Leukemia-induced Alterations of Serum Glycosyltransferase Enzymes

William J. Kuhns, Roderick T. D. Oliver, Winifred M. Watkins, and Pamela Greenwell

New York University School of Medicine, Department of Pathology, New York, New York [W. J. K.]; Imperial Cancer Research Fund, St. Bartholomew’s Hospital, London, United Kingdom [R. T. D. O.]; Lister Institute of Preventive Medicine, London, United Kingdom [W. M. W., P. G.]

ABSTRACT

Studies on blood group A and H glycosyltransferase enzymes in 54 patients with acute myeloid leukemia were carried out on serum derived from blood samples taken prior to treatment, and in 16 cases, further tests were performed during clinical remission and at the time of relapse. The enzyme assay procedures, using low-molecular-weight compounds as sugar acceptors and radioactive nucleotide sugars as the donor substrates, have been described by Chester et al. (Eur. J. Biochem., 69: 583, 1976). Abnormally low values of H enzyme (expressed as percentage of radioactive sugar incorporated into product; that is, 1 to 3%) were observed in practically all presentation sera, but the values reverted to normal levels (3 to 15%) at the time of clinical remission and then became low again with the development of drug resistance and clinical relapse. A enzyme levels measured in presentation sera which had demonstrated abnormal H enzyme were mostly within the normal range. In 2 of 5 A₁ patients’ sera and in all of three A₂ patients increases in enzyme levels were observed in remission as compared with presentation serum samples. The depression of biosynthetic enzymes in acute leukemia sera could not be accounted for on the basis of competitive inhibitors or catabolic enzymes. It is proposed that changes of serum glycosyltransferase enzymes reflect alterations in a leukemic cell population and that knowledge of these changes may be of value in prognosis in acute leukemia.

INTRODUCTION

Serum glycosyltransferase enzymes have been reported in humans and in a number of infrahuman species (7, 16, 18, 26, 31). Their origin and function in the serum is unknown (11). Presumably, these transferases are released from secretory cells and from disintegrating cells of various types (1, 2). It has been speculated that such enzymes may provide a repair mechanism for erythrocyte surface molecules which are subject to degradation during the circulating life span of the RBC (27). The reason for their alterations in certain cancers is at present unclear (9).

Changed cell surface glycoconjugates have been described in leukemia (21, 30), and there are numerous reports which indicate modification or loss of ABO blood groups in this condition (4, 22-24). Altered cellular and serum glycosyltransferase enzymes have also been demonstrated in the course of this disease (5, 9, 23) and in other abnormal states (19, 20, 32, 34). Abnormally low blood group gene-specified glycosyltransferases have been observed in cases of acute leukemia, and at times, they have been accompanied by blood group deficiencies or losses (23). Other investigators have found unusually high levels of certain serum transferases in untreated leukemia patients using high-molecular-weight acceptors (9).

The experiments described herein were carried out using the low-molecular-weight acceptor 2'-fucosyllactose (6) for A transferase assays and phenyl-β-D-galactoside for H transferase assays (3). The results of tests carried out on sera from 54 patients with acute leukemia and from normal blood donors revealed that the H and occasionally the A blood group glycosyltransferase enzymes were abnormally low in presentation blood samples derived from patients with acute leukemia. Transferase values frequently changed to normal during clinical remission induced by treatment and in most instances reverted to a low value during relapse. Sera from patients with chronic leukemia, reticulum cell sarcoma, and carcinoma exhibited values mostly within the normal range.

MATERIALS AND METHODS

Patients and Patient Material

Frozen blood serum samples from untreated patients with acute leukemia were kindly provided by Dr. David Galton, Royal Post Graduate Hospital, London, and Dr. P. L. Mollison, St. Mary’s Hospital, London. Studies by us on the temperature stability of these glycosyltransferases have indicated that their activity is maintained in frozen storage over prolonged periods of time. Specimens from patients who were serially studied at presentation, in clinical remission, and in relapse were made available through the kindness of Dr. Sylvia Lawler, Royal Marsden Hospital, London, and the late Dr. Gordon Hamilton-Fairley, St. Bartholomew’s Hospital, London. Serum specimens were also obtained from patients with acute leukemia, chronic leukemia, reticulum cell sarcoma, and carcinoma seen at the New York University, Bellevue Hospital, and Manhattan Veterans Administration Hospital, New York, N. Y. High levels of peripheral blood blasts were commonly encountered in patients with acute leukemia prior to the institution of antileukemic therapy. Sera for studies of normal transferase enzyme values were derived from whole blood specimens of volunteer donors.

Radioactive Nucleotide Sugars and Low-Molecular-Weight Acceptors

Labeled UDP-N-acetyl[14C]galactosamine (43 Ci/mol) was purchased from the New England Nuclear, Frankfurt, Germany, and GDP-L[14C]fucose (170 Ci/mol) was obtained from the Radiochemical Centre, Amersham, England. The low-molecu-
lar-weight group acceptor 2'-fucosyllactose used for A transferase assays [α-L-fucosyl(1 → 2)-β-D-galactosyl(1 → 4)-D-glucoside] was isolated from human milk (13). The low-molecular-weight acceptor for H transferase assays, phenyl-β-D-galactopyranoside (3), was obtained from the Sigma Chemical Co.

Assays for Serum Glycosyltransferases

A Transferases. The α-3-N-acetylgalactosaminyl transferases (A enzymes) were assayed at both pH 6 and pH 8 to provide the conditions required for the differentiation of the A1 and A2 gene-specified transferases (25, 28). Details of the reaction mixtures and incubation times are given in the charts. All sera were tested immediately following their reconstitution from the frozen state. Multiple or serial samples derived from a single patient were tested at the same time. At the end of the incubation, the reaction mixtures were streaked onto Whatman No. 40 filter paper and dried, and the total radioactivity was measured in the liquid scintillation counter. Neutral sugars were removed from charged components by resolution on ion-exchange papers as described (6, 28). The neutral sugars were chromatographed on Whatman No. 40 filter paper in Solvent A [ethyl acetate:pyridine:water (2:1:2 by volume, upper phase)]. Under these conditions, residual isotope-labeled nucleotide sugar (UDP-N-acetyl[14C]galactosamine) was readily separated from acceptor:sugar product. Radioactive peaks were detected with a radiochromatogram scanner and counted in a liquid scintillation counter. The A1 and A2 enzymes transfer N-acetyl-[14C]galactosamine to the acceptor substrate 2'-fucosyllactose to give a tetrasaccharide product with an Ractose of 0.5 in Solvent A (6). The radioactivity incorporated into the tetrasaccharide product was expressed as a percentage of the total radioactivity in the incubation mixture (3) and as μmol radioactive sugar incorporated into acceptor:substrate.

H Transferases. The α-2-L-fucosyltransferase (H enzyme) was assayed as described by Chester et al. (3). Details of the reaction mixtures and incubation times are given in the charts. The radioactive products were chromatographed on Whatman No. 40 paper in Solvent B [ethyl acetate:pyridine:water (10:4:3 by volume)], a mixture which permitted separation of isotope-labeled components into residual GDP-[14C]fucose and acceptor:sugar product. The product of [14C]fucose from GDP-[14C]fucose into endogenous acceptor without phenyl-β-D-galactoside. The reaction products were detected with a radiochromatogram scanner and counted in a liquid scintillation counter. To test whether there was competition between incorporation of [14C]fucose from GDP-[14C]fucose into endogenous acceptor substrates and the low-molecular-weight acceptor used in the H transferase assay, incubation mixtures were set up with and without phenyl-β-D-galactoside. The reaction products were broken down under the conditions used to assay the α-2-fucosyltransferase, 5.0 μmol of this substrate were incubated for 5 hr with 20 μl serum plus ATP, Tris-HCl buffer, pH 7.2, and NaNO3 at the concentrations used in the reaction mixture for the H transferase assay (see Chart 1). The product of [14C]fucosyltransfer, phenyl 2-O-[α-L-[14C]fucopyranosyl]-β-D-galactopyranoside (5000 cpm) was similarly incubated with 20 μl of serum and other constituents of the reaction mixture except GDP-[14C]fucose. At the end of the incubation time, the reaction products were examined by descending chromatography on Whatman No. 40 paper in ethyl acetate:pyridine:water (10:4:3 by volume) for 5 hr. The products obtained with phenyl-β-galactoside were visualized with alkaline silver nitrate (29). The radioactive products obtained with phenyl-β-D-galactoside were detected with a radiochromatogram scanner and counted in a liquid scintillation counter.

Incorporation of [14C]Fucose into Endogenous Acceptors in Control Sera and Sera from Leukemic Patients

Additionally, to determine whether the substrate for the H transferase assays, phenyl-β-D-galactopyranoside, was being broken down under the conditions used to assay the α-2-fucosyltransferase, 5.0 μmol of this substrate were incubated for 16 hr at 37°. ATP, Tris-HCl buffer, pH 7.2, and NaNO3 at the concentrations used in the reaction mixture for the H transferase assay (see Chart 1). The product of [14C]fucosyltransfer, phenyl 2-O-[α-L-[14C]fucopyranosyl]-β-D-galactopyranoside (5000 cpm) was similarly incubated with 20 μl of serum and other constituents of the reaction mixture except GDP-[14C]fucose. At the end of the incubation time, the reaction products were examined by descending chromatography on Whatman No. 40 paper in ethyl acetate:pyridine:water (10:4:3 by volume) for 5 hr. The products obtained with phenyl-β-galactoside were visualized with alkaline silver nitrate (29). The radioactive products obtained with phenyl-[14C]fucosyl-β-D-galactoside were detected with a radiochromatogram scanner and counted in a liquid scintillation counter.

Glycosidase Activities in Serum

α-L-Fucosidase and β-D-galactosidase activities in normal and leukemic sera were assayed with p-nitrophenyl-α-L-fucopyranoside and p-nitrophenyl-β-D-galactopyranoside, respectively. The nitrophenyl glycosides (0.33 μm; 50 μl) were incubated for 2 hr at 37° with 50 μl of serum and 500 μl of 0.1 M Tris-HCl buffer, pH 7.2. At the end of the incubation time, the reaction was stopped by the addition of 1.5 ml of sodium bicarbonate, and the absorbance was read in 1-cm light path cells at 420 nm in a Unicam AP. 600 spectrophotometer.
examined by descending chromatography on Whatman No. 40 paper in Solvent C [propan-1-01-ethy acetate:pyridine:water (5:1:1.3 by volume)] for 16 hr. In this solvent, the neutral endogenous products remain on the origin whereas UDP-fucose had an R_f value of 0.42, and phenyl 2-O-(α-L-[1^4C]fucosyl)-β-d-galactoside had an R_f value of 1.2.

RESULTS

Serum GDP-β-Fucose: β-α-Galactosyl-α-2-L-Fucosyltransferase from Normal Persons and from Patients with Cancers. The presence of α-2-L-fucosyltransferase (H enzyme) was demonstrated by the transfer of L-fucose from radioactive GDP-L-[1^4C]fucose to the low-molecular-weight derivative of D-galactose, phenyl-β-d-galactopyranosyl. The reaction mixture is given in Chart 1. The percentage of radioactive L-fucose incorporated into product (2-O-(α-L-fucosyl)-β-d-galactopyranoside) was variable over a 3- to 4-fold range as shown in Chart 1. Fifty-seven normal Group A sera yielded H enzyme values which ranged from 4.7 to 14.6% (0.25 to 0.79 µmol [1^4C]-fucose incorporated into acceptor substrate) and a mean value of 8.8% or 0.48 µmol incorporated (S.D., 0.6). Thirty-one Group A sera gave H enzyme values ranging from 7.8 to 19.2% (0.42 to 1.04 µmol incorporated) and a mean value of 13.5% or 0.73 µmol incorporated (S.D., 0.7) (Chart 1). The following were demonstrated in 10 A2 sera and 36 B sera: A2, 10.6 to 19.1 (0.58 to 1.03 µmol incorporated), mean, 13.7 or 0.74 µmol incorporated (S.D., 0.7); B, 4.5 to 14.0 (0.24 to 0.76 µmol incorporated), mean, 9.9 or 0.54 µmol incorporated (S.D., 0.6). H enzyme values in presentation samples from acute leukemia cases were low in comparison to normal values in Groups O and A patients. Thus, 31 sera from Group O patients with acute leukemia had H enzyme values from 0.4 to 2.5% (0.02 to 0.14 µmol incorporated) and a mean of 1.4% or 0.08 µmol incorporated (S.D., 0.07). The values in 21 sera from Group A patients with acute leukemia were as follows: range, 0.7 to 6.5% [0.04 to 0.35 µmol incorporated]; mean, 2.1% or 0.11 µmol incorporated (S.D., 0.09) (Chart 1). In patients with carcinomas, H enzyme values within the normal range were found in 7 of 10 sera from Group A patients and 11 of 16 sera from Group O patients. No changes in enzyme values were observed either following surgical resection of these lesions or following the institution of chemotherapy, and enzyme values from 3 patients with reticulum cell sarcoma were within the normal range.

Relationship of α-2-L-Fucosyltransferase Enzyme Levels to Peripheral Blood Blast Counts. Twenty-three sera were collected from patients whose blood was studied simultaneously for enzyme and for the presence of total leukocytes and blast cells. The studies were carried out at the time of presentation or prior to the initiation of treatment. The presence of peripheral blast cells was correlated with abnormally low H enzyme values. Blast counts from 12 to 95% were associated with enzyme values which ranged from 0.05 to 2% (0.0027 to 0.01 µmol) [1^4C] incorporation. Five cases in which initial blast counts ranged from 2 to 5% exhibited H enzyme values ranging from 3 to 6% [1^4C] incorporation (0.16 to 0.32 µmol incorporated).

Serum UDP-N-acetyl-α-D-galactosamine: α-L-Fucosyl(1→2)-β-D-Galactose-N-Acetyl-α-D-Galactosaminyl Transferase from Normal and Leukemic Individuals. Group A transferase enzymes were assayed by the transfer of N-acetylgalactosamine from radioactive UDP-N-acetyl-d-[1^4C]galactosamine to the tetrasaccharide 2-fucosyllactose. Details of the reaction mixtures are given in the Chart 2 legend. On the basis of agglutination reactions, the normal donors were classified as follows: 79 as A1 and 26 as A2. With sera from normal Group A1 donors, the amount of radioactive N-acetylgalactosamine incorporated into the tetrasaccharide product at pH 6 ranged from 8.0 to 25.6 (0.16 to 0.50 µmol [1^4C]-N-acetylgalactosamine incorporated into acceptor substrate) with a mean of 14.5% or 28 µmol incorporated (S.D., 0.8). With sera from normal A2 individuals, lower incorporation levels were noted, and as expected, the values were higher at pH 8 than at pH 6 (25). The values at pH 6 for 26 A2 sera ranged between 1.6 and 9.7% (0.03 to 0.19 µmol incorporated) with a mean of 4.5% or 0.09 µmol incorporated (S.D., 0.2) and between 3.7 and 13.8 at pH 8 (0.07 to 0.27 µmol incorporated) with a mean of 8.5% or 0.17 µmol incorporated (S.D., 0.6). Acute leukemia sera from Group A persons demonstrated presentation values within the normal or low normal range.

Serum GDP-β-Fucose: β-α-Galactosyl-α-2-L-Fucosyltransferase from Patients with Acute Leukemia Studied at Presentation, during Clinical Remission following Therapy, and in Relapse. Sixteen patients were available for serial investigations. Of these, 9 were Group A, and 7 were Group O. The H enzyme levels in the presentation sera were generally in the low or below normal range, but values either increased or reverted to normal at the time of clinical remission. The most striking increases at remission were observed for the H enzyme in group A individuals. Of 5 Group A patients whose sera at the time of relapse were available for examination, 3 exhibited...
marked lowering in transferase levels in comparison with remission values. The enzymes in the sera of 5 of 6 Group O patients showed slight decreases at relapse in comparison with remission values. It was of interest that α-2-L-fucosyltransferase attained higher levels in Group A patients in remission than in Group O persons, thereby reflecting the tendency towards higher H transferase levels in normal Group A individuals than in normal Group O individuals and untreated leukemic patients.6 Detailed chronological studies were carried out in 4 patients who were transfused at the time of presentation and whose sera were frequently assayed for H enzyme during clinical remission and relapse (Charts 3 to 6). In each of these cases, a close relationship was observed between clinical remission and H enzyme values in the normal range and, on the other hand, clinical and laboratory evidence of acute leukemia and abnormally low H enzyme values.

**Modes of Therapy in 14 Leukemic Patients Whose Serum α-2-L-Fucosyltransferase Levels Were Studied before and during Clinical Remission and at Relapse.** Details of chemotherapy were available in the patients studied sequentially for serum α-2-L-fucosyltransferase enzyme. Daunorubicin, cytarabine, thioguanine, and cyclophosphamide were all used in 15 of 16 patients. Other antimitabolites and/or immunotherapy (35) were also used in 10 of these patients. Charts 3 through 6 illustrate the sequence of therapy in relationship to peripheral leukocyte and blast cell counts and to the levels of serum α-2-L-fucosyltransferase in 4 patients. The reciprocal character of enzyme level versus peripheral blast counts is readily observed and may reflect the cytotoxic effects of chemotherapy at first remission or the emergence of a drug-resistant cell population at relapse.

**Serum UDP-N-Acetyl-O-Galactosamine: α-L-Fucosyl-O-Galactosyl α-N-Acetylgalactosaminy Transferase in Serially Studied Patients with Acute Leukemia.** Samples from 8 patients were available for serial studies. In 4 patients designated A1, small or no enzyme differences were demonstrated between assays (both pH 6 and pH 8) carried out on samples obtained at remission and relapse in comparison with presentation specimens. Sera from a fifth patient demonstrated an exceptionally high rise in remission in A3 enzyme (pH 6 values) which reverted at the time of relapse. In 3 patients designated A2 on the basis of the enzyme assay patterns at pH 6 and pH 8, increased levels of A transferase were found in each instance in the remission samples.

**α-L-Fucosidase and β-O-Galactosidase Activities in Normal and Leukemic Sera.** Leukemic and control sera were tested for α-L-fucosidase activity on the acceptor:sugar product formed in the H transferase assays, that is, phenyl 2-O-[^14]C]fucosyl β-O-galactoside. The radioactive acceptor:sugar complex used in each assay (1100 cpn/3 nmol) was derived by chromatographic separation from reaction mixtures as described under Chart 1 and then eluted in water and concentrated. Under the conditions used in the standard assay procedure, no liberation of[^14]C]fucose was observed when either 4 control sera or presentation and remission sera from 4 leukemic patients were tested for fucosidase activity, but a known source of α-L-fucosidase (33) was capable of liberating[^14]C]fucose from the acceptor:sugar product. In one case, samples from a patient with acute leukemia were tested with p-nitrophenyl-α-L-fucoside or β-O-galactoside as substrate. Results of tests and control were comparable, confirming results obtained by incubation of serum product or serum acceptor mixtures. This indicated that the low levels obtained in assays for α-2-fucosyltransferase were not attributable to breakdown of the product by fucosidase or by galactosidase.

Direct tests with 4 normal control sera and sera from presentation and remission sera from leukemic patients on phenyl β-O-galactoside acceptor itself failed to reveal any breakdown of the substrate since the latter functioned normally in enzyme assays following preincubation with serum samples. In these experiments, separate serum:acceptor mixtures incubated under conditions described in Chart 1 were carried out using normal or leukemic serum, or acceptor alone was incubated. Acceptor which was derived chromatographically from each incubation was utilized in H enzyme assays with a serum which in prior tests had been shown to contain a normal content of enzyme. Thus, the low α-2-L-fucosyl-transferase values in the presentation leukemic samples did not appear to be attributable to increased activity of β-galactosidase which was destroying the acceptor substrate phenyl-β-O-galactoside.

**Effect of Endogenous Acceptors on the Incorporation of[^14]C]Fucose into the Low-Molecular-Weight Acceptor Phenyl-β-O-Galactoside.** Reaction mixtures for the H transferase assay were set up in the presence and absence of the low-molecular-weight acceptor phenyl-β-O-galactoside, and the products were examined by chromatography in Solvent C which separated endogenous high-molecular-weight substances from other components of the reaction mixture including GDP[^14]C]fucose. The level of endogenous incorporation of[^14]C]fucose with presentation and remission serum samples from leukemic patients and with control sera was lower in the presence of phenyl-β-O-galactoside than in the absence of this acceptor (Chart 7; Table 1). The low-molecular-weight acceptor therefore appeared to be competing very effectively with the endogenous high-molecular-weight acceptors for the[^14]C]fucose transferred from GDP[^14]C]fucose. The decreased levels of α-2-L-fucosyltransferase activity in the presentation samples from the leukemic patients was therefore not related to the inability of phenylgalactopyranoside to compete with an increased level of endogenous acceptors.

The low-molecular-weight acceptor assay involves ethanol precipitation of the incubation mixture. Assays carried out on washed precipitates derived from normal and leukemic incubation mixtures failed to show any significant differences. The low levels of radioactivity in the precipitates indicated that this precipitable portion of the incubation mixture possessed little or no endogenous acceptor (Chart 7; Table 1).

**Effects upon Normal H Enzyme Activity of Mixtures of Normal and Leukemic Sera.** Enzyme assays were carried out as described previously except that for each assay mixtures were prepared which contained equal amounts of patients’ serum and normal Group O serum. The latter serum diluted with an equal quantity of 0.1 M Tris-HCl buffer at pH 7.2 served as a positive control. Tests of 10 presentation sera: normal serum mixtures failed to demonstrate suppression of enzyme in comparison with normal serum controls. It was concluded from these findings that sera from untreated patients with acute myeloid leukemia did not possess an inhibitor for normal H enzyme expression.

---

Relationship of Serum Glycosyltransferase to Total Serum Proteins. Total serum proteins were evaluated in 45 presentation samples from Groups O and A patients with acute leukemia, and the results were related to levels of $H$ enzyme in these persons. The relationship of total proteins to $A$ enzyme levels in 9 patients was similarly studied. Normal control sera from 60 Group O persons and from 10 Group A persons were examined for comparative purposes. The range in total protein in acute leukemic patients (5.7 to 8.1 g/dl) was greater than that observed in normal sera (6.6 to 7.9 g/dl). No correlations could be established between protein levels and $A$ or $H$ glycosyltransferase enzyme values.

DISCUSSION

The cause for a specific deficiency of $\alpha$-2-fucosyltransferase ($H$ enzyme) in acute myeloid leukemia is at present unknown. It is possible that gene suppression induced by leukemogenic agents causes abnormally low $H$ enzyme levels in presentation blood samples from patients with acute leukemia. Such a lesion is apparently reversible as judged by the change back to normal levels, which occur at the time of clinical remission. This situation appears to be temporary since deficiencies are again encountered with the onset of drug resistance and clinical relapse. Such alterations, more closely correlated with
Clinical laboratory findings and the schedule of treatment, may be of use in prognosis and in the formulation of altered drug regimens as suggested for fucosyltransferases by Khilanani et al. (9, 10).

Correction of anemia, although at times temporally related to changed enzyme levels, would appear not to be causally related. Massive blood transfusions equivalent to 2 exchanges have failed to alter abnormally low enzyme values in a patient tested for H enzyme within 24 hr of transfusion. We have also found serum H enzyme to be within the normal range in cases of carcinoma and chronic leukemia accompanied by profound anemia. Furthermore, analyses of specific cell types have indicated that mature erythrocytes possess an extremely low H enzyme content in comparison, for example, to granulocytes. Extracts prepared by us from equal numbers of normal granulocytes and acute myeloid leukemic leukocytes and tested for H enzyme indicated that leukemic cells were enzyme deficient in comparison to normal cells (14).

Since combination chemotherapy is ordinarily administered to these patients, temporal relationships referring to effects of a single agent upon H enzyme are difficult to achieve. However, low enzyme levels were demonstrated prior to any chemotherapy in presentation samples, and they increased upon clinical remission, presumably the result of chemotherapy. It is reasonable to believe that the drug-related reduction in tumor burden and repopulation by normal myeloid cells is closely related to
$H$ enzyme changes, particularly in view of our studies of cell extracts (cited above). It is difficult to rule out the possibility that low enzyme values in relapse were due to inhibition by chemotherapy. Specific experiments to assess the effects of these tumor agents would be required.

As stated above, deficiencies of $\alpha$-2-L-fucosyltransferase have been demonstrated in homogenates or extracts derived from acute leukemic leukocytes from Group O patients when compared with material derived from equal quantities of normal granulocytes (14). If correlations can be established with serum transferase enzyme levels, the latter may prove useful as a screening technique to designate alterations in a predominating cell population. The reciprocal nature of peripheral blast counts and serum transferase levels in the present studies are in support. The findings of Khilamani et al. (10) in regard to another form of fucosyltransferase suggest a similar relationship.

It would be of importance to know whether these findings are confined to acute leukemia, a question which should be resolved by additional observations on the glycosyltransferase levels in leukemia and other disease states. Limited tests with sera from individuals with carcinomas and chronic leukemias indicated values within the normal range, but detailed studies during blast crisis in the latter conditions have not yet been carried out. It is possible that defective glycosyltransferase enzymes represent only a few of many proteins altered in the course of acute leukemia (8) or that individual enzymes react differently in the presence of leukemogenic agents (9, 10). Studies of disorders other than acute myeloid leukemia but characterized by myeloid cell subpopulation shifts may be helpful in correlation of $H$ enzyme with cell morphology or cell type. It was of interest that within the group of glycosyltransferase enzymes studied in untreated leukemic patients deviations of $H$ enzyme from normal were far greater than were those observed for $A$ enzymes and that within the group of $A$ persons, values in $A_2$ patients were subnormal in contrast to those found in $A_1$ persons.

The interpretation that leukemogenic mechanism(s) suppresses $ABO$ genes is supported by observations that $ABO$ antigens are weakened or missing in acute myeloid leukemia (4, 22-24). The frequency of such a finding is difficult to assess, since quantitative scoring has not been customarily carried out. In instances where agglutination has been scored, the frequency of weakened $H$ antigen has been relatively high (15, 24) but still not comparable to the very high incidence of abnormal serum transferase enzymes observed in our studies. $H$ transferase appears to be derived entirely from the hematopoietic tissue since persons of the para-Bombay phenotype, whose RBC lack $H$ activity but who nevertheless secrete $H$

![Diagram: Chart 7. Assays were performed as described under Chart 1 and "Materials and Methods." Incubation products were examined by descending chromatography in Solvent B. Chromatographic separation of endogenous acceptor from nucleotide sugar was carried out using Solvent C (95% ethanol:1 M ammonium acetate, pH 7.0 to 3 by volume). Each calculation represents the result of 3 determinations.]

<table>
<thead>
<tr>
<th>Designation of $^{14}$C-labeled components</th>
<th>% of $^{14}$C Incorporation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Washed ethanol</td>
<td>Endogenous acceptor ethanol supernatant</td>
</tr>
<tr>
<td>Normal 1</td>
<td>56520$^{c}$</td>
</tr>
<tr>
<td>Normal 1$^{a}$</td>
<td>68444</td>
</tr>
<tr>
<td>Leukemia 1</td>
<td>69233</td>
</tr>
<tr>
<td>Leukemia 1$^{a}$</td>
<td>65017</td>
</tr>
<tr>
<td>Leukemia 1R$^{b}$</td>
<td>56251</td>
</tr>
<tr>
<td>Leukemia 1RA$^{a}$</td>
<td>61825</td>
</tr>
</tbody>
</table>

$^{a}$ Expressed as cpm.

$^{b}$ Phenyl-2-O-['$^{14}$C]fucosyl-$\beta$-p-galactoside.

$^{c}$ Assays performed as described under Chart 1 and "Materials and Methods." Chromatographic separation of $^1$C-labeled components was carried out using Solvent B. Extracts from washed precipitates were prepared by solubilizing each precipitate with 10x volume 0.1% Triton X-100 and extracting 16 hr at 4°C. The resultant solution was then utilized as putative endogenous acceptor. The residual [$^1$C]GDP-[L-['$^{14}$C]fucose peak derived from incubation mixtures was eluted in minimal volume and utilized as putative ethanolic supernatant endogenous acceptor. Chromatographic separation of endogenous acceptor from nucleotide sugar was carried out using Solvent C (95% ethanol:1 M ammonium acetate, pH 7.0; 7.5 to 3 by volume). Each calculation represents the result of 3 determinations.
substance in saliva, do not have any demonstrable H enzyme activity in their sera (17). On the other hand, hematopoietic tissue is believed to contribute only 20 to 30% of the Blood Group A and B glycosyltransferases present in serum and the remainder is derived from other sources (12, 36).

From such observations and from those which indicate low H enzyme levels in acute myeloid leukemic cells, we believe it possible that serum H enzyme losses in untreated acute myeloid leukemia reflect a dilution process on the basis of enzyme-deficient material shed from dead and disintegrating tumor cells (2). This process may be reversed in remission when the augmented enzyme yield from shed normal cell fragments is reflected as an increase in serum H enzyme. Such a process would not necessarily implicate erythrocytes or their precursors, but enzyme data on these cell forms would be required in proof.

A high-molecular-weight assay for a-2-L-fucosyltransferase was studied in leukemic sera by Khilanani et al., (9). The authors measured the levels in plasma of a transferase in 18 patients with acute adult myeloid leukemia at various clinical stages in conjunction with simultaneous bone marrow aspirations and biopsies. Desialysed fetuin, a high-molecular-weight acceptor, was utilized in these enzyme assays, and from previous studies involving the use of this acceptor, it was assumed that the enzyme assayed was the H gene specified a-2-L-fucosyltransferase. Patients in remission, however, had significantly lower levels of this enzyme than did nonresponding or relapsing patients, and elevated plasma levels of enzymes in presentation samples could be correlated with the percentage of marrow blast cells. These results are completely at variance with our findings utilizing the low-molecular-weight acceptor phenyl-β-D-galactoside. Kessel recently carried out electrofocusing patterns and found 2 forms of activity. The major activity which is elevated in acute myeloid leukemia focuses at pH 4.7, but the H gene-specified enzyme focuses at pH 5.1 and is subnormal in acute myeloid leukemia.

REFERENCES

Leukemia-induced Alterations of Serum Glycosyltransferase Enzymes


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/40/2/268

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pub@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.