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Isolation and characterization of an RNA–proteolipid complex associated with the malignant state in humans

(opalescence/KBr density gradient/lipoproteins/cancer diagnosis)

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ABSTRACT **An RNA–proteolipid complex was isolated from sera of patients with a variety of malignant disorders as well as from culture media of malignant cell lines. The complex, characterized by a relatively constant composition, contains 27S poly(A)$^+$ RNA and M_r 1250 oligopeptide(s) and is rich in phospholipids and glycosphingolipids. Serum lipoproteins (low and high density) differ from this complex in density, chemical composition, and immunological reactivity. The complex was detected in 94 of 96 cases of malignancy tested but not in any of 58 patients with nonmalignant disorders or in 46 healthy individuals.**

Altered lipoprotein metabolism has been implicated in the malignant state and several lines of evidence support such an association (1–3). Association of specific markers, such as oligopeptides of the proteolipid, are important in oncology from three points of view. First, whatever the origin of such markers (as long as the association is specific to the malignant state), these could serve as powerful tools for diagnostic and even prognostic purposes; second, such markers could mediate specific host–tumor interactions (immunologic, desmoplastic, or otherwise); and third, such markers could also be important probes in understanding the cell biology of the malignant state.

In the course of our investigations of alterations in protein metabolism in the malignant state, we have encountered a hitherto undescribed RNA–proteolipid complex, both *in vivo* and in cell culture. We report here the isolation and characterization of this complex and demonstrate its utility as a diagnostic tool in clinical oncology.

MATERIAL AND METHODS

Isolation of the RNA–Proteolipid Complex. Aliquots (3 ml) of sera or supernatants of cell culture media were mixed with 0.3517 g of KBr/ml (final density 1.225 g/ml) and were layered below a discontinuous KBr gradient [1.006–1.221 g/ml, with increments given elsewhere (4)]. The samples were centrifuged for 16 hr at 4°C at 105,000 × *g* to obtain a clearly demarcated opalescent band that could be readily distinguished from the neighboring lighter, orange, low-density lipoprotein (LDL) and the heavier, yellow, high-density lipoprotein (HDL_2) bands. All the lipoprotein fractions plus this opalescent fraction were individually collected by suction and dialyzed in benzoyl cellulose bags against isotonic saline solution (4°C, 24 hr, 3 changes). Presence of the apolipoproteins A and B was checked by immunodiffusion with monospecific rabbit antiserum (Behring, Marburg, FRG). For fractionation of the RNA–proteolipid complex into components, aliquots (1 ml) of each fraction were further dialyzed in benzoylcellulose bags against distilled water and lyophilized.

Gross Separation of the Proteolipid From RNA. The lyophilized samples were suspended in 5 ml of chloroform/methanol (2:1, vol/vol) and stirred for 3 hr at 22°C. Centrifugation at 10,000 × *g* for 10 min at 4°C yielded a pellet which was reextracted with 2.5 ml of chloroform/methanol (1:1.5, vol/vol). The organic-soluble phases were pooled and evaporated to dryness. The organic-soluble fraction (designated fraction 1), which was free of RNA, was further fractionated to examine its peptide and lipid content. The precipitate, fraction 2, was rich in RNA and was independently fractionated.

Fractionation of peptides and lipids in fraction 1. Fraction 1 was solubilized in 2 ml of chloroform/methanol (2:1); it then was added to 3 volumes of diethyl ether and kept at 4°C for 10 hr to precipitate a peptide-rich fraction, which was collected by centrifugation at 10,000 × *g* for 10 min at 4°C. The supernatant (L1) was analyzed for lipid composition directly (see below). Since the peptide-rich fraction would readily not dissociate from firmly bound glycosphingolipids and phospholipids, it was extracted with 2 ml of chloroform/methanol (1:1) acidified to pH 2 with HCl. The extraction was repeated twice, yielding a peptide precipitate (P1) and a pooled lipid extract (L2). The delipidated oligopeptides were collected by centrifugation and dissolved in distilled water for estimation and further characterization.

Fractionation of RNA-rich fraction 2. The insoluble fraction was extracted twice with 2 ml of 65% ethanol at 22°C for 2 hr. The soluble material was dried *in vacuo* and then was extracted twice with acidified chloroform/methanol (1:1) to obtain a peptide fraction (P2) and the soluble lipid fraction (L3). The ethanol-extracted pellet was dissolved in 100 mM NaCl, and RNA was further purified from peptides by three cycles of precipitation at −70°C with 2 volumes of ethanol. The pooled supernatants yielded another peptide fraction (P3).

Lipid Analysis. Fractions L1–L3 were pooled and analyzed on silica H gel (Merck), together with standard lipids (Sigma and Seromed, Munich, FRG), in chloroform/methanol/H_2O (60:35:8, vol/vol) and hexane/diethyl ether (70:30, vol/vol). Cholesterol was analyzed by gas chromatography with stigmasterol as a standard. Phospholipids isolated by chromatography were determined by the method of Bartlett (5). Glycosphingolipids (ceramide hexasaccharides) were determined by gas chromatography of the trimethylsilylated methyl glycosides as described (6).

Peptide Analysis. Polypeptide fractions P1–P3 were subjected to gel filtration on Bio-Gel P-2 and P-10 (Bio-Rad; mesh 200–400) in a 50 × 1 cm column precalibrated with

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Abbreviations: LDL, low-density lipoprotein; HDL_2, high-density lipoprotein.

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bacitracin, valinomycin, reduced glutathione, and histidylleucine (Serva, Heidelberg). The oligopeptides were eluted with 2% ethanol at a flow rate of 0.25 ml/min, and column eluate was monitored at 203 nm with a LKB Uvicord III. Protein was determined according to the method of Lowry *et al.* (7) with bacitracin as standard.

RNA Analysis. RNA was identified by UV spectrometry (λ_{max} at 254 nm) and hydrolysis by RNases T_1 and A. RNA was quantitated by UV spectrometry with tRNA (Sigma) as standard.

RNA isolated from the serum- or tissue culture medium-derived particles was solubilized (10 μg of RNA/200 μl) in binding buffer (0.2 M NaCl/0.2% $NaDodSO_4$/5 mM EDTA/10 mM Tris Cl, pH 7.5). Samples were applied to a poly(U)-Sepharose 4B column (0.9 × 10 cm; Pharmacia) previously equilibrated with the binding buffer (8). For binding, the sample was stirred with one-half of the column bed volume for 30 min at room temperature. The remainder of the poly(U)-Sepharose was left in the column. The column was repacked and washed with 5 bed volumes of binding buffer at a rate of 20 ml/hr at 20°C. Poly(A)$^+$ RNA was eluted with 5 mM EDTA/0.2% $NaDodSO_4$/10 mM Tris Cl, pH 7.5, at 20°C. The eluted RNA was precipitated with ethanol. Digestion with RNase or DNase (Sigma) was in 10 mM Tris glycine, pH 7.4/1 mM EDTA at 37°C for 2 hr at an enzyme/substrate ratio of 1:10. Molecular weight determination of RNA was performed by electrophoresis in composite 2.0% polyacrylamide/agarose gels (9) in 50 mM Tris glycine, pH 8.9, as well as in 0.1% $NaDodSO_4$/50 mM acetate buffer, pH 6.5. Ribosomal RNA (28S, 23S, 18S, 16S, and 5S) and tRNA (4S) (Boehringer Mannheim, Miles) were used as standards. Gels were stained with acridine orange or toluidine blue.

Aliquots (5 μg) of proteolipid-bound RNA or isolated RNA were incubated in 50 mM sodium phosphate buffer, pH 7.0/150 mM NaCl with 0.1 unit of human serum RNase C (Sigma) in a final volume of 100 μl. After incubation for 3 hr at 37°C, 200 μl of concentrated perchloric acid was added and the sample was chilled in an ice bath. After centrifugation at 1000 × *g* for 10 min, the absorbance of acid-soluble nucleotides was measured at 260 nm (10).

Light-Scattering Studies. The opalescence of the RNA–proteolipid complex was studied as a function of increasing concentrations of electrolytes and temperature. Aliquots (5 μg) of the complex were mixed with various concentrations of KBr, up to 1.5 M, or with sucrose at 10–50% at 4°C, 15°C, and 23°C. Turbidity was determined at 600 nm.

Handling of Human Sera. Samples of serum were prepared from 10 ml of fresh human blood obtained from patients or healthy donors who had fasted overnight. The sera were stored up to 24 hr at 4°C and were tested "blind" in two different laboratories. All cases cited were of confirmed diagnosis by histopathologic as well as clinical criteria.

Cell Cultures. Established cell lines HEp-2, KB, EB-2, HeLa, J111, HT-1080, RD, and malignant melanoma of the skin were purchased from Flow Laboratories and from Seromed. Secretion of the RNA–proteolipid complex by malignant cell lines (J111, HT-1080, and KB) was studied in the presence or absence of cytochalasin B (5 μg/ml) according to Couble *et al.* (11), and in the presence of 1 μM monensin as described by Tartakoff and Vassalli (12).

Immunological Methods. The antigens (RNA–proteolipid complex, HDL_2, and LDL) were dissolved at a concentration of 150 μg of RNA/ml. Antibodies were prepared as described previously (13). The antibodies against the RNA–proteolipid complex were purified further by adsorption of the IgG fraction with cyanogen bromide-treated Sepharose 2B (Pharmacia) coupled with serum of healthy donors (1 mg of protein equivalent to 3 ml of swollen activated beads) according to Porath (14). The unbound IgG fraction was used for further investigations. Rocket immunoelectrophoresis was performed according to the method of Laurell (15).

RESULTS

Identification of the RNA–Proteolipid Complex. In cases of malignancy as well as in malignant cell lines, the sera/media exhibited an opalescent band in KBr gradients corresponding to a mean density of 1.085 g/ml. Fig. 1 shows the opalescent band observed in cases of gastric cancer and malignant lymphoma, in comparison with the serum from a healthy donor. The resolution of the gradient was such that LDL (mean density 1.05 g/ml) and HDL_2 (mean density 1.12 g/ml) could be readily distinguished from the opalescent band. It was necessary to concentrate the medium from cultures of malignant cell lines 5-fold to observe the opalescent band. The minimum concentration of the complex that could be detected by centrifugation was 0.2 μg of RNA/ml.

Chemical Characterization of the Complex. The composition of the complex is similar whether it is isolated from sera of patients suffering with malignant disorders or from culture media of malignant cell lines (Table 1). Since the complex contains components like RNA and oligopeptides, it is, however, fundamentally different from components either of LDL or HDL_2, indicating that it indeed represents a distinct macromolecular complex.

Gel filtration of the peptide fraction isolated from the complex yields a major peak corresponding to M_r 1250; the same value is obtained from chromatography in the presence of 0.1% $NaDodSO_4$/0.1% cetyl trimethylammonium bromide.

With the exception of cholesterol esters, the lipid composition of the complex isolated from sera is comparable with that of the complex isolated from culture media. Phospholipids account for 35% of the complex by weight; in contrast, LDL and HDL_2 have about 20% phospholipids. The high content of large glycosphingolipids is an unusual feature of the complex.

Based on UV spectrometry, RNA purified from the complex was comparable to an authentic sample of RNA uncontaminated by peptides. Polyacrylamide electrophoresis of the RNA with appropriate standards yielded a single band corresponding to 27 S in either the presence or the absence of 0.1% $NaDodSO_4$. The 27S band disappeared on pretreat-

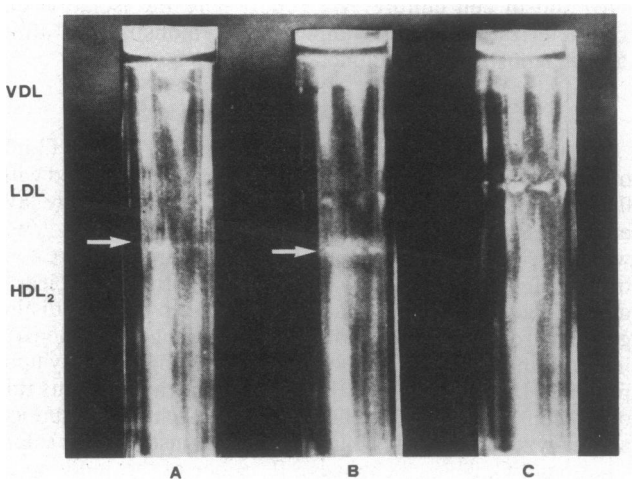

FIG. 1. KBr-gradient centrifugation of the sera of a patient with gastric cancer (gradient A), a patient with malignant lymphoma (gradient B), and a normal donor (gradient C). An 8.8-ml discontinuous KBr gradient with a density of 1.221–1.006 g/ml was added above 3 ml of serum adjusted to 1.225 g/ml with KBr. Centrifugation was at 105,000 × *g* for 16 hr at 4°C. The opalescent band (arrow) floating between LDL and HDL_2 regions was collected, dialyzed against isotonic saline solution and analyzed for composition. VDL, very low density lipoproteins.

Table 1. Composition of RNA–proteolipid complex in serum and in the media of cultured malignant cell lines

Component	% of total weight			
	RNA—proteolipid			
	In serum (n = 62)	Secreted by cells (n = 8)	LDL in serum	HDL_2 in serum
Oligopeptide	12.8–15.1	15.4–18.1		
RNA	11.5–11.8	13.9–14.3		
Cholesterol	2.3–3.9	4.2–6.5	8	8.5
Cholesterol esters	8.4–9.7	0.8–1.7	37	18
Phospholipids*	29.9–37.4	28.5–34.1	22	21.5
Glycosphingolipids†	24.1–37.6	28.1–32.2	NP	NP
Apolipoprotein A‡	>2			50
Apolipoprotein B‡	>2		25	

NP, not present.
*Sphingomyelin, phosphatidylcholine, and inositolphospholipids.
†Ceramide hexasaccharides.
‡Detected by the use of rabbit antisera (Behring).

ment with RNases T_1 and A but not on treatment with DNase, further substantiating that the species is RNA. Heating the RNA at 65°C for 3 min increased the absorption at 254 nm by 2-fold; this hyperchromicity is consistent with the presence of double-stranded stretches. Poly(U)-Sepharose was capable of binding 95–98% of the RNA (n = 6) in the presence of high ionic strength, showing that the species represents poly(A)$^+$ RNA.

The dependence of opalescence of the complex on ionic strength and temperature conditions is represented in Fig. 2. Turbidity increased as a function of increasing KBr concentration and decreasing temperature.

Resistance of the RNA–proteolipid complex to RNase C treatment was determined by separate incubation of the complex and the isolated RNA with the enzyme under physiological conditions. Whereas incubation of the isolated RNA led to subsequent appearance of perchloric acid-soluble nucleotides (63–67% mononucleotides), the RNA associated with the proteolipid complex was resistant to RNase C treatment (n = 7).

Immunochemical Studies. Antibodies were raised in rabbits against human RNA–proteolipid complex isolated from sera, against the complex isolated from culture media of malignant cells, and against LDL and HDL_2, independently. Antibodies (IgG) against the complex were rendered more specific by passage through CNBr-activated Sepharose columns previously reacted with pooled sera from normal donors. Fig. 3 shows the immunoelectrophoretic pattern of purified antibodies when run against the complex, either obtained from sera (*A*) or from culture medium (*B*). The lack of

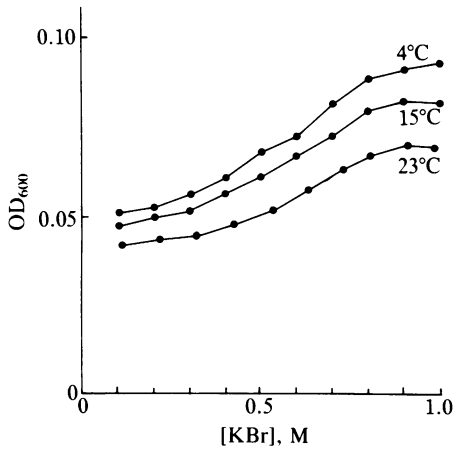

FIG. 2. Dependence of turbidity of RNA–proteolipid on salt concentration and temperature. Aliquots of RNA–proteolipid (5 μg/ml) were adjusted to the indicated KBr concentrations by dialysis. Turbidity was measured at 600 nm at 4°, 15°, and 23°C.

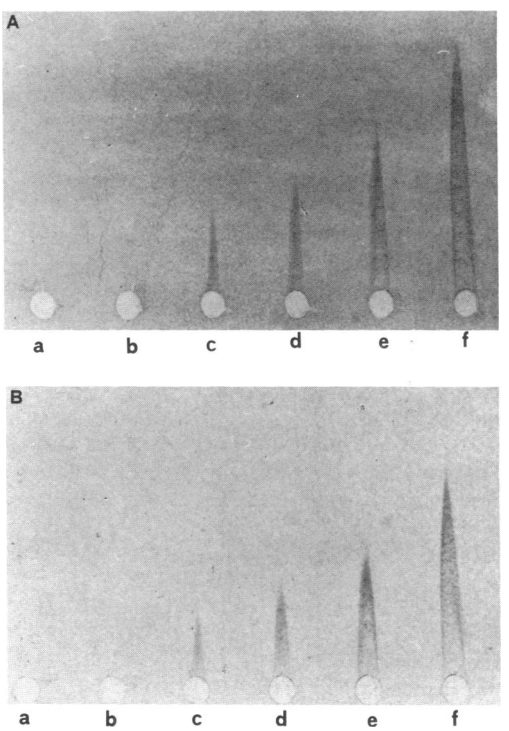

FIG. 3. Rocket immunoelectrophoresis of purified RNA–proteolipid complex. (*A*) Wells a and b: 3 and 5 μl of serum of healthy donors. Wells c–f: 5-μl samples containing (based on RNA) 0.2, 0.3, 0.4, and 0.5 μg, respectively, of RNA–proteolipid isolated from the serum of a patient with malignant lymphoma. (*B*) Well a: 5 μl of culture fluid (0.3 μg of RNA) of skin fibroblasts prepared from healthy donors. Well b: 5 μl of culture fluids (0.3 μg of RNA) of lymphocytes prepared from normal donors. Wells c–f: 5-μl samples containing (based on RNA) 0.2, 0.3, 0.4, and 0.5 μg, respectively, of RNA–proteolipid isolated from malignant cell line J111. The antibody solution was 4.5% immunoglobulins purified from rabbit antiserum to RNA–proteolipid by immunoabsorption with serum from normal human donors. Electrophoresis at pH 8.8 in buffer containing 24.5 mM barbital, 73 mM Tris, 0.3 mM calcium lactate, and 3 mM sodium azide was at 2.5 V/cm in the gel at 15°C for 18 hr. Gels were stained with Coomassie brilliant blue R. Anode at top for both *A* and *B*.

precipitation when the antibodies were run against LDL and HDL_2 (data not shown) further substantiates that the complex is distinct from the lipoproteins. Fig. 3*B* illustrates that antibodies raised against the complex isolated *in vitro* did not show the presence of antigenic material in the culture media of normal skin fibroblasts and lymphocytes.

Clinical Studies. It was considered essential to test a variety of malignant conditions for the presence of the RNA–lipopeptide complex in order to determine its potential use as a diagnostic tool. Table 2 summarizes the malignant disorders we have tested thus far. In each instance, the diagnosis was confirmed on both clinical and histological grounds. Since RNA content was relatively constant, we have expressed the circulating levels of the complex as μg of RNA (isolated from the opalescent band in each case) per ml of serum. We chose RNA for estimation of the complex because other lipoproteins do not have RNA and it would automatically eliminate any observer bias in the identification of an opalescent band. It should also be noted that in all instances, the opalescent band was clearly separated from neighboring lipoprotein bands even in conditions of abnormal lipoprotein metabolism. The circulating levels of the complex varied widely. This could be because the levels of the complex in serum are a function of the density of the malignant cells (live or decaying) or it could merely represent rapidly proliferating cells as well as differences in the clearance of the complex from blood. A variety of nonmalignant conditions were chosen to rule out chance association (see Table 2); the complex was detected in none of these conditions.

Established malignant cell lines were cultured and the rate of complex production was monitored (Table 3). The complex was detected as early as 6 hr after culturing, and the levels varied proportionately with the time of incubation and cell density (data not shown).

The RNA–proteolipid complex apparently represents a secretory product of the cells. Addition of cytochalasin B (at a concentration that is not toxic to the cells) to cultures of either established malignant cell lines or freshly prepared polymorphonuclear leukocytes from patients with myelogenous leukemia ($n = 5$) resulted in inhibition of secretion of the complex to <0.2 μg of RNA per 10^8 cells in 24 hr. For controls (cells incubated in the absence of the compound), apparent secretion ranged between 11.5 and 25.4 μg of RNA per 10^8 cells in 24 hr. Similarly, incubation with monensin, a compound known to slow down intracellular transport of newly synthesized secretory proteins within the Golgi complex (12), secretion of the complex was reduced to 0.2–0.8 μg of RNA per 10^8 cells in 24 hr. That the production of the complex by malignant cells is dependent on the cells' metabolism is further supported by the inhibition of its appearance in medium containing CN^- (data not shown).

A number of control experiments, performed under the same conditions, with polymorphonuclear leukocytes, T and B lymphocytes, and fibroblasts of healthy donors did not reveal production and/or secretion of the complex above basal values (data not shown). However, it is possible that very low levels of the complex could be present in the sera of normal individuals and not be detected by RNA isolation. However, we have not thus far succeeded in detecting the complex by rocket immunoelectrophoresis, even with 10-fold concentrated normal sera.

Table 2. Serum levels of RNA–proteolipid complex

Disorder or condition	No. of patients tested	Serum concentration of RNA–proteolipid*
Malignant disorders		
Myelogenous leukemia		
Acute	3	5.5–9.6
Chronic	10	2.1–4.2
Malignant lymphoma		
Hodgkin	6	4.4–21.3
Non-Hodgkin	10	2.1–16.1
Melanoblastoma	7	3.9–15.3
Plasmacytoma	5	1.3–3.6
Histicocytoma	1	22.6–24.0
Sarcoma		
Bone	1	10.0–11.2
Mediastinum	3	2.9–8.8
Pleural mesothelioma	1	20.0–22.6
Bronchus carcinoma		
Nonmetastatic	3	5.2–11.4
Metastatic	3	6.8–13.0
Esophageal carcinoma	2	6.2–8.1
Gastric cancer	5	2.9–7.3
Colonic carcinoma	5	1.8–4.2
Hepatic carcinoma	2	3.1–4.4
Pancreatic carcinoma	4	4.7–13.5
Gallbladder carcinoma	1	4.1–4.5
Hypernephroma		
Nonmetastatic	4	5.5–9.9
Metastatic	4	8.6–14.6
Urinary bladder carcinoma	1	4.4–5.3
Others‡	11	1.6–10.9
Choriocarcinoma	2	10.8–16.3
Inflammatory diseases		
Acute viral infections	5	<0.2
Chronic arthritis	5	<0.2
Lupus erythematosus	3	<0.2
Mixed connective-tissue disease	2	<0.2
Scleroderma	2	<0.2
Sarcoidosisq	3	<0.2
Liver cirrhosis	5	<0.2
Chronic bronchitis	2	<0.2
Disorders of lipoprotein metabolism		
Familial hypercholesterolemia		
Homozygous	2	<0.2
Heterozygous	4	<0.2
Secondary hyperlipoproteinemia		
Type IV	3	<0.2
Type V	5	<0.2
Pregnancy		
Trimester 1	5	<0.2
Trimester 2	4	<0.2
Trimester 3	6	<0.2
Disorders with tissue breakdown		
Myocardial infarction	4	<0.2
Peri- or myocarditis	4	<0.2
Myositis	3	<0.2
Tissue injury†	2	<0.2
Cerebral infarction	2	<0.2
Malnutrition		
Anorexia nervosa	3	<0.2

*Expressed in μg of proteolipid-bound RNA/ml of serum.
†Due to accidents (e.g., auto crash).
‡Teratocarcinoma, ovarian and uterine carcinoma, nasopharyngeal carcinoma, squamous-cell carcinoma.

Table 3. RNA–proteolipid production by tumor cell lines

Cell line	Origin	RNA–proteolipid, μg of RNA per 10^8 cells per 24 hr
HEp-2	Carcinoma of the larynx	16.4 ± 1.2
KB	Oral epidermal carcinoma	13.3 ± 1.6
EB-2	Burkitt lymphoma	18.5 ± 1.4
HeLa	Cervical carcinoma	11.2 ± 1.2
J111	Monocytic leukemia	26.8 ± 2.1
HT-1080	Fibrosarcoma	19.2 ± 2.0
RD	Rhabdomyosarcoma	14.3 ± 1.6
Unspecified	Malignant melanoma of the skin	19.8 ± 2.3

Cells (10^8) were incubated in Eagle's minimal essential medium plus 10% heat-inactivated fetal calf serum for 24 hr in tissue culture flasks in a 5% CO_2 atmosphere. Supernatants of each culture then were obtained by centrifugation at 500 × *g* at 4°C for 10 min and analyzed for proteolipid concentration by using a KBr gradient as described in *Materials and Methods*. Values are expressed as mean ± SD, $n = 5$.

DISCUSSION

The discovery of the RNA–proteolipid complex as a distinct opalescent band between the LDL and HDL_2 bands from the sera of patients suffering from malignant disorders was due to a fortuitous choice of centrifugation conditions. Although the presence of the complex raises more questions than it answers, one fundamental question refers to the origin of the components of the complex. The light-scattering studies have clearly indicated that the presence of electrolytes (e.g., KBr at higher concentrations but not sucrose) increases the opalescence; therefore, it is possible that this complex is a metaaggregate of preexisting complexes of lipid, RNA, and protein. The studies on the apparent secretion of this RNA–proteolipid complex by cultured malignant cells show that respiration is an absolute requirement for its appearance in the culture medium. Since the production and/or secretion is inhibited by cytochalasin B, CN^-, and monensin, the complex cannot be merely a degradation product of the cancer cells. We have attempted to identify this complex in normal cells of different kinds and in malignant cells of different kinds after homogenization in buffer alone as well as in a high concentration of KBr. We never detected any of this complex as an immunoprecipitate by using rocket immunoelectrophoresis of homogenates, even when the homogenates were concentrated 10-fold.

The high ionic strength of the KBr gradients combined with low temperature is apparently necessary for the opalescence of the band containing the complex, since no such band was observed in sucrose gradients. The possibility that the KBr gradient might have induced artifactual combination of the RNA with the protein and the lipid appears unlikely, since we found that serum RNase C cleaved the isolated RNA under physiological conditions but not RNA associated with the intact complex. These findings suggest that the RNA–proteolipid complex consists of RNA within a vesicular lipid complex; formation of such a complex would not be consistent with the exposure of RNA to lipids in serum under normal conditions. The high lipid content of this structure suggests that the role of the lipids has something to do with an occlusion of the RNA within, as may also be inferred from the RNase C-resistance experiments. The lipids may also have a role in the formation of the aggregates of the complexes which lead to opalescence in KBr gradients.

The physical location of the oligopeptide in the complex remains an enigma.

In all patients, the complex was estimated from the opalescent band obtained by ultracentrifugation. This band was never found associated with any lipoprotein disorder, including hyperlipidemia and malnutrition. This RNA-containing band could be resolved from LDL and HDL_2 by at least 5 mm under the conditions described. Even with abnormal lipoprotein metabolism, no band interfering with this area was detected. This band always contained RNA as estimated by UV spectrometry both of the purified putative RNA and of the products of its digestion with RNase T_1.

Detailed immunological characterization of this complex is still pending because the preliminary evidence indicates that the RNA is including within the vesicle. Until the antibody against the whole complex is resolved into antibodies against separate components, a clear statement of the immunochemistry cannot be made.

In conclusion, this RNA–proteolipid complex offers a potential diagnostic tool for the management of malignant disorders. We favor the view that the complex represents a specific secretory product of the tumor cells, which may mediate host–tumor interactions.

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