Behavior of Total Serum Complement in Hodgkin's Disease and Other Malignant Lymphomas

By ANTONIO ROTTINO AND ARTHUR L. LEVY

DATA on the level of total serum complement in the blood are to be found in the literature dating back to 1902. The bulk of the literature deals with the relationship of complement to infectious diseases, to immunochemical diseases, and to noninfectious inflammatory processes, only three references to its behavior in neoplastic disease having been found. The first work, that of Southam and Goldsmith, was a study of 42 patients with various malignant neoplasms and Hodgkin's disease, and values of complement were found not to differ from normal controls; also no lowering was noted of complement by radiation or nitrogen mustard used therapeutically. The two other investigators, Fischel and Schier, found complement to be elevated in a small number of cases of Hodgkin's disease.

Our study embraced patients with carcinoma, various lymphomas and Hodgkin's disease. The purpose was to determine whether the level of complement in the serum would indicate the nature of Hodgkin's disease, and it was hoped that significant immunologic data might emerge. We were encouraged to undertake this work by the better understanding of glomerulonephritis which followed upon study of the total serum complement.

PROCEDURE

Complement determinations were made on the sera of 67 normal, healthy blood donors and on sera from 65 patients with carcinoma, 14 with lymphosarcoma, 8 with chronic lymphatic leukemia, 2 with chronic myeloid leukemia and 4 with myeloma. These constituted the normal and disease control groups for 72 patients with Hodgkin's disease, from whom 217 blood specimens were taken at various times over a period of a year. Thirty-nine of the Hodgkin's disease patients were female, 33 male. As a group, the female patients were younger than the male patients, 26 being below 40 years as compared to 11 male patients below 40 years. Since the duration of disease ranged from less than one year to 13 years, we were able to study early, late, mild and severe stages of the disease.

The method used for determining the total serum complement was that described by Mayer et al., as summarized by Fischel et al. Sheep cells were treated as described by Kabat and Mayer.

In titrating the complement, human sera were used in 1:100 dilution, guinea pig sera in 1:500 dilution. Three tubes were set up for each sample. Two milliliters of sensitized cells containing 2.5 x 10⁶ cells per milliliter were first placed in each tube, then an amount of buffered saline calculated to give a final total volume of 7.5 ml. was added, and finally an amount of serum dilution varying from 1 ml. to 3 ml. was added to each tube. A

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blank of cells and saline only was set up simultaneously, as well as duplicate tubes of cells plus water with an optical density corresponding to 100 per cent hemolysis.

The milliliters of serum required to give 50 per cent hemolysis can be obtained from a graph of milliliters of serum vs. per cent hemolysis by interpolation or, more conveniently, by using the set of factors tabulated by Mayer et al.2 This amount of serum is said to contain one 50 per cent unit of complement; the reciprocal gives the number of complement units per milliliter of serum.

As recommended by Mayer et al., we used only analyses that gave between 20 and 80 per cent lysis, since this minimizes the effect of variations in the slope of the von Krogh equation which describes the S-shaped hemolytic curve. We found the slope of normal sera to be 0.2 ± 0.02, the same as that obtained by Mayer. This same value was found for the pathologic sera also.

RESULTS

Table 1 shows the distribution of values for the entire group studied. The titer of the 67 normal sera varied from 31.0 to 48.4, the average value 39.2 units per milliliter with a standard deviation of 4.1. Ninety-five per cent of the normal values fell within the range of 31.1 to 47.3 units (2 standard deviations). These results agree well with those of Fischel, Pauli and Lesh14 who reported an average of 37.7 ± 3.9 (standard deviation) for 59 normal sera. The complement was elevated in 116, or 54 per cent, of the determinations done on sera of 58 Hodgkin's disease patients; sera of 12 other Hodgkin's disease patients were never other than normal in respect to complement; and 2 Hodgkin's disease patients had below-normal values. Elevation of complement above normal proved to be not unique for Hodgkin's disease since it was also found in a high percentage of sera from patients with lymphatic leukemia, lymphosarcoma, multiple myeloma and carcinoma.

Three patients, two with Hodgkin's disease and one with chronic lymphatic leukemia, had low C' in the serum. None of the cancer patients or patients with other lymphoma had a value below 31 units. The fluctuation of values in the serum of one Hodgkin's disease patient whose complement was low is shown in table 2.

During the entire period in which C' determinations were made, and since

<table>
<thead>
<tr>
<th>Units of C'</th>
<th>11-20</th>
<th>21-30</th>
<th>31-40</th>
<th>41-47</th>
<th>48-50</th>
<th>51-60</th>
<th>61-70</th>
<th>71-80</th>
<th>81-90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>67</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>5</td>
<td>5</td>
<td>7</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Hodgkin's Disease</td>
<td>72</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Lymphatic Leukemia</td>
<td>8</td>
<td>46</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Myeloid Leukemia</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Lymphosarcoma</td>
<td>14</td>
<td>4</td>
<td>4</td>
<td>7</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Myeloma</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Cancer</td>
<td>65</td>
<td>65</td>
<td>5</td>
<td>22</td>
<td>11</td>
<td>18</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>
T able 2.—Fluctuation of Serum C' in One Patient Who Had Levels Below Normal

<table>
<thead>
<tr>
<th>Date</th>
<th>Units C'</th>
</tr>
</thead>
<tbody>
<tr>
<td>9-5-56</td>
<td>15.5</td>
</tr>
<tr>
<td>9-27-56</td>
<td>11.2</td>
</tr>
<tr>
<td>10-18-56</td>
<td>20.7</td>
</tr>
<tr>
<td>11-29-56</td>
<td>41.6</td>
</tr>
<tr>
<td>1-3-57</td>
<td>44.3</td>
</tr>
<tr>
<td>3-7-57</td>
<td>35.7</td>
</tr>
<tr>
<td>6-13-57</td>
<td>44.2</td>
</tr>
<tr>
<td>7-18-57</td>
<td>42.7</td>
</tr>
</tbody>
</table>

T able 3.—Change in Complement Level in Splenectomized Guinea Pigs

<table>
<thead>
<tr>
<th>G. P. No.</th>
<th>Before Splenectomy 6-11-56</th>
<th>11-15-56</th>
<th>12-20-56</th>
<th>After Splenectomy 2-21-57</th>
<th>6-12-57</th>
<th>Change*</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-5</td>
<td>284</td>
<td>338</td>
<td>342</td>
<td>347</td>
<td>347</td>
<td>22%</td>
</tr>
<tr>
<td>W-1</td>
<td>232</td>
<td>283</td>
<td>305</td>
<td>339</td>
<td>339</td>
<td>46%</td>
</tr>
<tr>
<td>W-4</td>
<td>223</td>
<td>312</td>
<td>302</td>
<td>314</td>
<td>314</td>
<td>41%</td>
</tr>
<tr>
<td>F. S.</td>
<td>339</td>
<td>307</td>
<td>355</td>
<td>376</td>
<td>376</td>
<td>49%</td>
</tr>
</tbody>
</table>

*Per cent change = \( \frac{\text{Maximum change}}{C' \text{ before splenectomy}} \times 100.\)

†This value was calculated using the mean C' value of 252 u./ml. found for 6 normal guinea pigs as the value obtained before splenectomy.

then, this patient's clinical status has been good, with no subjective or objective evidence of activity. Since he had had a splenectomy some time before we made the study of complement, we felt that absence of the spleen might be responsible for the abnormally low C'. We looked for and found another Hodgkin's disease patient who had had splenectomy six years previously; her serum C' was normal. We therefore studied C' in splenectomized guinea pigs by making determinations before and after operation. This revealed an increase rather than a decrease after splenectomy, and this increase has persisted for more than six months, as shown in table 3.

A second patient with Hodgkin's disease had a below-normal complement level of 18.3 units. In three months it rose to 46.3. This patient's Hodgkin's disease had been arrested for several years. At the time the test was done she had a draining rectal abscess not related to Hodgkin's disease.

A third patient with persistently low C', 21.8 units, had chronic lymphatic leukemia with a hemolytic crisis. Three months following splenectomy the serum complement was 27 units, even though the hemolysis of red cells was controlled by the operative procedure.

Correlation of Complement of Hodgkin's Disease Patients with Various Factors

No relationship between levels of complement and age, sex or duration of disease emerged, and none between total complement and erythropoietic status.

Relationship of total complement and clinical status. There were 23 patients who, from a clinical point of view, appeared to have arrested Hodgkin's disease, there having been no signs or symptoms for six months or more, nor any at the
time the complement was determined. Seventeen of these persons had a normal amount and six an elevated amount (48.1, 50, 51, 52, 57 units). The elevation caused us to suspect active disease despite subjective and objective appearances to the contrary.

**Complement of patients with clinically active disease.** Thirty-seven patients (19 females, 18 males) showed either subjective or objective evidence, or both, of active disease. By this is meant malaise, fever, pruritus, fatigue, weakness, weight loss, the presence of palpable nodes or spleen, or both. In this group there were 16 persons with normal and 21 with elevated serum complement. This would seem to indicate that activity per se, as defined above, is not the prime factor responsible for elevation of complement and that there is perhaps some other factor which is at times present and at other times absent. Whether or not this may be necrotic tissue, which is at times found in biopsied nodes and at other times not, remains to be determined.

**Relation of complement to therapy.** The complement of ten patients was determined after completion of a course of x-ray therapy, and of four patients after nitrogen mustard therapy. In none had it fallen below normal; in three it remained normal; and in others the value varied from 48 to 70 units. In some instances where multiple determinations were done before, during and after therapy, the level of complement was found to have actually increased after therapy.

**Complement and properdin.** There appears to be a reciprocal relationship between high complement and low properdin (chi-square = 5.3 P < 0.05).22

- 13 patients with high complement had less than 2 units properdin (44 per cent);
- 7 patients with high complement had 2 units properdin;
- 9 patients with high complement had normal properdin;
- 7 patients with normal complement had less than 2 units properdin;
- 37 patients with normal complement had normal properdin.

**Complement and sedimentation level of erythrocytes.** There is a definite association between these. Of 87 patients with elevated complement, 78 had a corrected sedimentation level above 16, and 9 a level of 15 or lower. Of 67 with normal complement, 38 had elevated sedimentation levels and 29 a 15 mm. fall. There is obviously no direct relationship between the complement and sedimentation level since it was above 15 in more than 50 per cent of those with normal complement. However, the incidence of sedimentation levels below 15 mm. for those with high complement is very low, 9/87, as compared with 29/67 for those with normal complement values.

**Complement and C-reactive protein.** There is association of positive CRP and elevated complement in a high number of cases. In 52 instances of elevated complement the CRP was positive 42 times, whereas in 55 instances of normal complement the CRP was positive 19 times only.

**Complement and serum proteins.** Total complement determinations and determination by the paper electrophoresis technic of total protein and protein fractions were done on one and the same sample of 23 sera taken from 16 patients. The results are shown in table 4. From this table we see that pa-
TABLE 4.—Relation of C’ to Total Serum Protein and Electrophoretic Fractions

<table>
<thead>
<tr>
<th>Proteins</th>
<th>Patients with Normal Protein Levels</th>
<th>Patients with Increased Protein Levels</th>
<th>Patients with Decreased Protein Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal C’</td>
<td>Increased C’</td>
<td>Normal C’</td>
</tr>
<tr>
<td>TSP</td>
<td>8</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Albumin</td>
<td>5</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Alpha 1</td>
<td>0</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>Alpha 2</td>
<td>4</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Beta</td>
<td>3</td>
<td>13</td>
<td>7</td>
</tr>
<tr>
<td>Gamma</td>
<td>2</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

*“Increase” means a serum level above 47 units.

†Electrophoretic studies and C’ determinations done on the same sample of serum.

Patients with normal TSP were apt to have high or low values in about equal numbers. Patients showing elevated beta were much more apt to have an increased level of C’ (chi-square = 8, P. < 0.05).

DISCUSSION

Unfortunately, there seem to be as many systems for titrating complement as there are investigators, and this means that results obtained in different laboratories cannot be compared directly with each other. Southam and Goldsmith,† for example, used the method of Kent, Bukantz and Rein,‡ a method which does not utilize Mg++, which Mayer et al.§ have shown must be added in sufficient quantity to obtain optimal hemolytic activity. Thus, it may be that the failure of Southam and Goldsmith to note elevated complement values is a reflection of the low level of magnesium in the hemolytic system used by them.

Mayer and collaborators§ have also shown in detail that factors other than magnesium, such as total volume of the system, total salt concentration, temperature and pH, greatly affect the quantity of complement found. Hence it is necessary to control and standardize each of these variables and to state so in published reports of work done. Since Schier¶ failed to specify the volume he employed, we were not able to compare our results with his.

We found that the greatest precision could be achieved by using Mayer’s system and believe it is to be recommended for measurement of complement. Fischel and collaborators‖ have well shown how the use of Mayer’s method resolved conflicting reports on complement levels in glomerulonephritis.

In 1926, Jungeblut and Berlot¶ suggested that the reticuloendothelial system might participate in the formation of complement. This conclusion was reached when they observed that complement dropped to low levels following blockade of the RE system with injections of India ink. In late, advanced cases of Hodgkin’s disease there is virtual destruction of the lymphoreticuloendothelial apparatus, yet we failed to obtain abnormally low titers in such patients. In the two instances where titers were low, the disease was not advanced, and in due course complement returned to normal.

Early work by Ehrlich and Morgenroth and Dick with poisons affect-
ing the liver suggested that this organ takes part in the formation of complement. In 1929, Goldner,27 and in 1953, Jordan28 stated that liver disease causes complement to drop. In 1955, Rice et al.29 found that complement decreased sharply when ethionine was fed to guinea pigs; autopsy disclosed marked fatty change. Gordon30 in the same year eviscerated rats and demonstrated progressive drop in complement to 10 per cent of normal. He suggested that loss of the liver was the chief cause of this drop. Mandel and Lange31 studied complement in the sera of patients with chronic liver disease and found the level to be normal. In Hodgkin's disease we have found the liver to be involved in varying degree in 47 of 104 autopsied cases. If it should be true that the liver participates in complement function, we have never seen the organ involved by this disease to the extent that this function was affected.

We found it difficult to interpret the meaning of levels of complement found in Hodgkin's disease, since in many instances an elevated level was found in patients in whom the disease process was in active phase. We believe this elevation to be indicative of activity, yet there were many other patients also in the active phase who did not have an elevated level. It is obvious that the elevation of complement must be related to some manifestation of the disease, but a manifestation that is present in some patients and not in others. The observation of Boltax and Fischel16 may throw some light on this aspect of the problem. They noted that early in myocardial infarction there was an increase of complement but that it gradually came back to normal, presumably when the infarct had become fibrous. This suggests the possibility that complement in Hodgkin's disease could be related to necrosis in diseased lymphoid tissue, since focal necrosis of the cellular elements or of the collagen of old fibrosed nodes is not uncommon in Hodgkin's disease.

In 1952, Pitner and Smith32 correlated increased complement in tuberculosis with decreased gamma globulin. In our study we found three patients with decreased gamma globulin; in two the complement was increased, and in one it was normal. We had 12 other patients with increased gamma globulin, and 6 of these had an increased and 6 a normal level of complement. We found that patients with normal gamma globulin were apt to have normal complement. All we can conclude from this is that when the gamma globulin level is abnormal, the complement level is more likely to be increased than normal.

In Hodgkin's disease an almost invariable finding is the ubiquitous eosinophile, which pervades the affected lymph nodes in some instances to a very marked degree. Some have interpreted this to mean that Hodgkin's disease has an allergic component. Assuming this to be true, we felt that if complement was found to be low, this would support the hypothesis that Hodgkin's disease is an autoimmune disease. However, our findings failed to reinforce this hypothesis; on the contrary, 58 of 72 patients had elevated complement. Though our statistics furnish no incontrovertible evidence as to the nature of Hodgkin's disease, it should be remembered that in rheu-
matic fever, currently classified as an autoimmune disease, the behavior of complement is similar to its behavior in Hodgkin's disease. And, having found a correlation between elevated complement, C-reactive protein and rapid sedimentation rate, it can be logically concluded that when the complement of the serum is above normal, Hodgkin's disease is in an active phase.

SUMMARY

Using the method of Mayer and collaborators for the determination of complement in the blood serum, 67 normal persons, 72 patients with Hodgkin's disease, 28 with other types of lymphoma and 65 with cancer involvement of various organs were studied.

Fifty-eight of 72 Hodgkin's disease patients were found to have an elevated level of complement, 12 a normal level and 2 a below-normal level. Elevated levels were found also in patients with other lymphomas and cancer.

An association between elevated complement, increased sedimentation level, decreased serum properdin, positive C-reactive protein and elevated beta globulin in the serum was noted.

The conclusion reached is that an elevation of complement in the serum indicates that Hodgkin's disease is in an active phase. As to the nature of the disease, the statistics assembled seem to give no clue.

SUMARIO IN INTERLINGUA

Le methodo disveloppate per Mayer e su collaboratores pro le determination de complemento in le sero de sanguine esseva usate in le studio de 67 subjectos normal, 72 patientes con morbo de Hodgkin, 28 patientes con altere typos de lymphoma, e 65 patientes con affectiones cancerose de varie organos.

Esseva constatate que 58 del 72 patientes con morbo de Hodgkin habeva elevate nivellos de complemento. Inter le remanente 14 patientes de iste gruppo, 12 habeva normal e 2 infra-normal nivellos de complemento. Nivellos elevate de complemento esseva etiam trovate in patientes con altere lymphomas e con cancere.

Esseva notate un association inter elevation de complemento, augmento del nivello de sedimentation, reduction de properdina seral, positivitate de proteina C-reactive, e elevation de globulina beta in le sero.

Le conclusion del studio es que un elevation de complemento in le sero de patientes con morbo de Hodgkin indica que le morbo es in un phase active. Quanto al natura del morbo, le statisticas hic colligite non pare offerer ulle clave.

REFERENCES

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