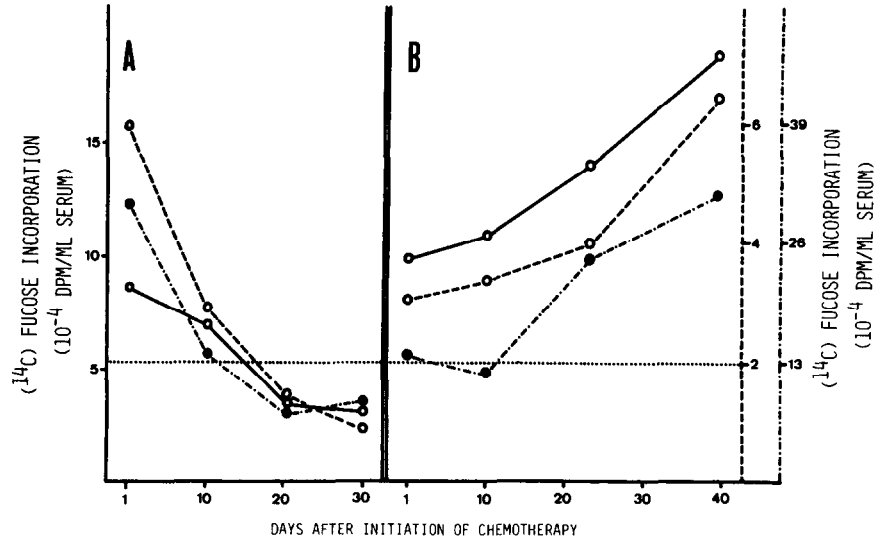

Fig. 6. Fucosyltransferase activities in patients undergoing chemotherapy. (A) Fucosyltransferase activities of a patient with a non-seminomatous metastasized germ cell tumor responding to chemotherapy. (B) Fucosyltransferase activities of a patient with lung cancer non-responding to chemotherapy..... Upper limit of the normal range ($\bar{x}$ + 2 S.D.); O——O endogenous acceptor of serum; O - - - - - O α_2-fucosyltransferase; ●- - - - - ● α_3-fucosyltransferase.

rates into the endogenous acceptor of serum were found in all cases and α_2-fucosyltransferase activities were elevated in 60% of patients.

Three out of 21 patients with testicular tumors and no occurrence of metastases after radical orchiectomy showed elevated α_2-fucosyltransferase activities, two of them also demonstrating elevated incorporation rates into the endogenous acceptor; α_3-fucosyltransferase activities remained within the normal range in these patients.

In 8 patients with Hodgkin's disease in drug-induced complete remission fucosyltransferase activities within the normal range were measured.

After successful chemotherapy a fall of fucosyltransferase activities could be observed. About 2 weeks after the beginning of therapy enzyme activities returned to normal (Fig. 6A). In non-responding patients an increase of fucosyl-transferase activities during and after cytostatic therapy was found (Fig. 6B).

DISCUSSION

Elevated serum fucosyltransferase activities were reported in up to 85% of cancer patients [11, 12], and 'shedding' of membrane-bound enzyme from the malignant cells into the blood has been suggested as a relevant factor [18]. In the present study increased incorporation rates into the endogenous acceptor of serum were found in 75% of patients with malignant diseases and α_2-fucosyltransferase activities were elevated in 67%, but increased α_3-fucosyltransferase activities were detected in only 49% of cancer patients.

Comparison of enzyme activities and tumor burden showed that increased enzyme activities occur in the early stage of the disease as well as in metastatic disease, especially high enzyme activities being found in patients with a rapid progression of disease. In patients with a low proliferation rate of the tumor low or normal fucosyltransferase activities were measured.

Khilanani *et al.* [9] reported increased α_2-fucosyltransferase activities in patients with acute adult leukemia. The authors could demonstrate a correlation of the disease status, percentage of marrow blast cells and plasma levels of the H-gene specified α_2-fucosyltransferase. In non-Hodgkin's lymphoma α_2-fucosyltransferase was reported to be elevated in non-responding patients to chemotherapy and was correlated with estimated tumor burden; normal enzyme levels were found in patients in remission [10]. In accordance with these findings we measured increased incorporation rates into the endogenous acceptor of serum and elevated α_2- and α_3-fucosyltransferase activities in patients with leukemia and non-Hodgkin's lymphoma. After chemotherapy α_2- and α_3-fucosyltransferase activities showed a similar pattern and returned to normal in patients responding to chemotherapy, whereas a slight fall only or even an increase in non-responding patients was found. Our results of a concordant pattern of α_2- and α_3-fucosyltransferase activities are in agreement with Bauer *et al.* [15] but contrast to the findings of Khilanani *et al.* [13], who reported normal α_3-fucosyltransferase levels in untreated leukemia and non-Hodgkin's lymphoma and substantial increases in enzyme levels 3 weeks after drug-induced remission. The authors suggested that the level of α_3-fucosyltransferase is correlated with regeneration of a normal bone marrow population after chemotherapy.

We could confirm the data of Bauer *et al.*, who found elevated α_3-fucosyltransferase activities in various tumors [11, 15], but this enzyme showed a lower sensitivity than α_2-fucosyltransferase [12].

Contrary to the findings of Bauer *et al.* [11, 12], who found elevated α_3-fucosyltransferase activities in patients with teratoma whereas α_2-fucosyltransferase was within the normal range, we measured increased α_2-fucosyltransferase activities in 60% of patients with disseminated testicular tumors whereas occurrence of metastases was indicated by high α_3-fucosyltransferase activities in only 17% of cases. Comparison of fucosyltransferase activities with the well-established tumor markers α-fetoprotein and the β-subunit of human chorionic gonadotropin in disseminated testicular cancer showed that, especially in patients with metastatic seminoma, a tumor which is always α-fetoprotein-negative and human-chorionic-gonadotropin-positive in only about 15% of cases, determination of fucosyltransferase activity (endogenous acceptor of serum, α_2-fucosyltransferase) might provide an additional marker in monitoring seminoma patients [19]. However, it should be mentioned that 3 out of 21 patients with testicular tumors after radical orchiectomy, known to be free of disease, offered elevated fucosyltransferase activities. Two of these false positive results might be explained by a concomitant viral infection.

Determination of fucosyltransferase activities in patients with non-malignant diseases showed that mild increases in fucosyltransferase activities are not a specific marker for the neoplastic origin of the disease and can occur in polycythemia and hemolytic anemia. The elevations of enzyme activities due to cell degradation results in not as high activities as they occur on basis of malignant cell proliferations. Fairly high incorporation rates into the endogenous acceptor of serum were measured in patients with rheumatoid arthiritis, but α_2-fucosyltransferase activities remained within the normal range; in 17% of cases increased α_2-fucosyltransferase activities were measured (Fig. 7). The increased incorporation rates into the endogenous acceptor might be due

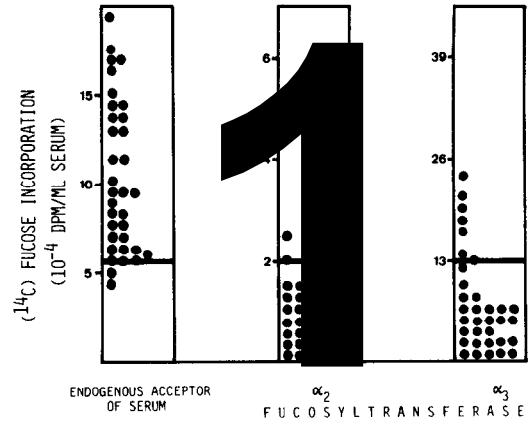

Fig. 7. Fucosyltransferase activities in 34 patients with rheumatoid arthritis. Incorporation of [^{14}C]-fucose into the endogenous acceptor of serum, α_2- and α_3-fucosyltransferase.

to alterations of plasma glycoproteins accompanying rheumatoid arthritis, which may serve as acceptors for [^{14}C]-fucose.

After successful chemotherapy fucosyltransferase activities fall to normal; in non-responding patients only a slight fall or even an increase in enzyme activity can be observed.

Our results indicate that elevations of fucosyltransferase activities are correlated to malignancy and the occurrence of metastases, and the determination of fucosyltransferase activities could provide a good control parameter for the success of chemotherapy. Due to the better sensitivity, especially α_2-fucosyltransferase might be useful in monitoring cancer patients. However, no specificity of fucosyltransferases could be observed; increased enzyme activities were found in all tumor groups studied and cannot be correlated to a certain tumor or a specific histologic type of a tumor.

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