The Life-span of Complement-sensitive and -insensitive Red Cells in Paroxysmal Nocturnal Hemoglobinuria

By Wendell F. Rosse

The survival of the complement-sensitive and complement-insensitive red cells in four patients with paroxysmal nocturnal hemoglobinuria (PNH) have been determined using either a cohort or a random label. The complement-sensitive cells have a very short survival (T 1/2) of less than 6 days by either technique. The complement-insensitive cells have a longer survival, with a large component of random destruction. The complement-sensitive cells are not changed into complement-insensitive cells. The proportion of complement-sensitive cells produced per day is very much greater than the proportion of these cells in the circulating blood.

The red cells of patients with paroxysmal nocturnal hemoglobinuria consist of at least two populations with respect to their sensitivity to lysis by antibody and complement. One population is lysed by very small amounts of complement in the presence of antibody (the "complement-sensitive" population). These cells are partially lysed in the acidified serum lysis test. The other population requires nearly as much complement for lysis as normal cells (the "complement-insensitive" population). These populations appear to be discrete and are not simply ends of a spectrum of complement sensitivity.

The existence of two populations that differ in their survival in the circulation had been suspected from study of the survival curves, using both the Ashby technique and 51Cr labeling. In some studies, these curves showed two components, one very short-lived, the other more nearly normal in its survival. In other studies, no such biphasic curve was seen. Kan and Gardner showed that the reticulocyte-rich fraction of cells in PNH had a shorter survival than the reticulocyte-poor fraction, again suggesting that cells of different longevity were present in PNH.

The purpose of the present paper is to relate the life span of each population to its sensitivity to complement lysis. We have calculated the life span of each population separately by two methods: (1) labeling the heme of a cohort of cells with glycine-2-14C and (2) labeling the cells randomly with diisopropyl fluorophosphate (DF32P). In each case, the populations were analyzed.
Table 1.—Brief Clinical Summary

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age and Sex</th>
<th>Present Symptoms</th>
<th>Duration of Illness</th>
<th>Transfusion Required</th>
<th>Percentage Complement-sensitive cells</th>
<th>Complement Sensitive Population</th>
<th>Complement Insensitive Population</th>
<th>C Lysis Sensitivity Titer (CLS Ha units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P.J.</td>
<td>24 M</td>
<td>Hemolytic anemia</td>
<td>5 yr</td>
<td>None</td>
<td>17</td>
<td>2.6</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>L.P.</td>
<td>66 M</td>
<td>Hemoglobinuria</td>
<td>9 yr</td>
<td>Occasional</td>
<td>17</td>
<td>1.6 *</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>A.G.</td>
<td>42 M</td>
<td>Hemolytic anemia</td>
<td>16 yr</td>
<td>Occasional</td>
<td>60</td>
<td>2.4</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>M.G.</td>
<td>43 M</td>
<td>Hemolytic anemia</td>
<td>3 yr</td>
<td>None</td>
<td>40</td>
<td>3.6</td>
<td>42</td>
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</tr>
</tbody>
</table>

* Minor normal population.
† Complement-insensitive population.

After differential lysis of the complement-sensitive population with anti-I antibody and limiting amounts of complement.

We have found that the complement-sensitive population of cells appears to be destroyed randomly and these cells have true half-time (T½) of 4-6 days. The complement-insensitive cells survive considerably longer but are also randomly destroyed to a degree greater than normal. In general, the rate of lysis is parallel to the sensitivity of the cells to complement.

MATERIALS AND METHODS

Four patients in whom the diagnosis of paroxysmal nocturnal hemoglobinuria had been made were studied, and summaries of the salient points of their clinical history are outlined in Table 1.

Complement lysis sensitivity tests were performed as outlined in reference 1. Differential lysis of the sensitive cells on a large scale was performed using the same methods, but adjusting volumes so that sufficient numbers of cells could be obtained, as in Reference 7.

Iron kinetic studies. First, 15 µCi of 59FeCl₂ was mixed with 10 ml of sterile human serum from a donor with no known antibodies and with a known high transferrin content. After incubation for 45 minutes at room temperature, the material was injected intravenously. At frequent intervals, samples were removed and the radioactivity due to 59Fe in the plasma was assayed in a well-type scintillation counter. Daily samples were taken over the next few days and the radioactivity incorporated into the red cells of whole blood and of each population (complement-sensitive and -insensitive) was determined. 8

Glycine-2-14C studies. First, 100 µCi or glycine-2-14C was injected intravenously. At frequent (daily to weekly) intervals, 7-ml samples of blood were taken, and the red cells were washed three times with saline and divided into two portions. The population of cells sensitive to complement in one sample were lysed by an anti-I antibody and limiting amounts of fresh human serum. The residual complement-insensitive cells were washed three times with buffered saline. Heme was extracted from the cells of whole blood and from the insensitive cells by the method of Fisher. 9 The heme, dissolved in pyridine, was plated in amounts ranging from 1 to 1.5 mg on planchette and the radioactivity due to 14C was determined with a low-background beta detector. Samples of this size were found to have no loss of counts due to internal absorption. The contribution of 59Fe to the detected radioactivity was corrected by recounting the samples 2 months later. Specific activity was expressed as counts per minute per milligram of heme. The blood hematocrit did not vary more than 4% during the study.

DF32P studies. The survival of each of the populations was determined with DF32P by the method of Cline and Berlin. 10 First, 200 µCi of DF32P in glycol was injected intravenously. Then samples were removed at daily intervals and the cells were washed three times in saline. A portion of the cells were lysed by antibody and limiting amounts of complement and the lysed membranes (of complement-sensitive cells) and the unlysed cells (of the complement-insensitive population) were separated by differential centrifuga-
Table 2.—Red Cell Production and Destruction Studies in PNH

<table>
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<tr>
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<tbody>
<tr>
<td></td>
<td>Percentage of Sensitive Cells in Circulating Blood</td>
<td>Percentage of Complement-sensitive Cells Produced</td>
<td>Glycine-2-¹⁴C T 1/2 (Days)</td>
<td>DF²²P T 1/2 (Days)</td>
<td>Glycine-2-¹⁴C T 1/2 (Days)</td>
<td>DF²²P T 1/2 (Days)</td>
</tr>
<tr>
<td>P.J.</td>
<td>17</td>
<td>53</td>
<td>5.7</td>
<td>—</td>
<td>100</td>
<td>38</td>
</tr>
<tr>
<td>H.P.</td>
<td>17</td>
<td>42</td>
<td>4.5</td>
<td>—</td>
<td>—</td>
<td>48*</td>
</tr>
<tr>
<td>A.G.</td>
<td>60</td>
<td>92</td>
<td>4.0</td>
<td>—</td>
<td>—</td>
<td>36</td>
</tr>
<tr>
<td>M.C.</td>
<td>40</td>
<td>—</td>
<td>3.5</td>
<td>—</td>
<td>—</td>
<td>16</td>
</tr>
</tbody>
</table>

* By day 52 of study; see text.
Red cells from whole blood and lysed cells from each population were plated on planchettes, dried, covered with a thin film of paraffin, and counted in the end-window beta emission counter. The results are expressed as counts per milliliter of red cells from the whole blood.

**RESULTS**

The red cells of patients P.J., M.G., and A.G. consisted of two populations of cells, one sensitive and one less sensitive to C. The red cells in the blood of patient H.P. were divided into three populations: 17% of the cells were sensitive to the lytic action of complement, about 72% were more sensitive than normal but less sensitive than the first population, and about 11% of the cells were normal in their sensitivity to C lysis. This population could not be accounted for by previous transfusions, the last of which (one unit) had been given 3½ months prior to the beginning of the test. In the heme synthesis and cell survival studies, the cells most sensitive to C were separated from the other populations for analysis.

The incorporation of $^{59}$Fe into the sensitive and insensitive cells of patients H.P. and A.G. was measured (Table 2). Maximal incorporation occurred at 5 days in both populations. The proportion of the total incorporated iron which was present in the C-sensitive population was larger in each case than the percentage of cells in that population in whole blood. The sensitive population in each case rapidly reutilized the iron lost due to early lysis, as shown by the fact that the radioactivity in this population fell, did not reach zero activity, and rose again. Hence, although these studies demonstrated the rapid loss of radioactivity in the complement-sensitive population, $^{59}$Fe could not be used as a cohort label for survival studies.

Cohort labeling with glycine-2-$^{14}$C was performed on patients P.J. and H.P. The study on patient H.P. could not be carried beyond day 52 because of the requirement for transfusion due to the development of angina pectoris, even though the hematocrit had not fallen. The results on patient P.J. are shown in

![Fig. 1.—The survival of the complement-sensitive and -insensitive red cells in patient P.J., as determined with glycine-2-$^{14}$C.](attachment:image)
Fig. 1. Glycine-2-14C was rapidly incorporated into the heme of circulating red cells of both populations. At the peak of incorporation for both populations, the proportion of radioactivity in the complement-sensitive population was greater than the proportion of that population in the circulating blood (Table 2). The amount of radioactivity in the complement-insensitive population appeared to decrease more rapidly than normal but some labeled cells remained in that population for at least 70-90 days. The T½ of this component of random destruction was determined for points between the peak of labeling and the beginning of finite destruction. The rate of decrease in the specific activity of these cells is greater for patient H.P., whose complement-insensitive cells are more sensitive to complement than those of patient P.J. (Table 2).

Red cell survival studies using DF32P were performed in patients M.G. and A.G. The rate of decline of radioactivity due to DF32P in whole blood and in each population are shown in Fig. 2 (patient M.G.). Similar curves were obtained for patient A.G. In each instance, the specific labeling of the insensitive population is much greater than that of the sensitive population. This probably is a reflection of the absence of detectable acetylcholinesterase on the red cell membrane in these cells, since DFP is preferentially bound to the active center of that enzyme. By this method, the survival of the complement-insensitive cells was somewhat shorter than normal and random destruction is prominent.

DISCUSSION

These studies indicate that the cells in PNH consist of a mosaic with respect to red cell survival as well as to complement sensitivity. One population, the complement-sensitive cells, is very short-lived. These cells appear to be destroyed by random processes, and the T½ of survival is between 4 and 8 days. The survival of the complement-insensitive population was not normal as measured by either method. Although the finite life span was nearly normal in patient P.J., there was a greater than normal rate of random destruction. Ap-
proximately 40% of the cells had been destroyed prior to the onset of destruction due to cohort loss. In normal patients, the randomly destroyed component accounts for a very small proportion of the hemolysis of red cells.12 A similar finding was seen in patient H.P., although the determination of finite life span was interrupted by transfusions that became necessary on the 50th day of the study. The DF12P studies also showed that the life span of this population was diminished and it appeared likely from the curves that this is largely due to a random loss of cells since the graph was linear on semilogarithmic plot.12

We have previously shown that the “complement-insensitive” cells usually have a somewhat greater sensitivity than normal to complement1 and a somewhat diminished content of acetylcholinesterase than normal.7 The increased C lysis sensitivity appears to parallel the degree of acetylcholinesterase deficiency. In the present studies, the shortening of the life span of the cells is again roughly parallel to the degree of the abnormality of the other two parameters. However, it is the markedly increased lysis of the sensitive population that accounts in large part for the severity of the hemolytic aspect of the disease.

The studies show that proportion of complement-sensitive cells produced by the marrow is greater than the proportion of these cells in the circulation. This is expected since, because of their shortened life span, this population of cells has a higher rate of turnover than the insensitive cells. This fact explains the previous finding that the percentage of cells sensitive to complement1 (or to lysis by acidified serum13) in the reticulocyte-rich layer after centrifugation was higher than in the reticulocyte-poor layer. In patients with a proportion of sensitive cells over about 40%, nearly all the cells made each day are in the sensitive population.

These studies also indicate that the cells in the sensitive population do not transform into complement-insensitive cells. If they did so, as the number of labeled cells in the complement-sensitive population decreased, the number of labeled cells in the insensitive population would increase, and the number of labeled cells in both populations should remain the same. In fact, as the number of labeled complement-sensitive cells decreases, the total number of labeled cells in the blood decreases and the number in the insensitive population does not increase once these cells have been delivered (day 11). This indicates that the characteristic cells in the sensitive population are defective when made and are destroyed within a few days without changing into longer-lived complement-insensitive cells.

Previous studies have shown that the presence of complement-sensitive cells is a characteristic finding in PNH and that the severity of the clinical course is, in general, related to the percentage of these cells in the blood. The present studies relate directly the complement sensitivity to the increased hemolysis observed in these patients. Since the clinical manifestations of the disease do not always involve hemoglobinuria, much less hemoglobinuria that is paroxysmal and nocturnal, the syndrome might better be named “complement-sensitivity hemolytic anemia.” The reasons for the sensitivity of the red cells to the lytic effect of complement remain to be elucidated but may help unravel the manner in which complement is able to destroy red cells.
REFERENCES


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