LETTER TO THE EDITOR

Detection of CD16^{low} Neutrophil Subpopulations

A strong expression of CD16 (FcγRIII) is usually found on resting neutrophils and natural killer cells, but it is weakly expressed or absent on eosinophils and monocytes. In patients with aplastic anemia (AA) and those with neutropenia, neutrophil subpopulations with low CD16 (CD16^{low}) expression can be found. We were interested in a method to detect this CD16^{low} subpopulation. We realized that, when a region R1 (neutrophils) is established on a forward versus side scatter dot plot, some eosinophils and monocytes are included, principally in samples obtained from children. After testing different analyses with a three-color staining procedure using the CD49d fluorescein isothiocyanate (Immmunotech, Marseille, France), CD16 R-phycoerythrin (Caltag, San Francisco, CA), and R-phycoerythrin-Cy5 (TC) (Caltag), we chose the one that more clearly defines the contaminated cells to be excluded. CD49d, a transmembrane glycoprotein, is expressed on eosinophils, monocytes, and a broad variety of cells, but not on neutrophils. CD16 antigen is a low-affinity receptor for immunoglobulin G, and it is anchored to the neutrophil membranes through glycophosphatidyl inositol (GPI). GPI anchorage is affected when a paroxysmal nocturnal hemoglobinuria (PNH) clone is present in some AA patients. This clone is reflected by the detection of CD16^{low} neutrophil subpopulations (1), in addition to the low expression of other GPI receptors such as CD55 and CD59. The loss of CD16 expression also could correlate to the presence of apoptotic cells in patients with neutropenia (2) and other diseases.

Samples were acquired on a FACScan Flow Cytometer (Becton Dickinson, Mountain View, CA) and analysis was performed with CellQuest software (Becton Dickinson). We used the following strategy. An R1 region was gated on a CD16 versus side scatter dot plot (Fig. 1) of cells from a normal donor (Fig. 1A) and a PNH patient (Fig. 1B). A second region (R2) was delimited on CD45^{+} and CD49d^{-} cells on the CD49d versus CD45 dot plot of R1 to exclude eosinophils, monocytes, debris, and enucleated cells (Fig. 1A2 and 1B2). Then a logical gate was defined with the operator *and* (R3 = R1 and R2) indicated in CellQuest Tools. The percentage of CD16^{low} neutrophil subpopulations was evaluated on the CD49d versus CD16 dot plot on R3 by drawing a quadrant (Fig. 1A3 and 1B3). Our reference value of CD16^{low} neutrophil subpopulations in R3 was equal to 2.45% (n = 19; mean ± 2 standard deviations). A higher percentage of this value was detected in two of 14 AA patients, and one of these two had PNH (Fig. 1B) according to the CD55 and CD59 results. We confirmed this when we studied the CD16 isoforms (3) by indirect staining with HNA-1a (Accurate Chemical & Scientific Co., Westbury, NY) and HNA-1b (anti-NA2; kindly donated by Dr. F. Garrido, Granada, Spain). The other patient had a null CD16 neutrophil phenotype. We also applied the R3 strategy in patients with a history of neutropenia. In four of eight patients, 10%, 7%, 43%, and 62% of CD16^{low} neutrophils were observed. In another two patients, we found a high level of apoptosis (78% and 80%) when using Annexin V.

In conclusion, the use of the R3 strategy is encouraged for the detection of a PNH clone evolution in patients with AA and with neutropenia, where the loss of CD16^{low} neutrophils is significant.

LITERATURE CITED

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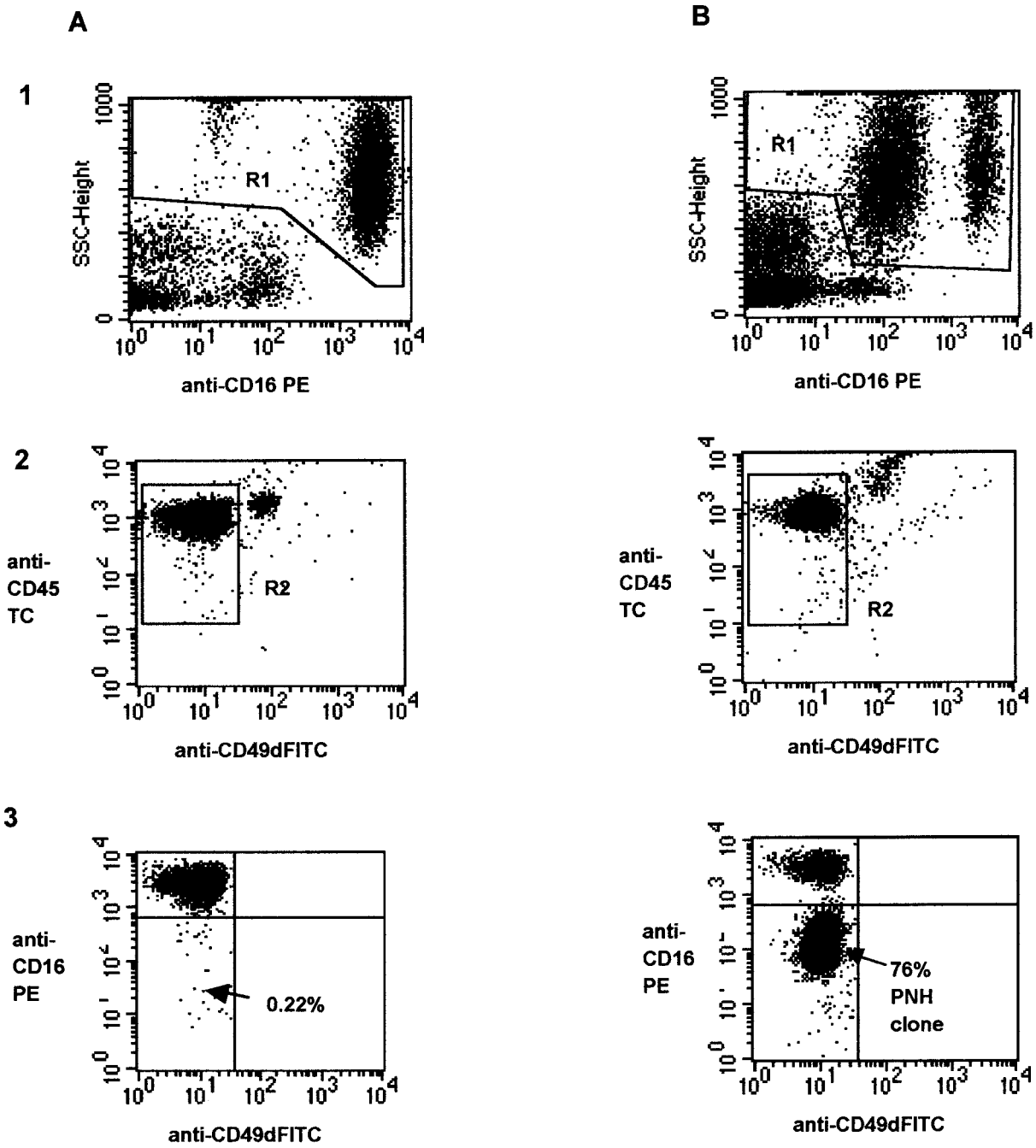

FIG. 1. Flow cytometric analysis of normal (**A**) and paroxysmal nocturnal hemoglobinuria (PNH; **B**) blood cells using the R3 strategy. **1:** The polymorphonuclear cell area (R1) was drawn on a CD16FITC versus SSC dot plot. **2:** A second region (R2; neutrophils) was delimited to include $CD49dFITC^{-}/CD45TC^{+}$ cells on a dot plot based on R1. A logical gate, R3, was defined as R1 and R2 with the use of CellQuest Tools. **3:** The percentage of the $CD16^{low}$ neutrophil subpopulation was obtained on the CD49dFITC versus CD16PE dot plot on R3 by drawing a quadrant. FITC, fluorescein isothiocyanate; PE, phycoerythrin; SSC, side scatter; TC, R-Phycoerythrin-Cy5.