

Acquired deficiency in C1-inhibitor associated with signet ring cell gastric adenocarcinoma: A probable connection of antitumor-associated antibodies, hemolytic anemia, and complement turnover

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Background: *Acquired deficiency in C1-inhibitor (C1-INH) associated with malignancy is often asymptomatic because clinical manifestations are not dependent on a critical complement threshold (in contrast to hereditary C1-INH deficiency). Increased complement consumption involving different kinds of antibodies is the postulated mechanism for this disease, but other factors must play an important role.*

Case report: *A 76-year-old woman with unremarkable medical history experienced three episodes of angioedema over 6 months. Investigations revealed a complement profile characteristic of acquired deficiency in C1-INH, a hemolytic anemia, and a signet ring cell adenocarcinoma (linitis plastica). A gastrectomy and a splenectomy were performed. The postoperative course was characterized by a complete disappearance of the symptoms of angioedema and hemolytic anemia. A local recurrence of the tumor 5 months later could not be resected. The patient died 17 months after the initial surgery was performed.*

Results: *Quantitative and functional analyses of the complement factors showed persistent excessive complement consumption. Markers of hemolytic anemia disappeared after tumor removal but recurred in the second part of the disease evolution. Immunohistochemical findings in tumor tissue showed loss of normal blood group antigens but expression of Le^a antigen, as well as C1q deposition.*

Conclusion: *To explain the whole clinical and laboratory picture, we hypothesize a connection between tumor immunohistochemical profile, complement consumption, and hemolytic anemia. Tumor cell surface antigens might lead to a permanent but asymptomatic complement consumption that is worsened and becomes clinically manifest by superimposed hemolytic anemia caused by cross-reactive antibodies to newly expressed blood group antigens on tumor cells. This hypothesis should be confirmed by other observations. (J ALLERGY CLIN IMMUNOL 1995;95:124-31.)*

Key words: *C1-INH deficiency, acquired, adenocarcinoma, gastric, anemia, hemolytic*

Clinical manifestations of acquired C1-inhibitor (C1-INH) deficiency are similar to those of hereditary angioedema. Both types of deficiencies can

Abbreviations used

C1-INH: C1 inhibitor
CEA: Carcinoembryonic antigen

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result in mucosal or subcutaneous swelling from increased vascular permeability caused by unopposed contact activation or activation of the complement cascade with subsequent mediator release.¹

The predominant form of hereditary angioedema is inherited in an autosomal dominant manner and is characterized by low levels of C1-INH, C2, and C4 but normal levels of C1q. It is due

to decreased synthesis and elevated turnover levels of a normal C1-INH molecule. The variant form of hereditary angioedema (15%) has normal or elevated levels of a functionally deficient C1-INH.² The acquired form, first described in 1972 by Caldwell et al.,³ is characterized by low C1-INH, C2, C4, and C1 (q r₂ s₂) levels and is usually associated with benign or malignant B-cell lymphoid proliferative disorders^{4,5} such as B-cell lymphoma,^{3,6} chronic lymphocytic leukemia,⁷ lymphocytic lymphoma,⁸ Waldenström's macroglobulinemia,⁹ or multiple myeloma.¹⁰ Some unusual associated malignancies have been described, such as rectal adenocarcinoma¹¹ and mammary carcinoma.¹² Various nonneoplastic conditions have also been described in association with C1-INH deficiency: *Echinococcus granulosus* infection,¹³ systemic lupus erythematosus,¹⁴ Churg-Strauss vasculitis,¹⁵ hepatitis B,¹⁶ and plane xanthomatosis.¹⁷ Sometimes, acquired C1-INH deficiency has been associated with antiidiotypic antibodies to monoclonal immunoglobulin, generally as a consequence of a lymphoproliferative disorder.¹⁸ A second type of acquired C1-INH deficiency has been described, and it is due to autoantibody against the C1-INH molecule, without any associated disease.^{19,20} Clinical manifestations result from the reduced availability of the molecule's reactive center and the likely unregulated activation of the kinin and complement pathways.¹⁹

The mechanism of acquired C1-INH deficiency in lymphoproliferative disorders has been ascribed to an increase in the metabolism of C1-INH and C1q.^{21,22} Another explanation has been proposed: activation of the complement system by tumor cells,^{23,24} which would explain why tumor removal is sometimes successful in restoring normal C1-INH levels.²⁴

The mechanism of the hemolytic anemia frequently associated with cancer is still disputed.²⁵ Modifications or reappearance of precursor forms²⁶ of blood group antigens have also been linked with cancer,^{27,28} have been shown to be of prognostic significance,^{29,30} and are probably essential for tumor growth and metastatization.³¹ This phenomenon has been particularly well studied in gastric cancers.^{32,33}

We present a recent observation of an acquired C1-INH deficiency associated with a gastric ring cell adenocarcinoma, hemolytic anemia, and modifications of blood group antigen expression on tumor cells. Because we have observed a connection between manifestations of hemolytic anemia and of C1-INH deficiency (angioedema), but not

between tumor burden and angioedema, we hypothesize that a clinically silent basic elevation of C1-INH turnover was initiated by the immunologic modifications caused by the tumor. A further elevation of C1-INH turnover on red blood cells because of hemolytic anemia caused by cross-reactive antibodies to newly expressed blood group antigens on tumor cells was necessary to precipitate clinical manifestations of C1-INH deficiency. This hypothesis might explain the whole clinical picture presented by our patient.

CASE REPORT

A 76-year-old Italian woman with B Rh positive blood type and unremarkable medical history except for chronic urticaria of 10 years' duration, experienced, in July 1989, a first episode of angioedema of the tongue and the neck, associated with dyspnea that lasted for 1 week. Results of routine blood analysis (hemogram, erythrocyte sedimentation rate, liver function tests) were normal. A second identical episode occurred in November, and a third episode occurred in December of the same year. The patient was then referred to our department for investigation.

Clinical history did not reveal any triggering factor, and results of physical examination were normal. Laboratory investigations showed an erythrocyte sedimentation rate of 88 mm/hr, a slight anemia (hemoglobin, 115 gm/L; hematocrit, 35%) and leukopenia (white blood cell count, 2.9 10⁹ cells/L) with normal platelet count. Analysis of the complement revealed a marked decrease in C1-INH (0.02 gm/L), C4 (<0.03 gm/L), and C1q (0.03 gm/L); whereas C3, the alternative pathway, and the membrane attack complex were normal. No autoantibody to C1-INH could be detected.

These clinical and laboratory findings suggested the presence of an acquired C1-INH deficiency. The patient was therefore hospitalized in January 1990 for further investigations in search of a malignancy. Detailed recent clinical history revealed only epigastric pain present for a week, weight loss of 6 kg within 6 months, loss of appetite, and low-grade fever. Physical examination at the time of admission was unremarkable except for slight jaundice, painful splenomegaly, and sharp pain in the epigastric area. Laboratory analysis revealed that the erythrocyte sedimentation rate (130 mm/hr) and the anemia (hemoglobin, 73 gm/L; hematocrit, 22%) had worsened. A slight alteration in results of the liver function tests was present (transaminase and bilirubin both at twice the upper normal level values). The result of the direct antiglobulin test was positive at a titer of 1:256 because of the presence of warm antibodies of the IgG-C3d type. No monoclonal gammopathy could be found, and results of the bone marrow examination were normal. An abdominal computed tomographic scan confirmed the splenomegaly (15 × 10 × 15 cm) and showed only an increase in gastric wall thickness. An esophago-

TABLE I. Evolution of complement profile

	Time (mo)					
	-1	Surgery	2	6	10	15
Concentrations						
C1-INH (0.11-0.26 gm/L)	0.02	0.04	0.05	0.03	0.03	0.06
C1q (0.10-0.25 gm/L)	ND	0.03	0.04	0.04	0.03	0.06
C4 (0.14-0.35 gm/L)	<0.03	0.02	0.01	0.02	ND	0.02
C2 (67%-127%)	ND	18	22	27	15	22
Functional tests						
C1-INH (68%-140%)	ND	34	32	5	22	64
C1q (75%-130%)	ND	22	27	13	10	29
C2 (62%-134%)	ND	12	1	3	1	3
C4 (53%-140%)	ND	12	ND	ND	1	39
CH50 (300-510 U/ml)	ND	11	ND	ND	ND	ND

ND, Not determined.

TABLE II. Evolution of hemolytic anemia and CEA serum level

	Time (mo)				
	-1	Surgery	5	10	15
Diminished C1-INH level	+	+	+	+	+
Angioedema	+	-	-	-	-
Erythrocyte alloantibody*	+	+	ND	-	+
Direct antiglobulin test	1/256	1/2048	ND	1/8	1/2
Specificity IgG-C3d	+	+	ND	+	+
CEA serum level ($n < 2.5$ ng/ml)	ND	0.7	2.4	1.0	21.0

ND, Not determined.

*Alloantibodies were of the panagglutinin type.

gastroduodenoscopy revealed the presence of a brachy-esophagus and an infiltration of the corpus wall, with an easily bleeding white mucosa. Gastric biopsy specimens established the diagnosis of a malignancy (see pathologic results section). A gastrectomy and a splenectomy were performed. Postoperative recovery was uneventful, and the patient was discharged 15 days later.

Follow-up was characterized by complete disappearance of the symptoms of angioedema, despite unchanged complement levels (Table I). Simultaneously, there was a complete disappearance of the signs and symptoms of hemolytic anemia with progressive, but transitory, disappearance of the warm antibodies (Table II). A local recurrence of the gastric tumor was diagnosed 5 months later but could not be resected. The patient's condition deteriorated progressively, and she died at home 17 months after surgery, with a voluminous tumor mass that interfered with eating and moving but without any recurrence of either hemolytic anemia or angioedema. No autopsy was performed.

METHODS

Pathologic analysis

Tissue for light microscopic examination was fixed in 10% phosphate-buffered formalin solution and embed-

ded in paraffin; 4 μ m sections were stained with hematoxylin-eosin. Periodic acid-Schiff and alcian blue staining techniques were also performed on gastric biopsy and surgical specimens.

Paraffin-embedded sections from the gastric tumor and metastatic lymph nodes were studied with monoclonal and polyclonal antibodies: panepithelial marker Lu-5 (Hoffman-La Roche, Basel, Switzerland; monoclonal, diluted 1:300), carcinoembryonic antigen (CEA) (Dakopatts, Copenhagen, Denmark; polyclonal, 1:250), common leukocyte antigen (CD 45) (Dakopatts; monoclonal, 1:15), Le^a (Chembiomed, Alberta, Canada; monoclonal, 1:150), Le^b (Chembiomed; monoclonal, 1:150), Le^x (Dakopatts; monoclonal, 1:40), CA 19-9 (sialylated Le^a) (Oris Industries S.A., Gif-sur-Yvette, France; monoclonal, 1:2.5), and blood group-related antigens A (Dakopatts; polyclonal, 1:30), B (Dakopatts; monoclonal, 1:30), and H (Dakopatts; monoclonal, 1:60).

Frozen sections from gastric tumor were also studied with monoclonal antibodies for the detection of IgM (Dakopatts; polyclonal, 1:160), IgG (Dakopatts; polyclonal, 1:400), IgA (Nordic, Tilburg, The Netherlands; polyclonal, 1:160), C3 (Dakopatts; polyclonal, 1:100), and C1q (Behring AG, Marburg, Germany; polyclonal, 1:80).

The various antigens, except for CA 19-9, were immunostained according the peroxidase-antiperoxidase method of Sternberger et al.³⁴ with diaminobenzidine as an indicator. Appropriate positive control tissues were processed identically. Negative controls were obtained by replacing the antibody at the first step with normal rabbit or mouse serum or by omitting the second antibody. Swine anti-rabbit IgG serum (1:30), rabbit anti-mouse IgG serum (1:60), and rabbit (1:60) and mouse (1:200) peroxidase-antiperoxidase complexes were obtained from Dakopatts. Enzymatic digestion was carried out before incubation with the various primary antibodies. Protease XXVII (Sigma P4789, St. Louis, Mo.; 1 mg/2 ml phosphate-buffered saline) was used for 10 minutes at 37° C. The CA 19-9 antigen was immunostained with avidin-biotin-peroxidase complex by the method of Hsu et al.³⁵ with an ABC kit (Vector Labs, Burlingame, Calif.).

Blood analyses

Blood from the patient and control subjects (healthy blood donors) was obtained by venipuncture and was collected in tubes with or without ethylenediaminetetraacetic acid. Blood collected with ethylenediaminetetraacetic acid was centrifugated immediately. Serum was obtained by centrifugation of whole blood after spontaneous clotting for 30 minutes at room temperature and for 1 hour at 4° C. Patient serum, normal human serum, and plasma samples were immediately frozen in aliquots and stored at -70° C. Aliquots were thawed only once, immediately before testing. Concentrations of serum plasma proteins were measured by radial immunodiffusion³⁶ or nephelometry.³⁷ C1-INH function was assessed by the formation of complexes between C1-INH and target proteases C1r.³⁸ Complement hemolytic titers were assessed by micromethods with antibody-coated sheep erythrocytes for evaluation of the classic and terminal activation pathway (CH₅₀ assay).³⁹ Evaluation of the alternative pathway activity (APH₅₀) was performed with rabbit erythrocytes at isotonic conditions and in the absence of Ca⁺⁺.^{40, 41} Functional C2 was assessed by the method of Joisel et al.⁴² Anti-C1-INH antibodies were assessed by the method of Alsenz et al.¹⁹

Antibodies and complement-mediating immune hemolytic anemia were detected by the Diamed-ID gel technique (Diamed, Cressier, Switzerland). CEA levels were measured by enzyme immunoassay (Hoffmann-La Roche, Basel, Switzerland).

RESULTS

Pathologic findings

Macroscopically, the wall of the fundus and the body of the stomach were considerably thickened. Microscopically, these regions were extensively infiltrated by a poorly differentiated adenocarcinoma (signet ring cell type) (Fig. 1). Tumor cells invaded the entire thickness of the digestive wall

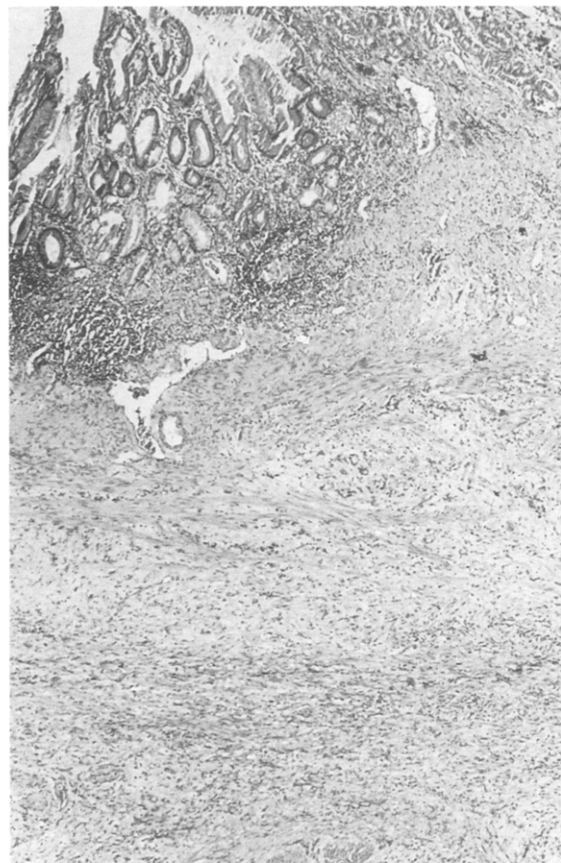


FIG. 1. The gastric wall was diffusely infiltrated by a poorly differentiated adenocarcinoma of the signet ring cell type. The overlying gastric mucosa showed changes of chronic atrophic gastritis with intestinal metaplasia. (Hematoxylin and eosin stain; $\times 33$.)

and extended into the peridigestive adipose tissues of the lesser and greater curvatures and the splenic hilar adipose tissue. Tumor cells contained cytoplasmic periodic acid-Schiff-positive, alcian blue-positive mucus vacuoles (Fig. 2). Ten of 15 perigastric lymph nodes were metastatic. The mucosa in the body and antrum showed lesions of chronic atrophic gastritis with intestinal metaplasia. The spleen, the omentum majus, the site of the liver biopsy performed perioperatively and the surgical margins of the gastrectomy specimen did not contain any tumor. No sign of a lymphoproliferative disease was detectable.

Immunohistochemical findings

The immunohistochemical findings are summarized in Table III. Tumor cells showed a strong reactivity with the antibody directed against C1q and epithelial marker Lu5, CA 19-9 and Le^a, but only a weak reactivity with the antibody directed

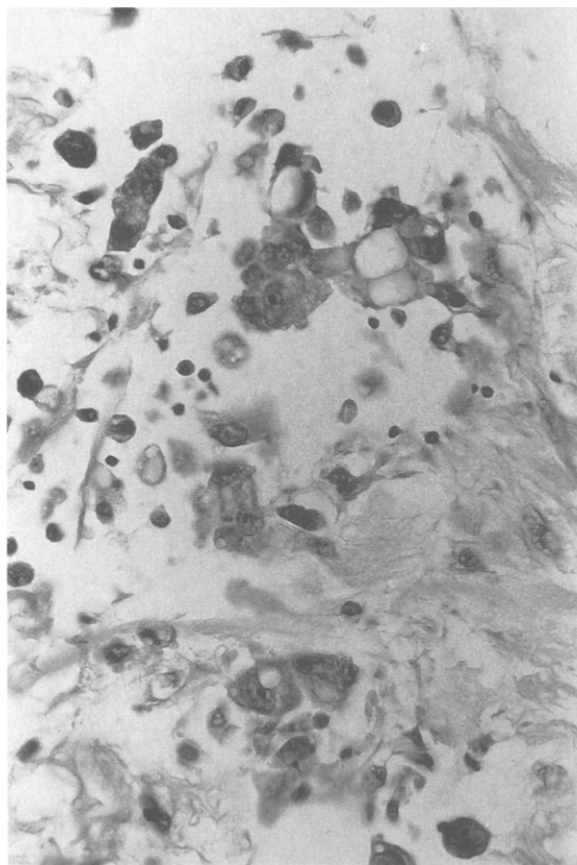


FIG. 2. Groups of carcinomatous cells exhibiting a signet ring appearance caused by cytoplasmic mucus accumulation. (Hematoxylin and eosin stain; $\times 33$.)

against CEA and no reactivity against blood group antigens. Normal gastric mucosal cells showed a strong reactivity against CEA and Lu5, a moderate reactivity against Le^a, and only a weak reactivity against blood group antigens B and H. The common leukocyte antigen (CD45) did not react with the tumor cells but demonstrated strong reactivity with the lymphocytes of the lymph nodes and gastric mucosa (internal controls). Red blood cells and endothelial cells reacted strongly with the antibody directed against the blood group antigen B and did not react with blood group antigen A, H, or any other marker.

Exclusion of a B-cell lymphoproliferative disorder

During the first two episodes of angioedema, the hemogram was normal; whereas during the third one, the white blood cell count showed a transitory leukopenia with a minimum of 2.8×10^9 cells/L in January 1990. Repartition showed only a slight excess in monocytes (13%), whereas lymphocytes

TABLE III. Immunohistochemical findings

Antigen	Gastric tumor	Lymph node metastases	Normal gastric mucosa
Lu-5	+++	+++	+++
CLA (CD45)	—	—	—
CEA	+	+	+++
CA19-9	+++	+++	+
Le ^a	+++	+++	++
Le ^b	+	+	+
Le ^x	—	—	+
Blood group-A	—	—	—
Blood group-B	—	—	+
Blood group-H	—	—	+
IgM	—		
IgG	Inconclusive		
IgA	+++		
C3	—		
C1q	++		

Determinations of IgM, IgG, IgA, C3, and C1q were performed on frozen sections only.

+, Weak reactivity; ++, moderate reactivity; +++, strong reactivity.

were either normal or slightly below normal range. Serologic markers, such as lactate dehydrogenase, were elevated only during the episode of autoimmune hemolytic anemia but remained normal after surgery (7 and 10 months). The same was true for the β_2 -microglobulin level.

The absence of B-cell lymphoproliferative syndrome was further assessed by normal serum protein electrophoresis and quantitative dosage of immunoglobulins and light chains. No monoclonal gammopathy could be detected by immunofixation both before and at 10 months after surgery.

Finally, bone marrow biopsy specimens failed to show any malignancy, and pathologic and immunohistochemical analysis of the resected stomach and spleen did not reveal lymphoma.

Blood analyses

Complement profile. Evolution of the different complement factors is shown in Table I. Quantitative levels did not change during the whole observation period, whereas functional tests revealed only an increase for C1-INH that nearly reached the lower normal limit shortly before the death of the patient.

Hemolytic anemia. Analysis of the time course of the hemolytic anemia showed an almost complete disappearance of the positive blood titer as determined by the direct antiglobulin test after surgery. No specific antibody against red cell com-

ponents, other than those associated with the patient's original blood and Rh groups, could be demonstrated. More specifically, no antibody against Le^a phenotype was present at any time during the disease course. In parallel, serum CEA levels increased progressively from the time of surgery to the time of death (0.7 to 21 ng/ml, $n < 2.5$) (Table II).

DISCUSSION

We had the opportunity to observe a 76-year-old woman with acquired deficiency in C1-INH that was complicated by hemolytic anemia. Investigations led to the discovery of a signet ring cell gastric adenocarcinoma (linitis plastica) (Figs. 1 and 2) with perigastric lymph node metastasis by the time of surgery.

Because the gastric tumor was unusual and unexpected in connection with acquired C1-INH deficiency, detailed investigations were carried out to make sure that there was no associated lymphoproliferative disorder.³⁻⁸ The findings obtained before and at the time of surgery did not reveal any sign of lymphoproliferation, either in the blood markers (lactate dehydrogenase and β_2 -microglobulin, immunoglobulin levels, immunofixation), in the bone marrow, liver biopsy specimens, or in the stomach and spleen tissue that was surgically removed. During the evolution of the disease, the blood markers for lymphoma remained constantly within the normal range.

Despite incomplete tumor removal, the postoperative outcome was characterized by a complete disappearance of angioedema but unchanged complement levels both in concentration and functional test results (Table I). Nevertheless, deposition of C1q in tumor tissue indicated a connection between C1-INH deficiency and tumor cell surface antigens. Thus two questions were raised: (1) how systemic complement consumption might occur and (2) why the absence of symptoms persisted despite tumor recurrence that was larger in dimensions than before surgery. We hypothesized that the answers to both questions were closely connected and might be related to the appearance of cross-reactive antibodies to tumor cell surface antigens.

In contrast to the situation observed in hereditary angioedema, in which a functionally critical level of C1-INH seems to be necessary to avoid symptoms,³⁶ in acquired C1-INH deficiency the synthesis of the active protein is normal or even increased, and minor changes in consumption probably govern the appearance of clinical signs of

this regulatory protein deficiency.^{12, 21} In several other patients with acquired angioedema we have observed that the lymphoproliferative disorder was apparently not a trigger strong enough to promote the clinical manifestations of angioedema. However, when these patients also experienced immune hemolytic anemia, acquired C1-INH deficiency became clinically manifest.⁴³ This was also the case for the patient described here. It could be postulated that the complement activation induced by changes in tumor cell surface structure and binding of anti-tumor cell surface antibodies results in a low C1-INH level that is still sufficient to maintain homeostasis because this activation takes place in a single organ and in lymph node metastases. When anemia is superimposed, an additional systemic complement consumption results, which might lead to angioedema.

The origin of the hemolytic anemia was sought by analysis of blood group antigen expression in tumor cells and metastases (Table III). They showed a loss of group B antigen in tumor cells with appearance of Le^a antigen expression in both tumor and normal gastric cells. Other authors have described frequent deletion of B blood group antigen and acquisition of Le type 1 antigen, as well as CA 19-9 (a marker of mucosecretion) in intestinal neoplasms.^{32, 33} This was also the case for our patient (Table III). The hemolytic anemia clearly seems to be associated with the tumor burden. Indeed, the antiglobulin antibody level, present when the gastric adenocarcinoma was discovered, declined and eventually disappeared after incomplete tumor removal but was detectable again when the tumor recurred (Table II). Other factors must have played a role because no antibody level to Le^a could be demonstrated at any time during the disease evolution. This again suggests ongoing complement consumption mediated directly by tumor cell surface antigens or complexes of tumor cell surface antigens and specific antibodies, thus explaining the absence of changes of the complement factor levels.

Because we could find CEA and CA 19-9, as well as Le^a with C1q localized in the proximity of the carcinomatous cells (Table III), and because the molecule of CEA itself has been shown to be able to carry ABH or Le blood group specificities,^{44, 45} cross-reactivity might also exist between CEA and anti-CA 19-9 antibodies, which may represent part of the panagglutinins that activate the complement cascade on red blood cells. This would explain the whole clinical picture.

Thus we can hypothesize that some connection

between tumor immunohistochemical profile, complement consumption, and hemolytic anemia might exist and lead to the clinical manifestations of acquired angioedema. Systemic complement consumption by red cells might accelerate a basic, already excessive, turnover of C1, and consequently, of C1-INH in tumor tissue. Others' observations are urgently needed to confirm our hypothesis and particularly to determine whether the observed deposition of IgA, and perhaps IgG, is directed against the CEA molecule. This might help to explain the pathophysiology of the hemolytic anemia associated with cancer, as well as tumor growth and metastatization.

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