Longer In Vivo Survival of CD59- and Decay-Accelerating Factor-Almost Normal Positive and Partly Positive Erythrocytes in Paroxysmal Nocturnal Hemoglobinuria as Compared With Negative Erythrocytes: A Demonstration by Differential Centrifugation and Flow Cytometry

By Seitoku Fujioka and Terao Yamada

Three populations of erythrocytes have been shown by flow cytometric analysis on complement regulatory proteins: CD59 and decay-accelerating factor (DAF) on erythrocytes in paroxysmal nocturnal hemoglobinuria (PNH). CD59 and DAF in PNH may be completely deficient in CD59- and DAF-negative erythrocytes, they may be decreased variably in partly positive erythrocytes, and they may be approximately normal in almost normal positive erythrocytes. Control erythrocytes are always CD59- and DAF-normal positive. CD59 and DAF-negative erythrocytes have been shown to be most sensitive to complement lysis in vitro. However, it has not yet been elucidated whether CD59- and DAF-almost normal positive and partly positive erythrocytes in a patient have a longer in vivo survival than negative erythrocytes. Blood from controls and PNH patients was separated in five fractions by differential centrifugation. CD59 and DAF on the fractionated erythrocytes were determined by flow cytometry using specific antibodies. Ratios of CD59- and DAF-almost normal positive and partly positive cells to negative erythrocytes were increased progressively from the top fraction to the bottom. The erythrocytes in the top fraction are younger and reticulocyte-rich, while those in the bottom are older and reticulocyte-poor. Hence, the present results indicate that CD59- and DAF-partly positive erythrocytes as well as almost normal positive erythrocytes in patients may have a longer in vivo survival than negative erythrocytes.

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IN VIVO SURVIVAL OF PNH-ERYTHROCYTES

Fig 1. Reticulocyte counts (0/00 of the erythrocytes) in fraction 1 (top) to fraction 5 (bottom) by differential centrifugation.

Table 1. CD59- and DAF-Positive Erythrocytes in Each Fraction by Differential Centrifugation

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Fraction No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
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<tr>
<td>CD59*</td>
<td>Patient 1</td>
<td>6.7±</td>
<td>9.8</td>
<td>14.4</td>
<td>20.4</td>
<td>25.1</td>
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<tr>
<td></td>
<td>Patient 2</td>
<td>56.1</td>
<td>63.7</td>
<td>67.0</td>
<td>72.4</td>
<td>77.7</td>
</tr>
<tr>
<td></td>
<td>Patient 3</td>
<td>11.7</td>
<td>12.1</td>
<td>15.5</td>
<td>18.8</td>
<td>29.9</td>
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<tr>
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<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>CD59</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>DAF*</td>
<td>Patient 1</td>
<td>3.8±</td>
<td>4.5</td>
<td>6.2</td>
<td>7.9</td>
<td>8.7</td>
</tr>
<tr>
<td></td>
<td>Patient 2</td>
<td>58.7</td>
<td>63.0</td>
<td>62.0</td>
<td>62.4</td>
<td>64.1</td>
</tr>
<tr>
<td></td>
<td>Patient 3</td>
<td>5.9</td>
<td>7.8</td>
<td>7.9</td>
<td>9.2</td>
<td>13.2</td>
</tr>
<tr>
<td>Control</td>
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<td>93.8</td>
<td>94.9</td>
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<tr>
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<td>97.3</td>
<td>96.0</td>
<td>96.1</td>
<td>97.5</td>
<td></td>
</tr>
</tbody>
</table>

*CD59- and DAF-partly positive erythrocytes in Patient 1 and 3. CD59- and DAF-almost normal positive erythrocytes in Patient 2. CD59- and DAF-normal positive erythrocytes in Control.

†Percentage.

Figures and tables are used to illustrate the distribution of CD59- and DAF-positive erythrocytes in each fraction obtained by differential centrifugation. The results indicate that CD59-positive and DAF-positive erythrocytes from the patients were increased progressively from fraction 1 to fraction 5. The peak of CD59-positive erythrocytes from the patients was decreased from fraction 1 to fraction 5. CD59-normal positive erythrocytes from the control were constant in each fraction. Equivalent results to CD59 were obtained for DAF on the erythrocytes from patients 1 and 2, although there were some difficulties to distinguish in patient 2. The peak of DAF normal positive erythrocytes from the control was not changed in each fraction. Peaks of CD59- and DAF-negative erythrocytes from the control were not shown in any fraction.

DISCUSSION

CD59-positive erythrocytes were increased progressively from fraction 1 (the younger erythrocyte fraction) to fraction 5 (the older erythrocyte fraction). This is more apparently disclosed by the fact that the ratios of the number of CD59-positive cells to the negative cells in patients 1, 2, and 3 increased from 0.07, 1.28, 0.13 in fraction 1 to 0.34, 3.48, 0.43 in fraction 5, respectively. The results indicate that CD59-positive erythrocytes in vivo may survive relatively longer than negative erythrocytes in PNH. A longer erythrocyte life span may be caused not only by the normal amount of CD59 on erythrocytes, as shown in patient 2, but also by the diminished amount of CD59, as shown in patients 1 and 3. It has been demonstrated that about 20% of the normal amount of CD59 is sufficient to protect cells from CoF-initiated lysis in vitro.13 The diminished amount of CD59 on erythrocytes in PNH may work effectively in vivo as well as in vitro. Similar results were obtained about DAF. Complement-insensitive erythrocytes (PNH I cells) surviving after reactive lysis in vitro have shown partial DAF deficits.14 The progressive left shift of the fluorescence intensity peaks of CD59 and DAF from fractions 1 to 5 in patient 2 in Fig 2 might result from partial loss during cell aging, probably due to less stability, suggested earlier about acetylcholinesterase.15,16 It has been reported that the in vivo life span of PNH II erythrocytes may be close to that of normal cells.17 In the present report, we demonstrate that erythrocytes with a normal amount or partial reduction of CD59 and DAF may have a relatively longer in vivo life span than negative erythrocytes in PNH. It has been well known that hemolysis is enhanced by using younger, reticulocyte-rich cell populations.18 These can be obtained from the top layer from routine centrifugation of red blood cells or differential centrifugation. The present results support this phenomenon, because the top layer, such as fraction 1, contains more CD59- and DAF-negative erythrocytes. The exact relationship between the amount of CD59 and DAF and the extent of complement hemolysis in vivo and in vitro should be elucidated.

ACKNOWLEDGMENT

We are grateful to Prof M. Tomita for supplying mouse anti-CD59 MoAb.
Fig 2. Flow cytometric profiles of CD59 and DAF on the erythrocytes in fraction 1 (top) to fraction 5 (bottom) by differential centrifugation. (A) Flow cytometric profiles of CD59. CD59-normal positive erythrocytes (Control) are equally distributed in each fraction of the control. CD59-negative erythrocytes are not shown in the control. CD59-partly positive erythrocytes (Patient 1) and CD59-almost normal positive erythrocytes (Patient 2) are increased progressively in fraction 1 to fraction 5 of PNH. CD59-negative erythrocytes are decreased reversely in fraction 1 to fraction 5 of PNH. (B) Flow cytometric profiles of DAF. The equivalent result to CD59 is shown.

REFERENCES


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