To the Editor:

In paroxysmal nocturnal hemoglobinuria (PNH), the enhanced susceptibility of the affected blood cells to the autologous complement has been explained by a deficiency in complement regulatory membrane proteins such as decay-accelerating factor (DAF) and CD59, which are anchored to the membrane through glycosylphosphatidylinositol (GPI). The lack of GPI-anchored membrane proteins has a diagnostic value in PNH. Recent studies showed a synthetic defect in the carbohydrate moiety of the anchor and the possible interruption site in the anchor synthesis, and, moreover, showed a responsible gene (PIG-A) for the impaired synthesis. Thus, the molecular mechanism of the abnormal hemolysis is now being rapidly clarified.

On the other hand, CDw52 (Campath-1 antigen) is a marker of lymphocytes and the anti-CDw52 monoclonal antibody (MoAb) has been applied to deplete lymphocytes from human bone marrow grafts to reduce graft-versus-host disease in bone marrow transplantation. The antigen was also detected in some monocytes but not in erythrocytes or platelets, whereas DAF and CD59 are expressed in all of the blood cells. Among well-known lymphocyte markers, CDw52 is distinct because of its membrane localization via GPI-anchor. To examine whether CDw52 is a useful candidate among GPI-anchored proteins for the discrimination of affected lymphocytes in PNH, we analyzed the membrane expression of CDw52 in normal and PNH lymphocytes by cytofluorometry, as described previously, with a fluorescein isothiocyanate (FITC)-conjugated anti-CDw52 MoAb (Campath-1H; Nippon-Wellcome, Osaka, Japan). Interestingly, the anti-CDw52 MoAb stained virtually all of the normal lymphocytes more intensively than the MoAbs to DAF or CD59 (Fig 1). In the lymphocytes obtained from 7 PNH patients, there appeared a CDw52-negative population (Fig 1). This population was detected more clearly than those populations negative for DAF or CD59 (Fig 1). Two-color analyses showed that CDw52-negative lymphocytes were mostly negative for DAF and CD59 (Fig 2). The CDw52-negative population was composed of lymphocytes, especially of CD2+ T cells (Fig 3). Interestingly, the population of CD2+ T cells was more than 90% in the 7 PNH cases, whereas it was 65% to 75% in healthy controls in the lymphocyte preparation (data not shown). The detection of the affected lymphocytes with anti-CDw52 MoAb is technically simple as compared with the conventional two-color analysis using both anti-GPI-anchored proteins (DAF or CD59) and lymphocyte markers. In conclusion, CDw52 is a new probe for the simple identification of the affected lymphocytes in PNH. The analysis of lymphocytes with the PNH phenotype may be of value not only in PNH studies, which often use cultured lymphocytes, but also in clinical diagnosis and evaluation of disease severity in the heavily transfused patient whose affected erythrocytes were hemolytically excluded or diluted by the transfusion with healthy erythrocytes.

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Fig 1. Cytofluorometric analysis of DAF, CD59, and CDw52 expression in lymphocytes. PNH 1 through 3 are representatives of 7 PNH patients. The healthy control is a representative of 5 healthy volunteers.
Fig 2. Two-color analysis of GPI-anchored proteins in lymphocytes.

Fig 3. Two-color analysis of PNH lymphocytes. To identify the CDw52-negative population in PNH (Fig 2), cells were labeled with MoAbs against CDw52 and CD2 (a pan-T-cell marker), CD19 (a marker for B cells), CD56 (a marker for natural killer cells), and CD13 (a marker for granulocytes and monocytes). PNH cells in Figs 2 and 3 were from the same patient, who was representative of 7 PNH cases who showed similar results.

REFERENCES


A deficiency in CDw52 (CAMPATH-1 antigen) of paroxysmal nocturnal hemoglobinuria lymphocytes [letter]

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