CORRESPONDENCE

EXPRESSION OF CD33 ANTIGEN ON NORMAL HUMAN ACTIVATED T LYMPHOCYTES

To the Editor:

CD33 antigen (glycoprotein M_r 67,000), detected by the monoclonal antibodies (MoAbs) MY-9 and Leu-M9, is in highest concentration on immature myelocytic cells and on monocytes in marrow and peripheral blood, and it is weakly expressed by mature granulocytes in vivo. However, it has not been detected on erythroid cells, platelets, or normal lymphocytes.[1,2]

We successfully detected the expressions of CD33 antigen on normal human peripheral $CD4^+$ T and $CD8^+$ T lymphocytes that were activated with anti-CD3 MoAb (OKT-3) plus recombinant interleukin-2 (rIL-2). The normal human peripheral $CD4^+$ T and

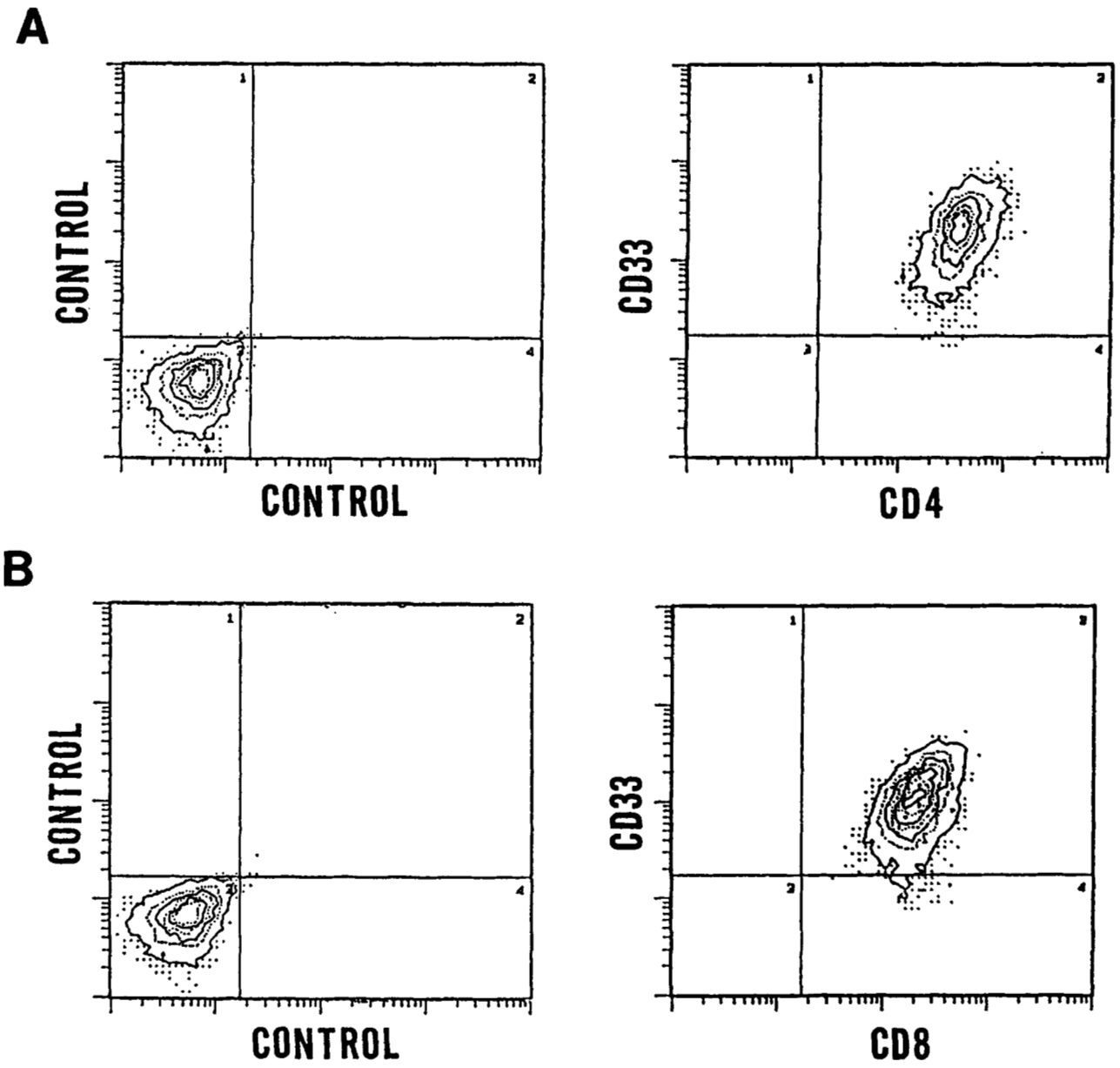

Fig 1. Typical expressions of CD33 antigen on $CD4^+$ T- and $CD8^+$ T-lymphocyte clones. $CD4^+$ T and $CD8^+$ T-lymphocyte clones from normal donor were established by the limiting dilution technique with repeated stimulations of immobilized anti-CD3 MoAb plus IL-2. The staining of CD4, CD8, and CD33 antigens were performed with fluorescein isothiocyanate (FITC)-conjugated anti-CD4 MoAb (Leu-3a), anti-CD8 MoAb (Leu-2a), and phycoerythrin (PE)-conjugated anti-CD33 MoAb (Leu-M9). Control profiles stained with FITC- or PE-conjugated isotype-matched (IgG_1) mouse control antibodies are also shown. Over 98% of each clone expressed CD33 antigen.

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$CD8^+$ T lymphocytes were separated by cell sorter, FACStar, from six normal volunteer donors (purities were more than 99.6%). The cells were suspended in RPMI-1640 medium containing 10% AB human serum and 200 IU/mL rIL-2 and cultured with immobilized anti-CD3 MoAb.[3,4] After the culture, expressions of CD33 antigen on activated $CD4^+$ T and $CD8^+$ T lymphocytes were measured by FACScan using anti-CD33 MoAb (Leu-M9, Becton Dickinson, Mountain View, CA; MY9, Coulter, Hialeah, FL).

Percentages of $CD4^+$ T lymphocytes expressing CD33 antigen were 1.3% ± 2.0% (+0 days [d]), 4.7% ± 4.2% (+3 d), 15.2% ± 7.6% (+5 d), 25.2% ± 11.2% (+10 d), 42.5% ± 11.3% (+20 d), and $CD8^+$ T lymphocytes expressing CD33 antigen were 0.8% ± 1.6% (+0 d), 1.8% ± 1.6% (+3 d), 5.1% ± 3.7% (+5 d), 13.6% ± 10.3% (+10 d), and 41.5% ± 13.8% (+20 d) (n = 6, mean ± SD). Both of activated $CD4^+$ T and $CD8^+$ T lymphocytes were positive for CD2, CD3, and T-cell receptor (TCR)-α/β antigens and released IL-2, IL-3, IL-4, interferon-γ, tumor necrosis factor-α, and granulocyte-macrophage colony-stimulating factor by the stimulation of anti-CD3 MoAb. To study the expressions of CD33 antigen on clonal level, we established 24 $CD4^+$ T-lymphocyte clones and eight $CD8^+$ T-lymphocyte clones from the normal peripheral blood by stimulation with immobilized anti-CD3 MoAb plus rIL-2. All clones were highly expressed CD33 antigen (Fig 1). These clones also expressed other T-cell markers and had an ability to produce the same cytokines as a bulk T lymphocytes by the stimulation of anti-CD3 MoAb.

T lymphocytes are triggered by antigens through T3-TCR complex or by an alternative pathway and proliferate in response to IL-2. Stimulation by anti-CD3 MoAb via T3-TCR complex is a physiologic signal transduction to the T lymphocytes. Therefore, T lymphocytes may express the CD33 antigen during inflammatory response in vivo, and it might be necessary to give attention for the analysis of leukocytes using anti-CD33 MoAb. Although the functions of CD33 antigen on T lymphocytes has not been clear, T lymphocytes might be good materials on which to study the functional analysis of CD33 antigen.

In conclusion, CD33 antigen are expressed not only on immature myelocytic cells and monocytes but also on activated T cells.

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REFERENCES

1. Andrews RG, Torok-Storb B, Bernstein ID: Myeloid-associated differentiation antigens on stem cells and their progeny identified by monoclonal antibodies. Blood 62:124, 1983

2. Bernstein ID, Singer JW, Andrews RG, Keating A, Powell JS, Bjornson BH, Cuttner J, Najfeld V, Reaman G, Raskind W, Sutton DMC, Fialkow PJ: Treatment of acute myeloid leukemia cells in vitro with a monoclonal antibody recognizing a myeloid differentiation antigen allows normal progenitor cells to be expressed. J Clin Invest 79:1153, 1987

3. Nishimura T, Nakamura Y, Takeuchi Y, Gao X, Tokuda Y, Okumura K, Habu S: Generation, propagation and targeting of human $CD4^+$ helper/killer T cells induced by anti-CD3 monoclonal antibody plus recombinant interleukin 2: An efficient strategy for adoptive tumor immunotherapy. J Immunol 148:285, 1992

4. Nakamura Y, Tokuda Y, Iwasawa M, Tsukamoto H, Kidokoro M, Kobayashi N, Kato S, Mitomi T, Habu S, Nishimura T: Large-scale culture system of human $CD4^+$ helper/killer T cells for the application to adoptive tumor immunotherapy. Br J Cancer 66: 20, 1992