Diagnostic and Prognostic Value of RNA-Proteolipid in Sera of Patients with Malignant Disorders following Therapy: First Clinical Evaluation of a Novel Tumor Marker

Andreas J. Wiecezorek, Vetury Sitaramam, Werner Machleidt, Kaspar Rhyner, André P. Perruchoud, and Lutz H. Block

ABSTRACT

The circulating level of a novel RNA-proteolipid complex associated with malignant diseases was critically evaluated as a tumor marker in clinical oncology. The complex, isolated from the sera of cancer patients, exhibited unvarying chemical composition regardless of the cell type and clinical staging. Clearance from blood was rapid with a half-life of approximately 2 days. Tumor mass could be correlated with the circulating level. After effective treatment the level fell and rose again 10 months prior to the conventional clinical diagnosis of relapse.

INTRODUCTION

We recently reported the isolation and the characterization of a novel RNA-proteolipid complex from the sera of patients with malignant disorders, as well as from the culture media of several malignant cell lines (1). The striking correlation between the malignant state and the detectability of this complex (94 positive of 96 independent cases of 27 types of malignancy, of which 92% were postdiagnostic) indicated the need to evaluate the diagnostic and prognostic value of this new marker as soon as possible. Of particular importance were the following queries, (a) Does the composition of the complex vary with the type of tumor? (b) Do the circulating levels depend on the tumor mass and/or cell type? (c) How sensitive is the marker as an indicator of relapse after remission following active therapy, compared to current oncological practice? (d) What is the kinetics of the complex in circulation?

We report here preliminary clinical data on the circulating levels of the complex with a specific bearing on the above questions. The circulating level of the RNA-proteolipid complex seems to fulfill several criteria for a potentially important clinical marker in clinical oncology, for both diagnostic and prognostic purpose. Further broad-based epidemiological studies are necessary before a final conclusion can be reached.

MATERIALS AND METHODS

Isolation of the RNA-Proteolipid Complex

Sera [3 ml] were mixed with KBr [0.3517 g; final density, 1.225 g/ml] and were layered below a discontinuous KBr gradient [1.006–1.221 g/ml, with increments as given elsewhere (2)]. The samples were centrifuged for 16 h at 4°C at 105,000 × g to obtain a clearly demarcated opalescent fraction. This fraction was individually collected by suction and dialyzed in benzoyl cellulose bags against isotonic saline solution (4°C, 24 h, 3 changes).

RNA Extraction and Determination

Routine methods for measurement of cellular RNA are not suitable for the extraction of RNA from the complex, since the small lipophilic peptides are too tightly bound to the RNA. Portions (0.5 ml) of the opalescent band were suspended in ethanol (1 ml) and centrifuged (6000 × g, 15 min, 23°C). To remove the residual lipids and lipophilic peptides, the resulting pellet was extracted twice with 2 ml chloroform:methanol:H2O (60:40:9, v/v) and finally with 2 ml chloroform:methanol (1:1, v/v), acidified to pH 3 with HCl, and again centrifuged. The pellet was extracted with 1 ml isotonic saline at 4°C overnight under sterile conditions. The RNA derived from 3 ml serum could now be cryoprecipitated by addition of 2 ml ethanol and isolated by centrifugation. It was then dissolved in 0.6 ml saline and the UV spectrum was read at 300–220 nm with a Beckman double-beam spectrometer. Solutions of yeast tRNA were used as standard.

Lipid Analysis

Combined solvent phases from the RNA isolation procedure were neutralized with NaOH and evaporated to dryness in a vacuum. The residue was dissolved in chloroform:methanol (1:1, v/v). Lipids were analyzed together with standards (Sigma; Seromed, Munich, Federal Republic of Germany) on Silica Gel H (Merck), in chloroform:methanol:H2O (60:35:8, v/v) and hexane:diethyl ether (70:30, v/v) as solvents. Cholesterol was analyzed by gas chromatography with stigmasterol as standard (3). Phospholipids were isolated by chromatography and determined by the method of Bartlett (4). In some samples, triglycerides were determined according to the method of Chernick (5).

For the isolation of the peptides and glycolipids, aliquots were applied to a cellulose column (10x1 cm) and eluted with 70% n-propyl alcohol at a flow rate of 0.2 ml/min. The peptide-containing fractions were quantified according to the method of Lowry et al. (6) with bacitracin as standard.

Glycolipids were identified after Silica Gel H chromatography using chloroform:methanol:H2O (60:40:8, v/v) as solvent and visualized by use of orcinol. Standard glycolipids were globoside and ceramide pentadecasaccharides from human erythrocyte blood group A prepared according to the methods of Stellner and Hakomori (7) and Hakomori et al. (8). The predominant glycolipid coincided with that of hexa-saccharide.

Quantitative analysis of the glycolipid fraction was performed by gas chromatography of the trimethylsilylated methyl glycosides, according to the method of Vance and Sweeley (9). The peptide fraction was further analyzed for its amino acid composition (see below).

Determination of Amino Acid Composition and Sequence of the Oligopeptides Associated with the Complex

The purified oligopeptides yielded a single hydrophilic peak in reverse phase high-pressure liquid chromatography. Oligopeptides were treated with 6 N HCl at 105°C for 24 h with and without performic acid oxidation (10). Tryptophan was determined in samples hydrolyzed in the presence of 2% thioglycolic acid (11). Amino acid analysis was performed using the Kontron Liquimat II with o-phthalaldehyde detec-
tion. Results were corrected for destruction and incomplete hydrolysis. The results are expressed as mol %. In an attempt at sequence analysis of the oligopeptide, the oligopeptide fraction was coupled to aminopropyl glass via its carboxyl group using N-hydroxysuccinimide-catalyzed carbodiimide activation (12). Degradation was performed according to the method of Edman (13).

Clinical Studies

Selection of Cases. Forty-eight cases of malignant disorders (Table 1) were studied over the last 4 years, selection of cases depending solely on availability, without any conscious bias. The clinical management was entirely independent of the current investigations.

Evaluation of Disease Status. Standard clinical protocols were used. The extent of the disease was determined on the basis of the complete history and on physical examination, hemogram, bone marrow aspiration, and cytocentrifugation for WBC morphology; and radionuclide or computer tomography of the liver, spleen, and bone. A few patients also underwent laparotomy for diagnostic or palliative purposes.

The malignancy was classified as localized, when an extensive clinical survey showed it to be anatomically localized in a single extranodal site, with or without positive regional lymphnodes on one or two adjacent lymphatic regions. In the case of tumors arising in the gastrointestinal tract or lung, the criterion for localization of the tumor was its amenability for grossly complete excision. Histopathological diagnosis was the basis of classification of tumors in all instances. Clinical staging was carried out for lymphomas according to the Ann Arbor classification (14).

Isolation of the RNA-Proteolipid Complex. The levels and the composition of the complex were studied in the 34 cases with an established diagnosis of lymphoma. The diagnosis was established according to the criteria of Rappaport (15): 9 patients with histiocytic lymphoma; 8 with diffuse mixed lymphoma; 7 with poorly differentiated lymphoma; and 10 with well-differentiated lymphocytic lymphoma (24 males, 10 females; age range, 15–73 years; mean age, 44 years). None received prior treatment; only 18 of 34 patients had constitutional symptoms such as fever, night sweats, or weight loss.

Short-Term Follow-up. A total of 18 cases were selected for short-term follow-up following therapy (with an overlap of 5 cases from the above group). They were assigned on clinical grounds to various modes of therapy: 5 cases to chemotherapy; 6 to radiation therapy; and 7 cases of localized tumor to surgery. Details of the individual cases are given in the legends to the relevant figures.

Determination of the Tumor Mass. Tumor cases were chosen on the following grounds: (a) those diagnosed to be solitary and nonmetastatic by computer-aided tomography or diagnostic ultrasound; (b) for the attempt to find out the relationship between tumor mass and circulating levels of RNA-proteolipid, the cross-sectional study required at least 3 widely different sites of tumor of the same histological type.

Long-Term Follow-up after Chemotherapy. In 16 cases, the patients were monitored for the circulating levels of the complex and for possible relapse following chemotherapy. The choice of cases was based on clinical evaluation of the possibility of a relapse, so that the effect of a relapse on the dynamics of the complex in circulation could be investigated in as many cases as possible. There were 8 surviving cases with a clinically identified relapse following successful therapy and remission of the tumor, in a total follow-up period of 40 months. The clinical diagnosis of relapse was that of the attending oncologist, based on routine clinical grounds in an ambulatory clinic.

RESULTS

Invariance of the Gross Composition of the RNA-Proteolipid Complex in Malignant Disorders. We reported earlier that the RNA-proteolipid complex appears to be a secretory product of the malignant cells and that it is not likely to be a degradation product (1). This was based on: (a) inhibition of progressive accumulation in culture media of malignant cell lines by cytochalasin B and monensin; and (b) immunological cross-reactivity of antibodies raised against the complex isolated from sera and the culture media of malignant cell lines. It was therefore essential to demonstrate that the composition of the chemically heterogeneous complex remained relatively constant in terms of its major constituents (RNA, oligopeptide, and lipids) and that possible contamination with apolipoproteins A and B was negligible.

As can be seen in Table 2, the values for RNA, oligopeptide, phospholipid, and glycosphingolipid have coefficients of variation of only 5% in all the cases. On the other hand, the apolipoproteins A and B have coefficients of variation in excess of 65%. The normal plasma constituents such as cholesterol and cholesterol esters have coefficients of variation of 8–16%, i.e., closer to RNA and peptide than to plasma contaminants such as the apolipoproteins. The extraordinarily low coefficients of variation for the components permit a clear distinction between contaminants such as apolipoproteins A and B and the constituents of the complex. If the complex were an artifact, one would not expect such a marked specificity of composition.

The complex appears to be a vesicular lipid structure with RNA and the peptide sequestered within. This is consistent with an exocytotic mechanism of extrusion of the complex from the malignant cells. The low level of contamination of the complex (as shown by low coefficients of variation for RNA, lipid, and oligopeptide and high coefficients of variation for the apolipoproteins A and B) suggests partitioning of plasma cholesterol with the preformed complex; this would account for the intermediate variance associated with cholesterol content. Thus the results strongly support the assumption that the RNA-proteolipid complex occurs as a distinct chemical entity in the sera of patients with a variety of malignant disorders.

The circulating level of the complex would depend on the rates of its appearance in blood and its disappearance due to uptake and degradation. In addition, the circulating levels could depend on the type, clinical staging, and size of the tumor. It is evident that a very large cross-sectional study would be required before any definitive conclusion could be drawn on this subject.

However, our data indicate that in general the circulating level of the RNA-proteolipid complex increases as the stage of the tumor increases. This is the case both when results for several patients are pooled (Table 2) and when repeated estimations were made in a single patient.3

Amino Acid Analysis of the Oligopeptide Associated with the Complex. Table 3 gives the amino acid composition of the oligopeptide (M, 1250; based on gel filtration), isolated from the RNA-proteolipid complex in 9 cases. If the oligopeptide were heterogeneous, the amino acid composition would vary greatly from case to case. However, amino acid analyses of the oligopeptides from patients with different malignancies at dif-

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3 Unpublished observations.
Table 2 Composition and circulating levels of the RNA-proteolipid complex in sera of patients with lymphomas at several clinical stages of the disease

<table>
<thead>
<tr>
<th>Histiopathological diagnosis and clinical stage</th>
<th>No. of patients</th>
<th>RNA</th>
<th>Peptide</th>
<th>Cholesterol</th>
<th>Phospholipid</th>
<th>Glycosphingolipid</th>
<th>Apoprotein A</th>
<th>Apoprotein B</th>
<th>Circulation levels (μg RNA/ml serum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histiocytic stage</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>2</td>
<td>1.16</td>
<td>12.9</td>
<td>3.8</td>
<td>6.9</td>
<td>31.2</td>
<td>32.3</td>
<td>1.3</td>
<td>9.7-12.6</td>
</tr>
<tr>
<td>II</td>
<td>2</td>
<td>1.22</td>
<td>12.7</td>
<td>3.5</td>
<td>7.3</td>
<td>30.1</td>
<td>33.0</td>
<td>1.2</td>
<td>13.8-16.2</td>
</tr>
<tr>
<td>III</td>
<td>1</td>
<td>1.10</td>
<td>13.3</td>
<td>2.9</td>
<td>7.0</td>
<td>31.5</td>
<td>32.3</td>
<td>1.1</td>
<td>14.7</td>
</tr>
<tr>
<td>IV</td>
<td>4</td>
<td>1.15</td>
<td>13.8</td>
<td>3.2</td>
<td>7.2</td>
<td>31.0</td>
<td>32.0</td>
<td>0.4</td>
<td>13.1-16.8</td>
</tr>
<tr>
<td>Mixed stage</td>
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<tr>
<td>I</td>
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<td>1.21</td>
<td>13.2</td>
<td>2.7</td>
<td>7.1</td>
<td>29.2</td>
<td>34.6</td>
<td>1.0</td>
<td>7.9-12.7</td>
</tr>
<tr>
<td>II</td>
<td>2</td>
<td>1.17</td>
<td>13.8</td>
<td>2.4</td>
<td>7.4</td>
<td>31.4</td>
<td>32.2</td>
<td>1.1</td>
<td>11.4-16.2</td>
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<tr>
<td>III</td>
<td>1</td>
<td>1.13</td>
<td>13.4</td>
<td>2.9</td>
<td>7.0</td>
<td>31.7</td>
<td>31.8</td>
<td>0.7</td>
<td>13.8-22.4</td>
</tr>
<tr>
<td>IV</td>
<td>3</td>
<td>1.24</td>
<td>12.8</td>
<td>3.2</td>
<td>7.8</td>
<td>29.1</td>
<td>33.5</td>
<td>0.2</td>
<td>14.4-28.6</td>
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<tr>
<td>Lymphocytic (poorly differentiated) stage</td>
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<td></td>
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<td></td>
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</tr>
<tr>
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<td>1</td>
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<td>13.9</td>
<td>1.9</td>
<td>7.6</td>
<td>32.8</td>
<td>31.3</td>
<td>0.8</td>
<td>11.8</td>
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<tr>
<td>II</td>
<td>1</td>
<td>1.11</td>
<td>14.4</td>
<td>2.4</td>
<td>8.5</td>
<td>30.9</td>
<td>30.9</td>
<td>1.3</td>
<td>9.4-17.4</td>
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<tr>
<td>III</td>
<td>1</td>
<td>1.12</td>
<td>14.5</td>
<td>2.3</td>
<td>8.1</td>
<td>29.2</td>
<td>32.1</td>
<td>0.1</td>
<td>11.2-26.6</td>
</tr>
<tr>
<td>Mixed stage</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>1</td>
<td>1.18</td>
<td>14.2</td>
<td>2.8</td>
<td>7.7</td>
<td>30.0</td>
<td>32.9</td>
<td>1.9</td>
<td>8.9</td>
</tr>
<tr>
<td>II</td>
<td>1</td>
<td>1.26</td>
<td>14.0</td>
<td>3.6</td>
<td>9.1</td>
<td>30.5</td>
<td>29.4</td>
<td>0.8</td>
<td>13.9-22.9</td>
</tr>
<tr>
<td>III</td>
<td>1</td>
<td>1.15</td>
<td>13.4</td>
<td>3.1</td>
<td>8.5</td>
<td>32.3</td>
<td>30.2</td>
<td>0.2</td>
<td>11.4-24.7</td>
</tr>
<tr>
<td>IV</td>
<td>6</td>
<td>1.15</td>
<td>13.4</td>
<td>3.1</td>
<td>8.5</td>
<td>32.3</td>
<td>30.2</td>
<td>0.2</td>
<td>11.4-24.7</td>
</tr>
<tr>
<td>Mean ± SD (n = 34)</td>
<td></td>
<td>11.8± 0.49</td>
<td>13.7 ± 0.69</td>
<td>30.0 ± 1.2</td>
<td>31.7 ± 1.41</td>
<td>0.68 ± 0.46</td>
<td>0.45 ± 0.38</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Sphingomyelin, phosphatidylcholine, and inositolphospholipids.
* Ceramide hexasaccharides.
* Detected by the use of rabbit antisera against apoproteins A and B according to the method of Laurell (22). Triglyceride assays were performed in some of the RNA-proteolipid preparations, and amount being comparable with that of free cholesterol.

Table 3 Amino acid composition of the oligopeptide associated with the complex

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Mol %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid</td>
<td>9.81-9.31</td>
</tr>
<tr>
<td>Threonine</td>
<td>4.9-5.02</td>
</tr>
<tr>
<td>Serine</td>
<td>9.8-14.05</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>14.7-15.1</td>
</tr>
<tr>
<td>Proline</td>
<td>12.7-15.9</td>
</tr>
<tr>
<td>Glycine</td>
<td>14.2-16.57</td>
</tr>
<tr>
<td>Alanine</td>
<td>6.4-6.9</td>
</tr>
<tr>
<td>Valine</td>
<td>3.8-4.2</td>
</tr>
<tr>
<td>Cystic acid</td>
<td>0</td>
</tr>
<tr>
<td>Methionine</td>
<td>0</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>0</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>2.96-3.3</td>
</tr>
<tr>
<td>Leucine</td>
<td>5.1-5.5</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>1.86-2.1</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>8.97-9.26</td>
</tr>
<tr>
<td>Lysine</td>
<td>1.5-1.9</td>
</tr>
<tr>
<td>Histidine</td>
<td>1.4-1.6</td>
</tr>
<tr>
<td>Arginine</td>
<td>3.4-3.76</td>
</tr>
</tbody>
</table>

* Determined after hydrolysis in 2% (v/v) thiohypocolic acid. Data are derived from 9 individual cases.

fertst stages show that the amino acid composition is comparable in all of them.

These data suggest that the complex could be associated with a specific oligopeptide which appears to be acidic. Furthermore, in an independent experiment, the amino acid composition of the oligopeptides was comparable in a pooled sample from 8 different cases. Sequence analysis of the oligopeptide was attempted. However, Edman degradation was unsuccessful, indicating a blocked NH2-terminal end. Thus, the specificity of the oligopeptide can be established only when the sequence has been successfully determined.

Correlation between Tumor Mass and Circulating Levels of the Complex. Since the circulating levels of the complex would depend both on its rate of secretion and its rate of clearance, it was of interest to determine the level of the complex as a function of tumor mass.

Ideally, such a study should be carried out on a longitudinal basis, the circulating levels of the complex and the tumor being monitored in the same patient. The chief hindrance to such a study was in obtaining and maintaining contact in a reasonable time frame with an adequate number of cases fully resistant to tumor therapy. As an ethically acceptable alternative, a cross-sectional study was attempted in 12 freshly diagnosed cases in the hope that the circulating levels would bear a relatively constant relationship to each type of tumor. Data in Fig. 1 show that such a relationship is clearly discernible in the 4 types of malignancy tested. These results also show that different types of tumors contribute differently to the circulating levels, consistent with different rates of secretion or clearance.

The rank order of serum complex level for a given mass volume of tumor was malignant melanoma > oat cell carcinoma > immunoblastoma stage III > immunocytoma stage III. The data were consistent with a zero order rate constant ($s$) for synthesis, which depends on the type of tumor, and a first order rate constant for clearance ($k$) in all instances such that the change in the circulating levels can be represented by

$$\frac{dC}{dt} = s - kC$$

$s$ relates to the cell type and mass of the tumor. The observed logarithmic relationship indicates that there is a finite threshold for the detection of the mass of the tumor depending on its capability to secrete the complex. In reality, the circulating levels should be governed by the dynamic model for growth that each tumor follows, the logarithmic relationship being only a general approximation. A common mechanism for clearance was suggested by the observation that while the tumor volume varied by 3 orders of magnitude the circulating levels of the
complex varied only within 2 orders of magnitude.

Kinetics of the Clearance of RNA-Proteolipid Complex in Circulation following Different Modes of Therapy. If the circulating levels of the complex are to be a reliable index of the tumor mass, the half-life of the complex in blood should be quite short compared to tumor growth per se. In discrete tumors that can be removed surgically, the rate constant of clearance of the complex from circulation can be measured and compared with the rate resulting from other modes of therapy such as chemotherapy and radiation, wherein the tumor primarily undergoes progressive remission.

While different tumors could secrete different amounts of the complex, the rate constant of clearance could be the same in all cases within the limits of biological variation. Thus important information on the pharmacokinetics of the circulating complex could be obtained following therapy. Fig. 2 shows the time course of circulating levels following chemotherapy, radiation, and surgical ablation of the tumor. In each instance, the data between 0 and 95% change in the circulating levels approximated to a single exponential profile at P < 0.001. The half-lives of the circulating complex were: chemotherapy, 12.9 ± 4.1 (SD) days (range, 8–18 days); radiation, 11.1 ± 3.0 days (range, 7–14 days); and surgery, 2.3 ± 0.5 days (range, 1.5–3.0 days) (n = 5, 6, 7, respectively).

Tumors found by extensive clinical survey to be localized were chosen for surgical ablation. The rapid exponential decay in the circulating levels of the complex following a complete excision of the tumor would be the best approximation to the true half-life of clearance of the complex. The coefficient of variation in the half-life was also the least in this group (~21%).

The longer half-lives after chemotherapy and radiation reflect tumor regression, and not the half-life of the circulating complex. The half-life after surgical ablation, however, was consistent with a single dominant mode of clearance. The larger variance associated with chemotherapy and radiation would also include variable contributions of the therapeutic response.

Prognostic Value of the Circulating Levels of the Complex in Cases of Relapse. The short half-life of the circulating levels of the complex compared to the time required for remission of a tumor following chemotherapy and radiation and the correlation between the tumor volume/mass and the circulating levels suggested that the circulating levels of the complex could be a sensitive index for the onset to relapse. The circulating levels were therefore followed up on a long-term basis and correlated with the independent clinical diagnosis of relapse of malignancy. In patients who did not have clinical relapses the serum concentration of RNA-proteolipid complex was 0.2–0.8 μg RNA/ml (n = 6) (i.e., at the limits of detection) over a 40-month period. Fig. 3 illustrates 8 representative cases in which a relapse was clinically identified during the 40 months of follow-up. In each instance an increase in the levels of the complex preceded the routine clinical diagnosis of relapse in an ambulatory clinic by 4–10 months. Attempts to study the levels of the RNA-proteolipid complex in cases which did not respond to radiation or chemotherapy were severely limited since most of the available cases were lymphomas being treated in ambulatory clinics. In the few cases where it was possible to monitor the RNA-proteolipid levels, this was only for a short duration and the levels did not decrease (data not given). An evaluation of the hematological and other general parameters such as serum enzymes (e.g., lactic acid dehydrogenase) did not show any pattern consistent with the clinical course; therefore, only RNA-proteolipid levels are reported here.

DISCUSSION

One of the primary aims of the present clinical studies was to establish the chemical identity of the complex, both in terms of macrocomposition and in terms of the coefficient of variation in a relatively large sample.

The method of estimation of the complex was biochemical, involving a routine, somewhat tedious isolation of the complex by centrifugation in a KBr gradient. The patients were from an
For a tumor marker: (a) rapid decay on surgical removal of the detection limits of 0.2 μg/ml. 

4; acute pancreatitis, n = 2; and sarcoidosis, n = 5), besides investigated 45 cases of various disorders (including chronic tissue damage (n = 15), and malnutrition (n = 1), we further lipoprotein metabolism (n = 14), pregnancy (n = 15), conditions or tissue injury. Since the previous publication (1), which in hence, their omission in the present report. A continuing and characterization of the antigenic determinants) before routine use; 

The RNA-proteolipid complex satisfies the following criteria for a tumor marker: (a) rapid decay on surgical removal of tumor; (b) satisfactory correlation with tumor mass; (c) feasibility of early diagnosis of relapse; and (d) correlation between response to therapy and the circulating levels as a measure of tumor remission. The rapid exponential decay is of particular importance in understanding the fate of the circulating complex, i.e., uptake or degradation. Since the mechanism of clearance of the complex from the patients is still unknown, a suitable animal model is required. 

At the time of completion of this paper, we became aware of independent confirmatory reports by Wright et al. (16) and Fossell et al. (17). These authors also identified a proteolipid associated with the malignant state, using high resolution 1H-NMR signals of spin–spin (T2) relaxation time, traceable to isotropic neutral lipids identified with triglycerides (15, 18, 19). They suggested that the 1H-NMR signals were associated only with metastasizable malignant cells. They could detect the signals in animal models in solid, malignant tumors but not in benign ones, and also possibly on viral transformation, e.g., Epstein–Barr virus. 

Despite the claim by these authors that there are gross similarities between the composition of the proteolipid which they isolated and ours, significant differences remain to be resolved. These include the triglyceride content, which does not exceed 5% in our preparations, and RNA content. The high apolipoprotein B and triglyceride content as well as low RNA content in their preparations could be due to contamination, although absence of statistical evaluation and adequate sample size renders a judgment difficult and must await better concordance in the methodology used by the two groups. 

The possibility of the use of 1H-NMR was also indicated by the subsequent results of Mountford et al. (20), who identified the proteolipid by NMR in plasma of a patient with borderline ovarian tumor. As was correctly observed by others (21), the diagnosis of tumor by tumor-specific markers has not been particularly successful. The RNA-proteolipid must also await a fuller investigation of its chemistry and biology despite the promising results at the minipathological level given in this paper. 

Further investigations will focus on the RNA and peptide components of the proteolipid complex and their possible roles in cancer. 

REFERENCES 


The abbreviation used is: NMR, nuclear magnetic resonance. 

L. Wright, personal communication.
RNA-PROTEOLIPID AND CANCER

Diagnostic and Prognostic Value of RNA-Proteolipid in Sera of Patients with Malignant Disorders following Therapy: First Clinical Evaluation of a Novel Tumor Marker

Andreas J. Wieczorek, Vetury Sitaramam, Werner Machleidt, et al.


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