

SERUM LEVELS OF SECOND COMPONENT OF COMPLEMENT IN CANCER PATIENTS¹

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Awareness that advancing cancer is often accompanied by defective immunologic responses, and accumulating evidence that immune reactions may influence the course of cancer, has stimulated the study of various mechanisms of immunologic reaction in patients with cancer. As part of such a program we have determined the serum level of the second component of complement in a group of cancer patients.

In a previous study, total serum complement (C') titers in a group of cancer patients had the same mean value as in healthy controls, although the range was broader (1). However, the hemolytic phenomenon by which complement is assayed is a complex process involving many serum constituents (2-4). Therefore, the "total complement titer" is generally determined by that component which is present in the lowest effective concentration, and deviations in the concentration of complement components may occur which are not detected by the usual assay for total activity. More important, the participation of the various complement components may not be identical for all immunologic reactions. For example, Nelson (3) reports that of nine components of complement which are required for sheep cell hemolysis, only four are required for specific immune phagocytosis. Thus the level of each of the components of the complement system becomes of interest.

Depressed levels of the second component of complement (C'2) have been found in patients with systemic lupus erythematosus (5) and hereditary angioneurotic edema (6). A drop in C'2 has also been found to be an early and sensitive indicator of the homograft rejection reaction in patients bearing homotransplanted kidneys (7). Since these immunologic situations may possibly have a counterpart in defense reactions against autochthonous cancer, the present study of C'2 levels in cancer patients was undertaken.

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METHODS

The cancer patients were hospitalized in the James Ewing or Memorial Hospitals. They represent a wide range of diagnoses and clinical conditions and many were receiving chemotherapy or radiotherapy. The few patients with diseases other than cancer were also from these Hospitals. The healthy persons were personnel of this laboratory.

Blood was collected from the antecubital vein in "Vacutainers" (Becton, Dickinson and Company, Rutherford, N.J.). The tubes were kept at room temperature for ½ hr to allow clot retraction to start and were then placed in the refrigerator for up to 4 hr. After discarding the clot, the bloods were centrifuged at 0°C and the clear serum was distributed into four or five ampules containing approximately 1 ml each. The ampules were flame-sealed, labeled, and stored at -70°C in a mechanical freezer. They were thawed not longer than 5 min before the C'2 titration.

The technique of C'2 titration was that described in detail by Austen (6), to whom we are indebted for instruction in the technique. The general principle of the titration includes three steps: First, sheep erythrocytes (E) are sensitized with a rabbit antiserum (A) and then reacted with guinea pig serum under conditions which limit the fixation of complement to the first and fourth components (C'1 and C'4). The resulting product is designated EAC'1,4. Second, this EAC'1,4 preparation is reacted with the test serum under conditions which permit the binding of C'2 but not the third component of complement (C'3). The extent of this reaction is limited by the amount of C'2 in the serum under study. Third, these EAC'1,4,2 cells are reacted with guinea pig serum in the absence of divalent cations. This prevents any further reaction of C'2 but allows the several components of C'3 to react. Since the complexing to the sensitized erythrocyte system can occur only if C'2 has already reacted, the completed process of complement fixation and consequent hemolysis is directly proportional to

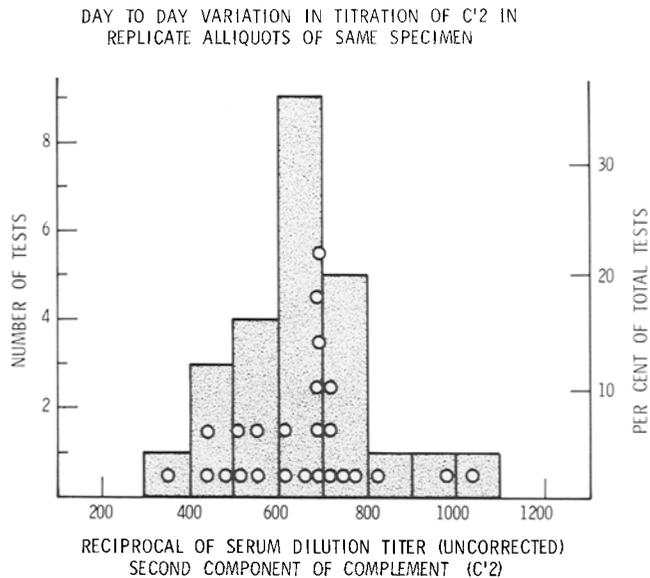


Figure 1. Actual titers of C'2 activity as determined in tests done on different days on individually stored aliquots of the same serum specimen. This spread of results reflects variations due to technical manipulation and due to biological variation in the reagents. It illustrates why C'2 titers are expressed in relation to a simultaneously tested reference serum rather than as serum dilution titers.

the amount of C'2 in the test serum. With occasional exceptions, each day's run included four serum specimens—the reference standard, one cancer serum, one healthy control serum and one other.

C'2 titers are expressed as that serum dilution which gave 63% hemolysis. (This is a convenient endpoint because it can be designated as unity on a logarithmic scale by plotting per cent of non-hemolyzed cells as natural logs, $\log 0.37 = -1.00$.) Per cent hemolysis was determined with the aid of a spectrophotometer. Extrapolation to the 63% hemolysis level from the observed amount of hemolysis at the serum dilutions actually tested was accomplished by plotting the per cent of non-hemolyzed cells, expressed as a natural logarithm, on the vertical axis of semilog graph paper and serum dilution on the horizontal (logarithmic) axis.

RESULTS

On three occasions a single specimen of serum was tested in duplicate simultaneous runs. Identical results were obtained twice and on the other occasion the duplicate samples gave titers which differed by 20%. These results indicate a fairly good reproducibility of the manipulative aspects of the technique.

The day-to-day reproducibility of the method was studied by performing multiple titrations of aliquots of a single serum specimen from a healthy donor on many days. The minimum amount of serum (expressed as serum dilution) required to produce the standard amount of hemolysis in repeated test runs of this serum specimen in 25 studies over a period of 6 months is indicated in Figure 1. The spread of results from 1:350 to 1:1040 for the serum is similar to that reported by Austen and Beer (6) and reflects variation in different preparations of the EAC'1,4 reagent. This rather wide range of values as determined in identical serum specimens necessitates the use of a standard reference serum against which all actual titers can be adjusted to provide a basis for comparison. This was accomplished by including a standard reference serum in every test and expressing the titers of test sera as a percentage of the standard. The serum shown in Figure 1 served as this reference standard, and to facilitate analysis of the present studies all other C'2 titers are expressed as per cent of this normal reference standard. However, in order to make possible the direct comparison of our results with those obtained in other laboratories we have also presented our data as "adjusted titers" with reference to the "FKA" standard of Dr. Austen's

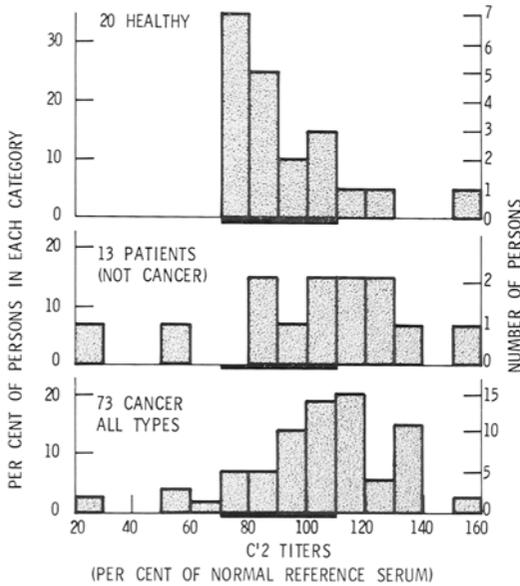


Figure 2. Distribution of serum C'2 titers (expressed as per cent of the standard reference serum) in healthy persons, cancer patients, and patients with diseases other than cancer. The thick black line on each abscissa is the range which includes 85% of the healthy control series. Note that more than one third of the cancer patients had C'2 levels above this range, and that both cancer patients and noncancer patients occasionally had very low C'2 levels.

laboratory. To make this possible, our reference serum was tested in Dr. Austen's laboratory and a ratio of the titer of our reference serum to his FKA reference standard was established (580:640). Thus our serum dilution titers can be "adjusted" to the FKA standard by multiplying by the ratio 640:580. Similarly, when our titers are expressed as per cent they can be converted to "adjusted serum dilution titers" by multiplying the FKA reference titer (1:640) by our percentage titers \times 0.01.

In order to determine the biologic variation of C'2 titers from day to day in healthy persons, serum samples were obtained from a healthy young woman on four occasions during a period of 9 months, and two sera were obtained 1 month apart from a healthy man. The woman's titers ranged from 79 to 100% (adjusted titers 1:455 to 1:580), and the man's titers were 65 and 74% (1:375 and 1:427).

In order to determine the normal range of C'2, sera were obtained from 20 healthy persons, 13 women with an average age of 34 (range 19 to 57),

and 7 men with an average age of 39 (range 30 to 59). Results are presented in Table I and in the top bar graph of Figure 2. If more than one serum was obtained from an individual or if a serum specimen was titered more than once, an average value was used so that each person is represented only once. This healthy group showed a mean C'2 titer of 90% (1:520) with a range from 71 to 158 per cent (1:417 to 1:916). This compares with a range of 1:325 to 1:600 and mean of about 1:480 (80%) in the 10 normals studied by Austen (6) using this same technique, and is similar to the values obtained by Townes and Stewart (5) using a system in which all the complement reagents were of human, rather than guinea pig, origin. The distribution shows the mode near the lowest value (75%), the median slightly higher (85%) and the mean even higher (90%) due to a sharply skewed distribution curve. The two highest titered sera (123% and 152%) were from Negro women. The only other Negro in the healthy control group was another woman, whose titer was 91%. No Negro men were studied. Females had higher C'2 levels than males (96% vs. 76%), even if only white persons were considered (88% vs. 76%). These differences were not attributable to chance grouping in the day-to-day titrations, but they cannot be assumed to be biologically significant because, in this small number of persons, such a difference could easily have occurred by chance. There was no apparent relation between C'2 level and age.

The 73 cancer patients had generally higher C'2 levels than healthy persons, as reflected in mean, median and modal values (Table I). However, the difference between the means of the cancer patient group (104%) and the healthy control group (92%) is not statistically significant. (The difference between these means is less than twice the standard error of this difference.) All but two of the cancer patients were white. One of the Negroes was a 31-year-old female with ovarian cancer and hepatic cirrhosis who had a C'2 titer of 71% (1:410) and the other was a 72-year-old man with rectal cancer metastatic to lungs who had a C'2 titer of 152% (1:880). Thirty-nine of the 73 cancer patients were females. There were more men in all diagnostic categories except cancers of the breast or female genital tract. The females averaged 50 years of age (range 3 to 70) and the males averaged 47 (range 12 to 72). As in the healthy control group, the average of C'2 titers was higher in female than in male cancer patients.

TABLE I
Summary of C'2 data by major diagnostic categories

Diagnosis	No. of Persons	C'2 Titers (%)				
		Range	Mode ^a	Median	Mean	S.D.
Healthy	20	71-152	75	85	92	±20
Sick; not carcinoma	13	28-154	Diffuse	109	102	±33
Leukemia	8	71-114	105	100	95	
Lymphomas ^b	29	24-157	135	107	102	±32
Other sarcomas	4	72-126	Diffuse	100	102	
Adenocarcinoma, breast	9	64-139	105	109	111	
Other adenocarcinoma	13	67-152	115	110	106	
Epidermoid carcinoma of the cervix	4	96-131	Diffuse	105	110	
Other epidermoid carcinomas	6	72-139	Diffuse	115	108	
All cancers	73	24-157	115	105	104	±26

^a Modes are expressed as midpoint of the 10 unit interval representing the most individuals. Medians and means are recorded to nearest unit.

^b Lymphoma category includes lymphosarcoma, reticulum cell sarcoma, Hodgkin's disease, and multiple myeloma. See Figure 3 for further breakdown.

Mean titers were 108% and 97% respectively. This difference, however, was due entirely to the five lowest titers which all occurred in males with lymphoma, so the difference is not related to sex *per se*.

Although the averages are similar, the distribution curve of C'2 titers is strikingly different between cancer and control groups (Fig. 2), being skewed to the right in the healthy population and skewed to the left in the cancer group. That is, high C'2 titers were more frequent in cancer patients than in healthy controls although none of the titers exceeded the maximum of the control group.

In contrast to the generally high C'2 titers among the cancer patients, 7 patients had C'2 titers below the minimum titer of the healthy controls. In 2, the titers were just below the control range—69% in a patient with endometrial cancer and 64% in a patient with mammary cancer. The other 5 were below 60% and all of these were from patients with lymphosarcoma or reticulum cell sarcoma. Eleven other patients with lymphosarcoma or reticulum cell sarcoma had C'2 titers widely scattered within the normal range (Fig. 3). In contrast, among 13 patients with Hodgkin's disease or multiple myeloma, which are also primary neoplasms of the reticulo-endothelial system, there were no low C'2 titers; in fact, the lowest titer among these 13 patients was above the average of the healthy control group and 9 of the 13 had titers above 110% level.

Further breakdown of the data according to the histologic types and primary sites of cancer failed to reveal any impressive deviations from the total cancer group; in fact, the distribution of C'2 titers in the carcinoma group (adenocarcinoma plus epidermoid carcinoma) almost perfectly duplicated the total cancer population (Figs. 3, 4).

In addition to this analysis according to basic diagnosis, clinical data were reviewed for particular consequences of cancer or forms of treatment to see if C'2 titer might be affected, and patients who had C'2 titers above and below the usual range were singled out for special review.

Of the five patients who had C'2 levels below 60% (all had lymphomatous neoplasms), two died on the same day that the serum sample was obtained, one died 5 days later and another died 11 days later. The other one died 8 months later, and lived only 10 months after clinical onset of his disease, but was in fairly good general condition when the serum sample was obtained. In contrast, the group of 13 patients who had C'2 levels over 130% included 5 lymphoma patients who were without evidence of active disease or who were in fairly good general condition and able to attend the out-patient clinic in spite of disease which required maintained treatment. However, the group with high C'2 levels also included one lymphosarcoma patient who died 7 days after the blood sample was obtained, and a patient with epidermoid cancer of the skin who died 22 days after her blood was obtained. Two other patients

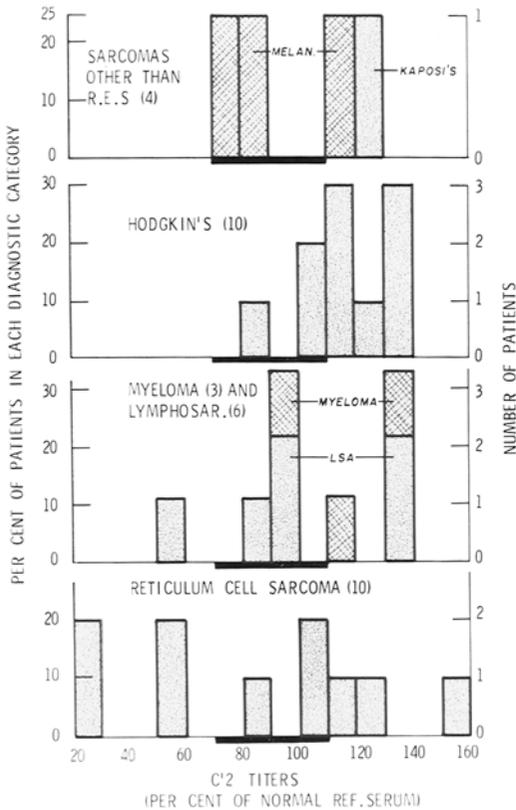


FIG. 3

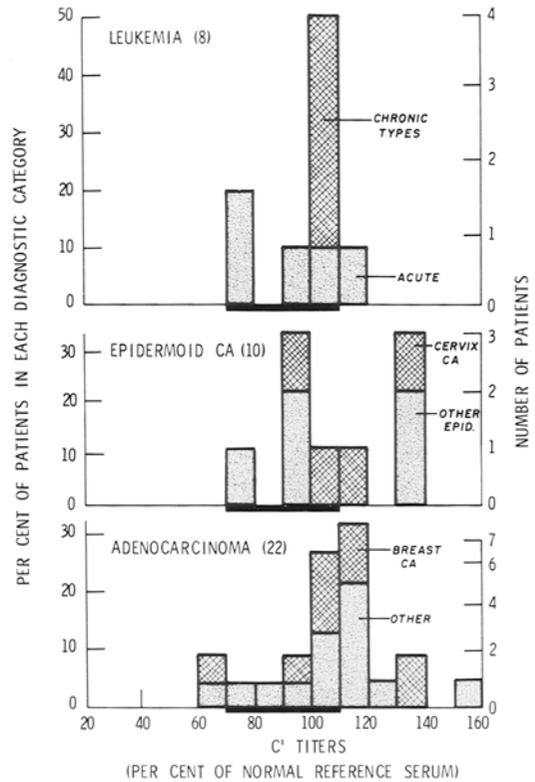


FIG. 4

Figures 3 and 4. Distribution of serum C'2 titers in patients with malignant neoplasms of various types. The thick black line on each abscissa is the range which includes 85% of the healthy control series. Note the preponderance of high titers in patients with carcinomas, and extreme variability among patients with lymphomatous neoplasms.

who had epidermoid carcinoma which ran a fulminating course, with wide dissemination and death within 3 and 6 months after clinical onset, had normal C'2 levels (72 and 76%) in sera collected within a month before death, and another patient who died of widely disseminated cervix cancer 3 days after a serum sample was obtained, had a C'2 level of 70%. Thus there was a frequent, but not a consistent, relationship between C'2 level and immediate prognosis. Attempts were made to define this relationship with respect to pattern of disease and types of treatment.

Two patients had blood urea nitrogen levels over 100 mg/100 ml and had C'2 titers of 97 and 116%. Four patients whose serum uric acid was elevated (between 6.5 and 8.5 mg/100 ml) had C'2 levels ranging from a below normal level of 57% to a high level of 152%. Two had obstructive

jaundice with serum bilirubin levels of 14.2 and 31.0 mg/100 ml, serum alkaline phosphatase levels of 17.7 and 8.2 Bodansky units; and C'2 levels of 71 and 50%, respectively. One patient had hypercalcemia (serum calcium 12.0 mg/100 ml) and a C'2 level of 97%. Two patients had *Proteus* septicemia, and C'2 levels of 70% and 116%. Thus with the possible exception of hyperbilirubinemia, none of these complications of cancer appeared to affect C'2 levels.

Paper strip electrophoretic studies of serum proteins were available for 7 of the cancer patients, on sera drawn within a week before or after the C'2 determination. Four of the 7 patients had C'2 titers between 130 and 139%. These had normal or near normal levels of β - and γ -globulins and total proteins, and one of them had elevated α -1 and α -2 globulins (0.67 and 1.21 g/100 ml, respectively). Three of the 7 patients had low C'2

titers (40, 50 and 71%). All 3 showed hypogammaglobulinemia (0.12, 0.53 and 0.58 g/100 ml) but there was no consistent deviation in their other globulins. The patient with the lowest C'2 titer had normal α -1, α -2 and β -globulin levels. The patient who had a 50% C'2 titer had a low β -globulin (0.28 g/ml) but normal α -globulin levels, and the patient who had 71% C'2 had a low β -globulin (0.35 g/ml) and extremely high α -1 (1.67 g/ml) and α -2 (1.39 g/ml) globulin levels.

Eleven of the cancer patients from whom sera were obtained for C'2 titration were on treatment with anticancer drugs. These included 2 on treatment with cytosine arabinoside, 2 on fluorouracil, 1 on bromomethoxyfluoro-deoxyuridine, 2 on duanomycin, 1 on Vincristine, and 3 on prednisone. Another 2 patients were receiving leukopenogenic doses of x-ray therapy. In addition to these patients on anticancer therapy, 1 patient was on coumarin as an anticoagulant, 1 on digitoxin, and another on allopurinol because of hyperuricemia. Among these 16 patients all C'2 levels fell between 72 and 124%—the normal range. (Unpublished studies in this laboratory have also shown that total C' hemolytic activity of serum was not significantly altered by prolonged and intensive treatment of 4 cancer patients with cortisone or hydrocortisone.)

Only 13 patients with non-neoplastic disease were included in this study. Their C'2 titers (Table I and Fig. 2) averaged higher than the healthy controls and had a wider range, thus resembling the cancer patients, but the differences are not statistically significant. The 1 patient who had lupus erythematosus had very low C'2 titers (26% and 31%) in the 2 serum specimens which were tested, a finding which is consistent with previously published reports (5). A patient with a histologically confirmed diagnosis of dermatomyositis—another disease in which there is thought to be an autoimmune reaction—had a normal C'2 of 98%. Another patient with a clinical picture of multiple myopathy of unknown cause had a low C'2 titer of 50%.

This non-cancer group included 3 patients with diabetes mellitus complicated by severe renal, retinal or peripheral vascular complications. One had a C'2 titer of 109%. Another who had been hypophysectomized because of the intractable diabetes, had a C'2 of 89%, and the third who had also been hypophysectomized and who in addition had gangrene of the foot requiring

amputation a few days later, had a C'2 titer of 135%. Two other patients had severe infections. One who had widespread *Proteus* infections had a C'2 titer of 116%. The others had staphylococcal pneumonia and cystitis and had a rather low titer (64%) of C'2. It may be significant that the latter patient also had a drug rash, penicillin antibodies and a positive Coombs test—reactions which might be expected to bind complement. The highest C'2 titer in the group of non-cancer patients was 154% in a 74-year-old white woman with cardiac failure due to arteriosclerotic heart disease. The other 4 patients in the non-cancer group had C'2 titers between 83% and 123%. Their diagnoses included tophaceous gout, myocardial infarct, duodenal ulcer, influenza and paraplegia (traumatic).

Thus these limited studies of non-neoplastic diseases failed to disclose any characteristic which correlates with C'2 levels, or which might explain the few deviations above and below the usual range of C'2 values in the cancer patients, other than conditions in which there are presumed to be autoimmune reactions.

The hypotheses that low C'2 levels might reflect immunologic reactions against the neoplastic disease or its infectious complications, or that high C'2 levels might reflect a non-specific stimulation of host defense mechanisms, cannot be supported by the available data but neither are they refuted. It is of interest that these C'2 data resemble the data on total complement hemolytic activity obtained in an earlier study (1) in that several individual patients had C' activity greater than or less than the healthy controls, although averages were identical in cancer patients and controls.

SUMMARY

The levels of the second component of complement (C'2) in the serum of cancer patients were determined. The average of C'2 titers in the cancer group was slightly higher than in the healthy control group but the differences were not statistically significant. Among patients with lymphosarcoma and reticulum cell sarcoma, a few had very low C'2 levels, and all patients with Hodgkin's disease and multiple myeloma had moderately high levels. The biologic significance of these differences is obscure. They are not explained by such variables as technique, sex, age or therapy. Impending death was often, but not consistently, associated with low C'2 levels. In a

small group of patients with various non-neoplastic diseases the only abnormality of note was a very low C'2 titer in a patient with lupus erythematosus.

ADDENDUM

Recent information made available to the authors by Drs. K. F. Austen and M. M. Mayer indicates that the titration of human C'2 (C'2^{hu}) by use of indicator cells (EAC'1,4) prepared with guinea pig complement (C'1^{gp} and C'4^{gp}) requires the participation of human C'4 (C'4^{hu}). This possibility was suggested by Nelson (3) and has been recently confirmed by Austen and Russell (8). Apparently, C'1a^{gp}, transferred from the reactive sites (SAC'1^{gp},4^{gp}) on the indicator cells, reacts with C'4^{hu} and forms SAC'1a^{gp},4^{hu} sites early in the T_{max} period; the stoichiometric titration of C'2^{hu} is then due primarily to its interaction with the newly formed SAC'1a^{gp},4^{hu} sites rather than with SAC'1a^{gp},4^{gp} sites. The advantage in using EAC'1a^{gp},4^{gp} cells is that they are stable for several days, whereas current methods for preparing EAC'1a^{hu},4^{hu} cells require that the intermediate be freshly prepared each day (9).

Since the level of C'4 in normal human serum is much higher than the level of C'2, it is doubtful

that C'4^{hu} would ever be a limiting factor in this reaction, so this newer information on the mechanism of the reaction does not alter the interpretation of this clinical study.

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