Circulating Immune Complexes Detected by ^{125}I-C1q Deviation Test in Sera of Cancer Patients

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ABSTRACT The presence of circulating immune complexes in freshly drawn sera of patients with various forms of malignancies was detected by the ^{125}I-C1q deviation test of Sobel et al. More than 50% of the 459 cancer sera showed a high inhibition of ^{125}I-Clq uptake by sensitized sheep erythrocytes when compared with sera of 50 healthy laboratory personnel. The levels were compared with levels of total hemolytic complement and immunochemical determinations of Clq and C3. A correlation between high levels of circulating immune complexes and low levels of Clq was suggested.

These immune complexes were separated by sucrose density gradient ultracentrifugation at low pH and were found to be heavier than 19S. Fluctuation of levels of immune complexes was evident when serial samples from the same patient were tested. Decrease of levels of immune complexes and a concomitant increase of C1q were detected after Calmette-Guérin bacillus and autologous tumor cell treatment in some melanoma patients.

INTRODUCTION

Circulating soluble immune complexes and hypocomplementemia are associated with several clinical disorders. These include systemic lupus erythematosus, serum sickness reactions, scleroderma, Sjögren syndrome, mixed cryoglobulinemia, and Australia antigenemia. We have observed hypocomplementemia in 10% of sera tested from 6,000 patients with various forms of malignancies, a finding which could be of potential significance in the understanding of the immune response to tumor antigens. One patient with chronic lymphocytic leukemia (CLL),[1] hypocomplementemia, and cryoglobulinemia was studied in greater detail. Of special interest in this patient was the presence of high levels of antilymphocyte antibodies which were cold reactive and could be detected by both C-dependent cytotoxicity and indirect immunofluorescence. Free lymphocyte surface antigen was also demonstrated by gel diffusion with specific rabbit antilymphocyte antiserum. Sucrose density gradient ultracentrifugation at low pH (3.5) demonstrated that the antibody was 19S (1).

The existence of circulating immune complexes in sera of cancer patients has recently been reported by us (2–4) and by Theofilopoulos et al. (5). These investigators used the Raji cell radioimmune assay. In our studies we showed that sera of patients with low C1q levels (<10 μg/ml) determined by the Mancini technique (normal values 17–23 μg/ml) were strongly positive by the precipitation test described by Agnello et al. (6). These sera with low C1q levels also inhibited the uptake of ^{125}I-C1q by the C1q deviation test described by Sobel et al. (7). In the present study we have (*a*) investigated the presence of immune complexes in sera of a large number of patients with various forms of malignancies; (*b*) compared the levels of total hemolytic complement (C) and C components (C1q, C3) with detectable immune complexes; (*c*) isolated the immune complexes by sucrose density gradient ultracentrifugation

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[1] *Abbreviations used in this paper*: AHG, aggregated human gamma globulin; BCG, Calmette-Guérin bacillus; C, total hemolytic complement; C1q, INH%, inhibition of ^{125}I-C1q uptake; CH_{50}, serum dilution at which 50% of the indicator erythrocytes are hemolyzed; CLL, chronic lymphocytic leukemia; DTT, dithiothreitol; DT, deviation test; EA, erythrocyte antibody; SVB^{++}, sucrose veronal buffer.

at low pH; and (*d*) shown that fluctuation of immune complexes can occur after therapy.

METHODS

Patient sera. Blood for complement studies and detection of immune complexes was obtained from patients at Memorial Hospital, New York with various forms of malignancies. The patients in this study in general were from the Outpatient Clinic or had been admitted to Memorial Hospital for surgery. The blood samples from the latter group of patients were drawn just before surgery and in general these patients were free of infections or fever. Studies of patients who had been treated will be presented with the Results. Blood samples were delivered on ice from the clinics and wards, kept at 4°C for 2–3 h, clarified by centrifugation, aliquoted, and kept at −80°C. Our experience demonstrates that it is critical that these samples be used for determination of immune complexes within 2 wk and that they not be thawed at intervals.

Preparation of ^{125}I-C1q. C1q was isolated from normal recalcified plasma by precipitation with calf thymus DNA (Worthington Biochemical Corp., Freehold, N. J.) according to the method of Agnello et al. (6). The purity of the isolated C1q was tested by the double immunodiffusion method of immunoelectrophoresis and Ouchterlony with 0.01 M EDTA gel with monospecific antisera prepared against human IgG, IgM, and IgA (Behring Diagnostics, Somerville, N. J.), against C1s, C4 (Behring Diagnostics), and C3 (prepared in our laboratories according to the method of Mardiney and Müller-Eberhard [8]). In some preparations a slight contamination with IgG and IgM but not with the other proteins tested was found. These preparations were not used for our study.

Radioiodination with ^{125}I (New England Nuclear, Boston, Mass.) was performed with lactoperoxidase (Calbiochem, San Diego, Calif.) according to the method of Marchalonis (9). The labeled C1q was then exhaustively dialyzed against pH 7.2 phosphate-buffered saline for 38 h with three-times renewal buffer to remove the nonprotein-bound isotope. The final dialyzing buffer showed negligible radioactive counts and the ^{125}I-C1q precipitate in 10% TCA showed 90–95% of the total radioactive counts.

The radioiodinated C1q was aliquoted in 50-μl portions. Each portion containing 40–50 μg (0.1–0.5 μCi cpm/μg C1q) was stored in a liquid nitrogen tank until ready for use. For each test the samples of ^{125}I-C1q were thawed and diluted in 10% sucrose veronal buffer (SVB^{++}), pH 7.2, to a final concentration of 1 μg C1q/10 μl.

Preparation of aggregated human gamma globulin (AHG). Human IgG was purified from Cohn fraction II (American Cyanamid Co., Pearl River, N.Y.) by DE-52 cellulose (Whatman Chemicals, Div. W. & R. Balston, Maidstone, Kent, England) column chromatography and 0.05 sodium phosphate buffer at pH 7.85 described by previous methods (10). The aggregation of IgG was carried out according to previously described methods (11).

Preparation of sensitized sheep erythrocytes for the ^{125}I-C1q deviation test. Sheep cells (4×10^8/ml) were sensitized with IgG hemolysin. The antibody was first isolated from a commercial preparation of anti-sheep hemolysin (Becton-Dickinson & Co., Rutherford, N. J.) as follows. 10 ml of hemolysin was first dialyzed against 0.05 M sodium phosphate buffer at pH 7.85 and then applied to a DE-52 column (3×26.5 cm). The IgG was eluted with the same buffer in 5-ml fractions. The IgG appeared in the first protein peak at OD_{280} and gave a single band when analyzed by immunoelectrophoresis against goat anti-rabbit whole serum and goat anti-rabbit IgG (Cappel Laboratories, Downington, Pa.).

Varying concentrations of the purified IgG hemolysin were then added to a constant amount of sheep erythrocytes. The amount of erythrocyte antibody (EA) (IgG) mixture that bound 55–45% of ^{125}I-C1q was used for our experiments. In general, the IgG hemolysin dilution ranged from 1/60 to 1/90.

^{125}I-C1q deviation test (DT). The C1q DT was performed according to the method of Sobel et al. (7) with 4×10^8 IgG sensitized cells per milliliter. The ratio of bound ^{125}I-C1q to EA was adjusted to 45–55% with various concentrations of hemolysin. To obtain reproducible results in this test, it is critical to keep the serum samples at a constant temperature and to test them within 2 wk. In our experience we have found that some normal sera which were initially negative in the C1q DT became positive in the test after 3 mo.

The presence of DNA in serum samples was determined according to the method of Kunitz (12). 50 sera with a high inhibition of ^{125}I-C1q were tested for reduction of this inhibition after incubation with DNAse (Worthington Biochemical Corp.) as follows. 50 μg of DNAse was added to 0.2 ml of serum at 37°C for 2 h. The serum was then centrifuged at 7,000 *g* for 20 min and tested by the C1q DT. The same samples were tested under the same incubation conditions without DNAse.

The presence of endotoxin in patient sera was tested by the Limulus test described by Levin et al. (13). The Limulus lysate and *Escherichia coli* endotoxin was obtained from Dr. S. Watson (Woods Hole, Mass.).

Determination of C and C components. Buffers for the measurement of total hemolytic C and C components have been described elsewhere (14). Total hemolytic C was analyzed in a Technicon Auto Analyzer (Technicon Instruments Corp., Tarrytown, N. Y.) enabling us to study 50 sera per day. The remaining components C1q and C3 were determined immunochemically according to previously described methods (15). Antisera to C1q and C3 were prepared as described before (8, 16).

Sucrose density gradient ultracentrifugation. Sucrose density gradient ultracentrifugation experiments were performed with 10–40% sucrose in 0.2 M glycine buffer and 200 μl of serum sample in Spinco L2-62B Beckman centrifuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) at 108,000 *g* for 15 h at 7°C. In some experiments the centrifugation was carried out for shorter periods of time. After centrifugation, 0.25-ml fractions were collected in 5-ml test tubes. Each fraction was tested by the C1q DT with 0.02 M EDTA SVB^{++}. Rabbit IgM, IgG hemolysin (Cordis Laboratories, Miami, Fla.), and ^{125}I-C1q were used as 19S, 7S, and 11S markers, respectively.

Reduction and alkylation of sucrose gradient fractions. Reduction of sucrose gradient fractions which gave high inhibition of ^{125}I-C1q uptake was carried out according to the method of Heusser et al. (17) by treating the fractions with different amounts of dithiothreitol (DTT), (Sigma Chemical Co., St. Louis, Mo.) for 30 min at 37°C. Alkylation was performed with a 2.5-fold molar excess iodoacetamide solution (Sigma Chemical Co.) above the amount of DTT used for 15 min at 37°C. The treated samples were dialyzed against pH 7.2 veronal buffer overnight and then tested by the C1q DT.

Preparation of ^{125}I-IgG and IgG complexes. Rabbit ^{125}I-IgG was prepared as described above. Immune complexes with rabbit ^{125}I-IgG and goat anti-rabbit IgG (heavy chain) (Behring Diagnostics) were prepared at slight antigen excess according to previously described methods (18).

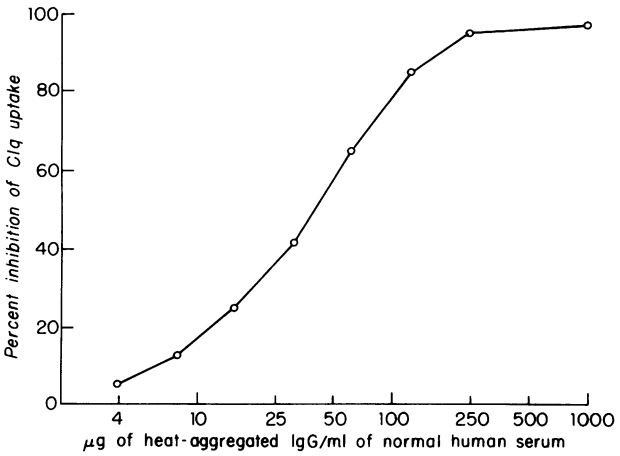

FIGURE 1 Uptake of ^{125}I-C1q by heat-aggregated gamma globulin. Percentage inhibition of C1q uptake (C1q INH%) was calculated to micrograms of heat-aggregated human IgG per milliliter (μg/ml eq AHG).

RESULTS

Quantitation of heat-aggregated human IgG by the ^{125}I-C1q DT. Various concentrations of heat-aggregated human gamma globulin were added to fresh normal human serum. Each mixture was diluted 1:4 in SVB^{++}, heated to 56°C for 30 min, and then tested by the C1q DT.

As presented in Fig. 1, a 95% inhibition of ^{125}I-C1q uptake (C1q IMH%) was obtained by 250 μg/ml of AHG, and 7% C1q IMH% was obtained by 4 μg/ml of AHG. This standardization was performed with every new batch of radiolabeled C1q and AHG. Normal sera without AHG was used as control (C1q INH% = 0). The concentration of C1q-reactive proteins or immune complexes in sera could thus be expressed as microgram equivalents of heat-aggregated human IgG (μg eq AHG/ml) based on this standard curve.

Detection and quantitation of immune complexes in sera of patients with malignancy. Sera from 128 patients with melanoma, 91 with breast cancer, 50 with head and neck tumors, 48 with gynecological malignancies, 41 with lung cancer, 37 with colon and rectal cancer, 19 with CLL, 16 with ovarian tumors, and 28 with miscellaneous tumors were tested by the ^{125}I-C1q DT for the presence of immune complexes. The results were expressed in terms of micrograms of equivalent protein to AHG based on the standard curve (Fig. 1). Immune complexes were detected in sera of a high number of patients (Table I). The presence and amount of complexes did not appear to be specific for a particular malignancy (Fig. 2). However the amount of immune complexes was widely scattered. In several cases the levels of immune complexes were as high as those seen in sera of 10 patients studied with active systemic lupus erythematosus.

Comparison of serum levels of C, C1q, and C3 with the amount of circulating immune complexes in sera of cancer patients. A comparative study of total hemolytic serum dilution at which 50% of the indicator erythrocytes are hemolyzed (CH_{50}), C1q, C3, and the amount of circulating immune complexes was made from sera of 459 cancer patients (Table II). As shown in the table, the mean values of C, C1q, and C3 in malignant sera were higher than normal. The correlations of C, C1q, and C3 with immune complexes are shown in Table III, and the results fall within five ranges (≥2 SD, ≥1 SD, normal, ≤1 SD, and ≤2 SD). The percentage of sera with elevated levels of immune complexes (>12 μg/ml) and the mean values of immune complexes in each group are presented. 453 sera were tested for total CH_{50}, 440 were tested for C1q levels, and 446 were tested for C3 levels. The values determined were compared with levels of immune complexes. As shown in the Table, 44–57% of sera with elevated levels (≥ + 1 SD or ≥ + 2 SD) of all three components or with levels below −1 SD and within the normal range had high levels of immune complexes. 63–77% of sera with reduced levels of total C, C3, and C1q, particularly with levels

TABLE I
Immune Complexes in Sera of Patients with Various Forms of Malignancies

Type of malignancy	Number of patients	Mean AHG/ml
	n	*μg eq/ml*
Melanoma	128	15.0 (0–175.0)
Breast	91	13.0 (0–140.0)
Head and neck	50	16.0 (0–72.0)
Gynecological	48	14.0 (0–105.0)
Lung	41	15.5 (0–150.0)
Colon and rectal	37	12.0 (0–48.0)
CLL	19	8.4 (0–46.0)
Ovarial	16	5.8 (0–29.0)
Pancreas	4	26.0 (17.5–51.0)
Chronic myelocytic leukemia	4	14.0 (8.5–20.5)
Bone	4	23.0 (0–81.0)
Bile duct	2	17.3 (12.5–22.0)
Erythroleukemia	2	8.3 (0–16.5)
Hepatoma	2	5.5 (0–11.0)
Stomach	2	29.0 (0–58.0)
Sarcoma	2	9.5 (0–19.0)
Acute myoblastic leukemia	1	8.5
Acute lymphoblastic leukemia	1	0
Bladder	1	33.0
Gall bladder	1	7.8
Parotid	1	18.0
Reticulohistocytosis	1	0
Normal (healthy individuals)	50	4.5 (0–12.0)
Systemic lupus erythematosus	10	60.0 (4.0–115.0)
Multiple sclerosis	17	7.0 (0–31.0)

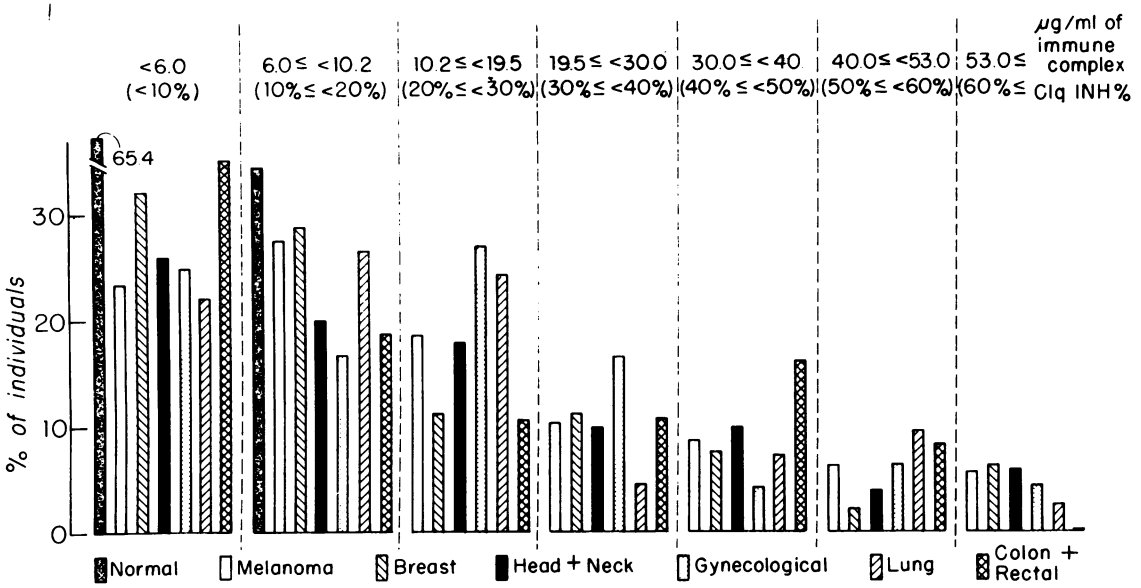

FIGURE 2 Distribution of immune complexes in sera of cancer patients. Normal value from sera of 50 healthy adults was 0–12 μg/ml eq AHG.

below 2 SD of all three components, had high levels of immune complexes. When levels of immune complexes were expressed as mean values, sera with low levels of Clq had elevated levels of immune complexes. Statistical analysis of these studies was not possible because the number of samples with low levels of C and C components was insufficient.

Comparative studies on the amount of complexes associated with changes in C levels were also carried out whenever possible in serial samples of patients. It was seen that fluctuations of complexes occurred which correlated with changes in C levels. Two examples are presented (Fig. 3). As seen in the serum of patient G. M., the amount of complexes decreased dramatically on the 3rd day from 93 to 19% (210 μg eq AHG/ml to 11 μ eq AHG/ml) with a concomitant increase of Clq levels from 15 μg N/ml to 22 μg N/ml. On the 49th day the levels of immune complexes were again elevated with significant decreases of C3 and total C levels. With patient N. K. (Fig. 3) the levels of immune complexes were found to be increased on the 22nd day (from 4 μg eq AHG/ml to 40 μg eq AHG/ml) with a decrease of C3 and total hemolytic levels, but when measured on the 83rd day, the increased levels of immune complexes correlated with decreased levels of Clq only. These data indicate that increased levels of immune complexes in some instances cause a decrease in levels of C and of C components. These observations are consistent with our findings that although in some instances elevated levels of immune complexes correlate with decreased levels and C and C components, in other instances elevated levels of immune complexes are also detected in the presence of normal C values. The reasons for these observations are not clear to us at present but are currently under investigation.

Detection of DNA and endotoxin. To rule out the presence of DNA and endotoxin, substances which are known to interfere in the Clq DT the following experiments were carried out.

Treatment with DNAse of 50 malignant sera with high levels of immune complexes indicated that with the exception of serum of one patient, the Clq reactants determined in cancer sera were not due to the presence of DNA.

20 sera samples with high levels of immune complexes were tested by the Limulus test described by Levin et al. (13) for the presence of endotoxin, with a Limulus lysate with extremely high sensitivity to endotoxin (range > 0.04 ng/ml). Positive controls included a preparation of *E. coli* endotoxin. The presence of endotoxin was not detectable in any of the serum samples tested. The serum samples were diluted serially to dilute out the inhibitor which is often present

TABLE II
Complement Levels (Total CH_{50}, Clq, and C3) of Cancer Patients

	Number	Mean	SD	Normal
CH_{50}	453	123.2	35.7	81–95 U CH_{50}/ml
Clq	440	25.4	5.3	18.1–23.3 μg N/ml
C3	446	131.4	36.0	84–130 mg/100 ml

TABLE III

Comparison of Levels of C, C1q, and C3 with Levels of Immune Complexes

	Level of CH_{50}, Clq, and C3	≥ +2 SD	≥ +1 SD	Normal	≤ −1 SD	≤ −2 SD
CH_{50}	No. sera, *n*	173	83	160	28	9
	Sera with high level of immune complexes, %	49.7	53.0	43.1	53.5	66.6
	Mean of immune complexes, *μg eq AHG/ml*	13.7	12.0	12.7	18.2	15.3
Clq	No. sera, *n*	194	81	140	14	11
	Sera with high level of immune complexes, %	44.3	51.8	45.7	57.1	72.7
	Mean of immune complexes, *μg eq AHG/ml*	12.0	12.7	14.5	17.0	28.5
C3	No. sera, *n*	114	87	220	17	8
	Sera with high level of immune complexes, %	53.5	45.9	43.6	52.9	62.5
	Mean of immune complexes, *μg eq AHG/ml*	15.2	13.5	12.0	18.8	18.2

in human serum and which often interferes with the clotting of the lysate.

In addition, when several dilutions of *E. coli* endotoxin were tested by the C1q DT, it was shown that more than 50 μg/ml of endotoxin inhibited the uptake of ^{125}I-C1q. Below that range the endotoxin did not interfere in the uptake of radiolabeled C1q by EA. These data strongly suggest that in most of our serum samples tested, the presence of C1q reactants was not due to the presence of DNA or endotoxin.

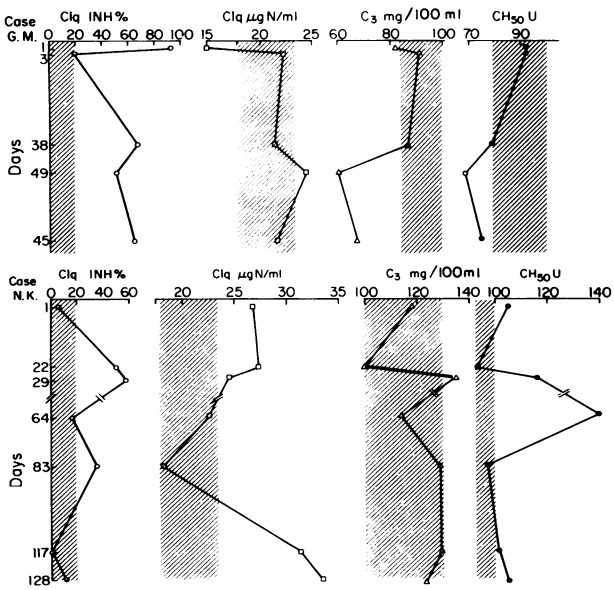

FIGURE 3 Study of levels of immune complexes, C1q, and C3 of two patients with melanoma (G. M. and N. K.) at different days. Correlation with levels of C1q, C3, and total hemolytic complement.

Isolation of soluble immune complexes by sucrose density gradient ultracentrifugation. Three serum samples, two from patients with melanoma and one from a patient with cancer of the stomach, all with high levels of circulating immune complexes (50–75 μg eq AHG/ml), were fractionated by sucrose density gradient ultracentrifugation on 40–10% sucrose gradient in 0.02 M glycine buffer at pH 7.2, 4.5, 3.5, and 2.5, respectively, for 15 h at 108,000 *g*. Controls included normal human serum and three markers (19S, 11S, and 7S) in separate tubes treated under identical conditions. The fractions were collected in 0.25-ml volumes. Each fraction was then tested by the C1q DT. At pH 7.2 a pellet was present in both the gradient tubes containing the patient's serum (Patient F) and in the freshly drawn serum of a healthy donor. No pellets were present in the tubes containing the markers. The pellets were redissolved in 0.3 ml isotonic saline solution and tested by the C1q DT. As shown in Fig. 4, there was a high inhibition of ^{125}I-C1q uptake with redissolved pellets in saline from the patient's serum, when compared with pellet washings from normal serum. The uptake of ^{125}I-C1q in this experiment by untreated EA was 60%. At pH 4.5 complexes were detectable in a broad region heavier than 19S and at pH 3.5 a dissociation of complexes was observed in the 19S region. With sera of patients H, T, and N (Fig. 5) the complexes were heavier than 19S. No complexes were detected when normal human serum was centrifuged at pH 4.5 or 3.5. These sucrose gradient fractionation experiments were very similar to patterns observed with gradient

fractions of serum of a patient with CLL where at low pH we were able to isolate complexes consisting of cold reactive lymphocyte antibody (1), also demonstrable by C1q DT, and to sucrose gradient fractions of rabbit IgG goat anti-rabbit IgG complexes prepared in slight antigen excess (Fig. 6). Although it is beyond the scope of this paper to identify the type of antibody present in the isolated complexes in various cancer sera studied, we were however able to show that antibody was present by the following experiment.

Sera containing high levels of immune complexes were subjected to sucrose density gradient ultracentrifugation at pH 3.5. The fractions (>19S) that inhibited the uptake of ^{125}I-C1q by EA were pooled, reduced, and alkylated and then retested by the C1q DT. As seen in Table IV, the inhibition of uptake of ^{125}I-C1q in the treated sample decreased. This decrease was dependent upon the amount of reducing agent used.

Changes in levels of circulating immune complexes after treatment. During the course of our studies we were able to investigate serial serum samples of four melanoma patients undergoing therapy. The therapy was as follows. Tumor cells were removed from the patient and gently minced in RPMI-1640 medium (Grand Island Biological Co., Grand Island, N. Y.). The cells were irradiated to 10,000 R, mixed with Calmette-Guérin bacillus (BCG) (supplied by Research Foundation, Chicago, Ill.), and injected intradermally, usually every 2–3 wk, until the supply was exhausted. Blood was drawn periodically for C studies. As shown in Fig. 7, in one patient (M. C.) there was a consistent and dramatic drop of levels of immune complexes concomitant with a drop of C1q levels 7–9 days after treatment. This was observed on two separate occasions. This drop was most marked on the 49th day (19.5–6.0 μg eq/ml AHG) and on the 111th day (74–15% μg eq/ml AHG) with a concomitant increase of C1q from 20.5 to 26.5 μg N/ml and 23.5 to 31 μg N/ml, respectively. These are significant changes and do not reflect day-to-day errors. The levels of immune complexes and C1q increased 9–10 days after the combined tumor cell BCG therapy. A similar depression of immune complexes was also observed in three other patients (Figs. not presented). However, the correlation between immune complexes and C1q was not always as clear as described for patient M.C.

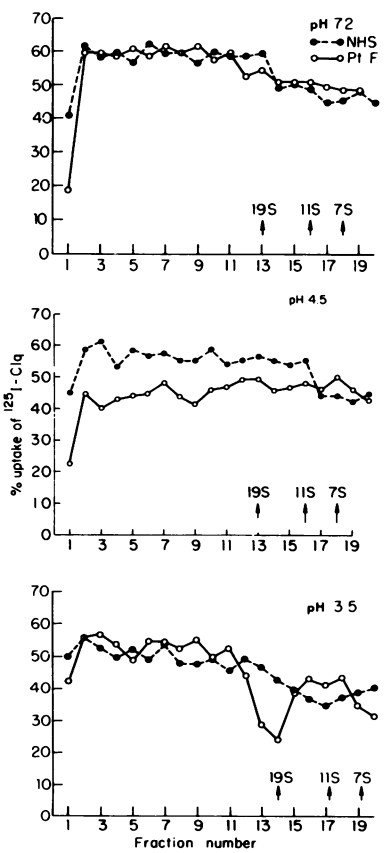

FIGURE 4 Sucrose density gradient ultracentrifugation at pH 7.2, 4.5, and 3.5 of serum of a patient with melanoma compared with serum obtained from a healthy adult. The fractions were tested with ^{125}I-C1q for presence of immune complexes.

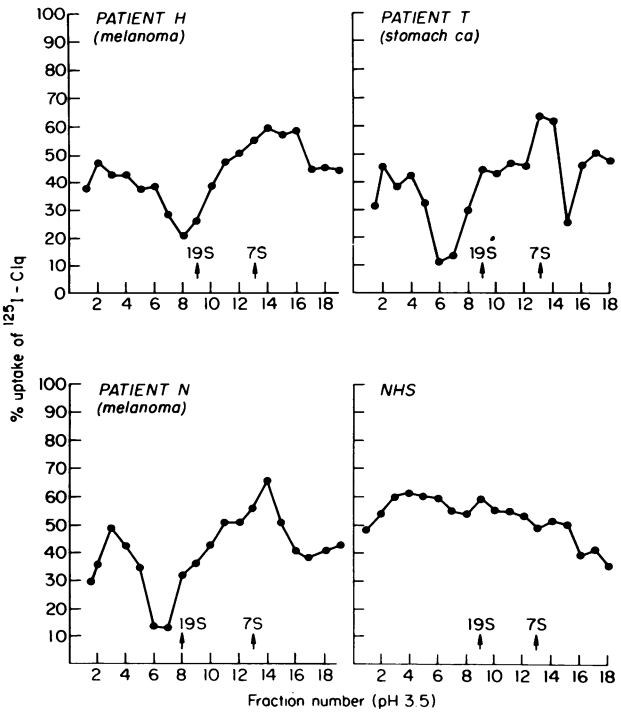

FIGURE 5 Sucrose density gradient ultracentrifugation of three malignant sera, two with melanoma and one with stomach cancer (ca), and one normal.

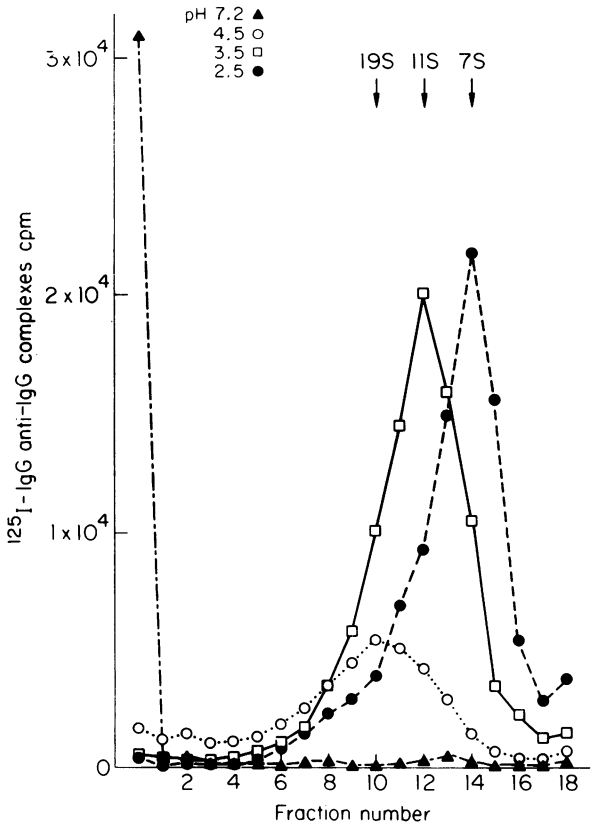

FIGURE 6 Isolation of IgG anti-IgG complexes at different pH by sucrose density gradient ultracentrifugation.

DISCUSSION

We have screened sera for complement studies in cancer patients for the past 3 yr and have observed that 10% of the sera are hypocomplementemic. One patient with marked hypocomplementemia associated with recurrent and nonhereditary angioedema, cryoglobulinemia, and CLL was studied in detail (1). C1q, C1r, C1s, C4, and C2 levels were dramatically reduced on three different occasions. Comprehensive laboratory examination revealed an elevated IgM and a cryoglobulin. The pattern of activation resembled that seen in immune complex disease. A further similarity to immune complex disease was the presence of a strong C1q reactant in the patient's serum measured by both the immunodiffusion technique of Agnello et al. (6) and by the more sensitive technique of Sobel et al. (7). The patient's serum had a strong lymphocytotoxic antibody which was specifically enriched in the cryoglobulin. In addition both the serum and the cryoglobulin demonstrated lymphocyte membrane antigens. The accumulated evidence indicated that immune complexes consisting of IgM antilymphocyte antibody and lymphocyte membrane antigens were present in the serum of this patient.

TABLE IV
Reduction and Alkylation of Immune Complexes

DTT	Uptake of ^{125}I-C1q	
	Normal	Patient N
mM	%	
0	50.2	39.7
1	51.5	47.5
5	52.6	49.0
10	53.2	50.4

The study of this patient was extended to include other cancer patients. We showed that although 10% of the patients were hypocomplementemic, over 50% of the patients' sera had circulating immune complexes when tested by the C1q DT (2). The quantity of complexes was sometimes as great as that detected in patients with systemic lupus erythematosus. DNA and endotoxin, substances known to interfere in the C1q DT, were negligible in sera of 50 cancer patients tested with high circulating immune complexes. The presence of Forssman antibody, if any, also did not interfere in our test since our sheep cells were already hypersensitized, and based on our calculations, a 10% increase of ^{125}I-C1q uptake over the 50% uptake on sensitized sheep cells would require 100 μg/ml of IgG antibody.

When the levels of immune complexes were compared with levels of total C, C1q, and C3, a comparison could be made in general between elevated levels of immune complexes and C1q and sometimes with C3 levels. This observation was not always consistent, as in some instances immune complexes were also shown to be present in sera of patients with high

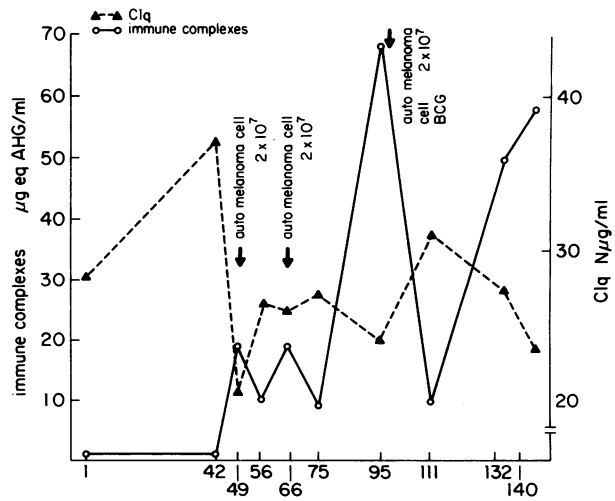

FIGURE 7 Levels of immune complexes and serum C1q of serial samples of a patient with melanoma after treatment with autologous tumor cells and BCG.

levels of C1q and C3. The reason for this observation is not clear at present but is currently under investigation.

The largest group of patients studied was that with melanoma. Whenever possible, serial samples from these patients were also tested. A fluctuation of levels of immune complexes was found in some instances. This fluctuation appeared to be most dramatic and consistent in sera of patients after treatment with irradiated autologous tumor cells and BCG. In one patient (M. G.) there was a consistent drop of levels of immune complexes concomitant with an increase of C1q levels 7 days after treatment. This was shown three times. An increase of immune complex levels followed by a decrease of C1q levels occurred 91 days after the combined tumor cell-BCG treatment. A similar depression of immune complexes was observed in three other patients after tumor cell-BCG therapy. The correlation, however, between immune complexes and C1q was not always as clear as described for M. C. It has not been possible in these studies to determine whether the changes noted in immune complexes and complement components are related to changes in extent of disease or other treatment. It should be possible with further studies not only to say that such abnormalities occur frequently in patients with cancer, but also to state which types and stages of cancer are most likely to be associated with abnormalities. In studies reported by Sobel et al. (7), patients with Dengue hemorrhagic fever were characterized according to severity of disease into Grades I–IV. Sera from patients with disease Grade IV exhibited the highest degree of C1q deviation, and those from patients with Grade 1, the lowest. These data suggest that immune complexes in sera of these patients are pathogenically significant.

Sucrose density gradient ultracentrifugation at different pH values was used to analyze some of the immune complexes. At pH 7.2 higher levels of ^{125}I-C1q inhibiting substances were present in the pellet of patient serum than in the pellet from normal serum. At pH 3.5 the immune complexes from the patient's serum sedimented at a region 19S. This was not observed with normal serum. These data suggest to us that complexes which before centrifugation were of heavy molecular substances gradually dissociated at low pH to a lighter molecular size. Reduction and alkylation of these isolated complexes showed that with increasing amount of reducing agents added, the ^{125}I-C1q inhibition decreased suggesting that antibody was present in these complexes.

Additional evidence for the presence of circulating immune complexes in malignancy has been recently reported by other investigators (5, 19, 20) with different techniques. The occurrence of immune complexes in a high number of cancer patients could be of potential significance in the understanding of the immune response to tumor antigens, particularly in the light of observations by several investigators in experimental animals. It has been shown that immunity can be induced against many experimentally induced animal tumor antigens when transplanted into syngeneic hosts (21–23). This immune response may have a diminished effect in the rejection of the tumor transplant by the presence of circulating humoral factors, i.e., blocking factors. It has been suggested that the blocking factors can be antibody (24–26) but there is greater evidence that blocking is mediated by immune complexes (24, 27, 28). Whether the circulating antigen-antibody complexes in sera of cancer patients have blocking effects will be elucidated by further studies.

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