

## Impaired Expression of Interleukin 2 Receptor and CD45RO Antigen on Lymphocytes from Children with Acute Lymphoblastic Leukemia in Response to Cytomegalovirus and Varicella-Zoster Virus

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**The expression of CD25 and CD45RO in lymphocytes from patients with acute lymphoblastic leukemia in response to cytomegalovirus and varicella-zoster virus antigens was analyzed by flow cytometry. It is suggested that the defects in the immune response to cytomegalovirus and varicella-zoster virus in patients with acute lymphoblastic leukemia on chemotherapy are in the expression of CD25 and in the switching of CD45RA cells to CD45RO cells.**

Patients with acute lymphoblastic leukemia (ALL) receiving chemotherapy have deficient cellular and humoral immunities (3, 4). This immunodeficiency increases the patient's susceptibility to frequently lethal bacterial, fungal, and viral infections (13). Cytomegalovirus (CMV) and varicella-zoster virus (VZV) infections in immunocompromised children, including those with leukemia (7, 8, 11), recipients of bone marrow transplantations (19, 23), and AIDS (6, 17), become serious. We have reported that the expression of the interleukin 2 receptor (IL-2R) ( $\alpha$  and  $\beta$ ) increases on T cells and that switching of CD45RA-positive cells to CD45RO-positive cells parallels activation of T cells in response to CMV and VZV antigens (14, 20). These increases in IL-2R (CD25) and CD45RO antigen expression are observed mainly on CD4<sup>+</sup> cells, and IL-2R $\beta$  is expressed on resting T cells. Thus, we consider expression of CD25 and CD45RO antigen as a reliable indicator of immunity to CMV and VZV. In the study described here, we investigated the expression of CD25 and CD45RO antigen on peripheral blood mononuclear cells (PBMCs) from patients with ALL in response to CMV and VZV antigens.

Twenty-three patients (ages, 3 to 12 years; mean age, 8.4 years) with standard-risk ALL were studied. All patients had been in complete remission for 3 months to 9 years at the time of the study. Eleven patients were on chemotherapy, and 12 patients were off chemotherapy. All patients were treated according to the chemotherapy protocol for standard-risk ALL composed of daily oral 6-mercaptopurine, weekly oral methotrexate, and consolidation therapy including vincristine and prednisolone every 3 months. All patients were confirmed to be seropositive for CMV and VZV by an immunofluorescence test (16, 27). No patients had clinical symptoms, signs, or laboratory data suggesting active CMV or VZV infection. Studies were also performed on 11 healthy children (ages, 5 to 16 years). PBMCs were separated by Ficoll-Hypaque (Sigma, St. Louis, Mo.) gradient centrifugation and were suspended in RPMI 1640 medium (GIBCO, Grand Island, N.Y.) with 20% autologous plasma at a concentration of 10<sup>6</sup>/ml. CMV (AD169

strain), VZV (Kawaguchi strain), and control antigens were prepared by the method described previously (14, 20, 28). A volume of 1.0 ml of cell suspension containing 10<sup>6</sup> cells was cultured with CMV, VZV, or control antigen (optimal dilution, 1:100) in round plastic tubes (12 by 75 mm; Becton-Dickinson, Mountain View, Calif.) at 37°C in 5% CO<sub>2</sub> for 6 days. The supernatant was removed, and the cells were washed with phosphate-buffered saline (PBS) three times and were then resuspended in PBS with 0.01% sodium azide (Sigma). A total of 2 × 10<sup>5</sup> to 5 × 10<sup>5</sup> cells were incubated with 10  $\mu$ l of fluorescein isothiocyanate (FITC)-conjugated anti-Leu 3 (CD4) and phycoerythrin (PE)-conjugated anti-CD25 (IL-2R $\alpha$ ) or FITC-conjugated anti-Leu4 (CD3) and PE-conjugated anti-Leu45RO (CD45RO) (Becton-Dickinson) monoclonal antibodies for 20 min at 4°C and fixed with a fluorescence-activated cell sorter (FACS) lysing solution (Becton-Dickinson) and washed twice with FACS flow solution (Becton-Dickinson). These immunofluorescence-stained cells were analyzed on a FACScan (Becton-Dickinson) by using Consort 30 software (Becton-Dickinson). Lymphocytes were identified by gating forward- and side-scatter parameters and were verified by LeucoGATE Simultest (Becton-Dickinson). Cells with intensities exceeding the upper threshold for the negative control were considered positive.

The percentages of CD25-positive CD4<sup>+</sup> cells and CD45RO-positive cells among the lymphocytes cultured with CMV and VZV antigens increased significantly compared with those of lymphocytes in healthy children ( $P < 0.05$ , paired  $t$  test). There was no significant change in the percentage of CD25-positive CD4<sup>+</sup> cells and CD45RO-positive cells in response to CMV and VZV antigens in patients who were in the group on chemotherapy (Fig. 1 and 2). A significant increase in the percentage of CD25-positive CD4<sup>+</sup> cells and CD45RO-positive cells in response to CMV antigen was observed in patients who were off chemotherapy ( $P < 0.05$ , paired  $t$  test). The percentage of CD45RO-positive cells in response to VZV antigen in patients who were off chemotherapy did not change. The mean percentage of CD25-positive CD4<sup>+</sup> cells cultured with VZV antigen was higher than that of CD25-positive CD4<sup>+</sup> cells cultured with control antigen in patients off chemotherapy. However, the percentages increased significantly of CD25-positive CD4<sup>+</sup> cells and CD45RO-positive cells cul-

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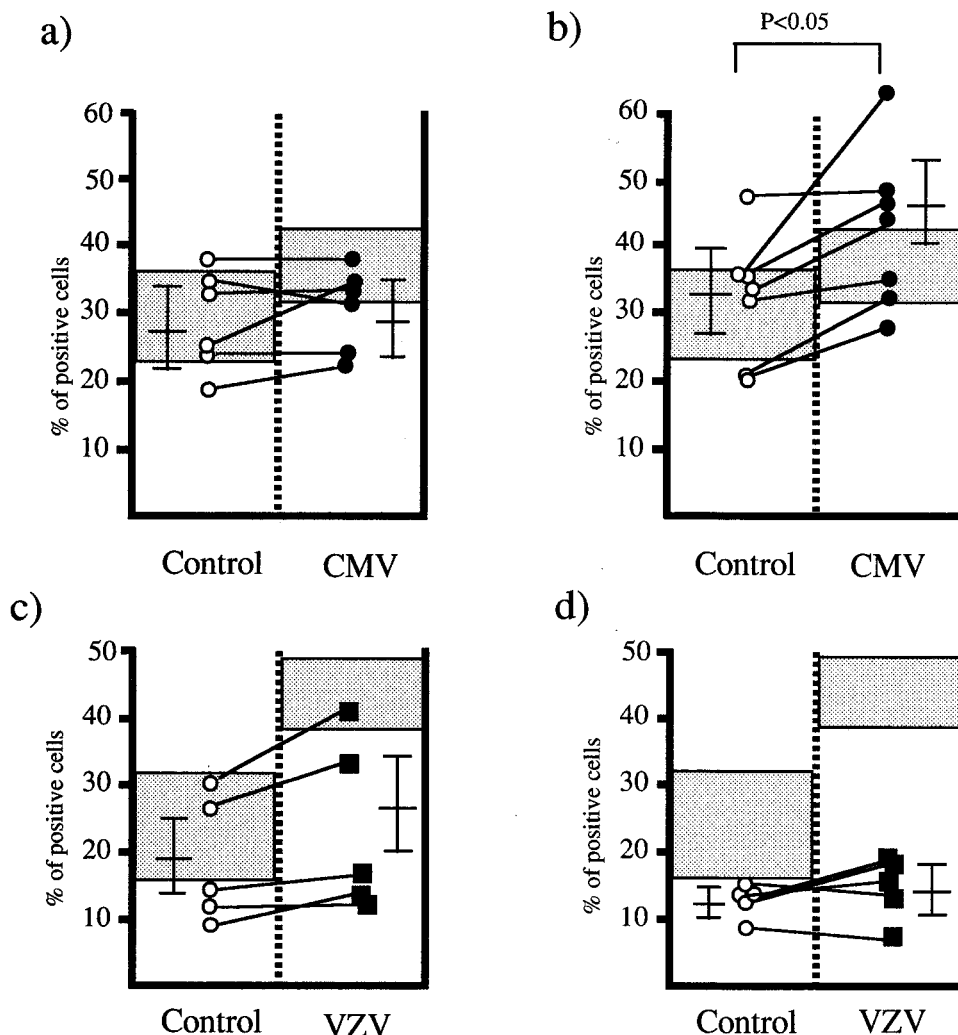


FIG. 1. Percentage of CD25-positive CD4<sup>+</sup> cells cultured with CMV, VZV, or control antigen. PBMCs were cultured with CMV (a and b; ●), VZV (c and d; ■), or control (○) antigen for 6 days. The percentage of CD25-positive CD4<sup>+</sup> cells was analyzed by flow cytometry. Patients were on chemotherapy (a and c) or off chemotherapy (b and d). Data represent the percentage of CD25-positive CD4<sup>+</sup> cells (mean  $\pm$  standard error). ■, values in healthy children.  $P < 0.05$  compared with control antigen (paired *t* test).

tured with VZV antigen in patients off chemotherapy were less than the levels in healthy children.

It is considered that the development of severe CMV and VZV infections in immunocompromised patients is related to a defect in cell-mediated immunity (5, 9, 18, 22). We consider that the expression of IL-2R on CD4<sup>+</sup> cells, as analyzed by flow cytometry, is a reliable indicator of helper T-cell function against CMV and VZV. We found that patients with ALL receiving chemotherapy have impaired expression of IL-2R and impairment of the switching of CD45RA-positive cells to CD45RO-positive cells in response to CMV and VZV antigen. Activation of resting T cells requires synthesis and secretion of IL-2 and expression of IL-2R (28). It is unclear whether or not the decreased level of IL-2 production in response to CMV and VZV antigens was due to the impaired activation of T cells, because no IL-2 activity was detected in the supernatants of PBMCs from healthy adults cultured with CMV and VZV antigen (14). T cells from peripheral blood express either CD45RA antigen (naive cells) or CD45RO antigen (memory cells) (1, 24, 26). Activation of CD45RO-positive memory T cells is also important in recovery from a viral infection. In-

creases in the number of CD45RO-positive cells in response to CMV and VZV antigens were not observed in patients receiving chemotherapy. Our results suggest that the defect of switching of CD45RA-positive cells to CD45RO-positive cells in response to CMV and VZV antigens is one of the abnormalities of the immune response to CMV and VZV in patients with ALL. In patients who are off chemotherapy, the percentages of CD25-positive CD4<sup>+</sup> cells and CD45RO-positive cells among lymphocytes cultured with CMV antigen increased significantly. However, the response of lymphocytes to VZV antigen in almost all patients off chemotherapy is still poor. It is difficult to explain this phenomenon, but the immunosuppressive effects on lymphocytes responding to VZV antigen may persist for a longer period.

The mechanism of these immunological abnormalities in response to VZV and CMV antigens is unclear. Immunological abnormalities in response to CMV and VZV antigens are most likely caused by systemic chemotherapy. The chemotherapeutic agents not only decrease the fraction of responding T cells but also suppress the function of T cells. We did not find a correlation between IL-2R expression and the absolute num-

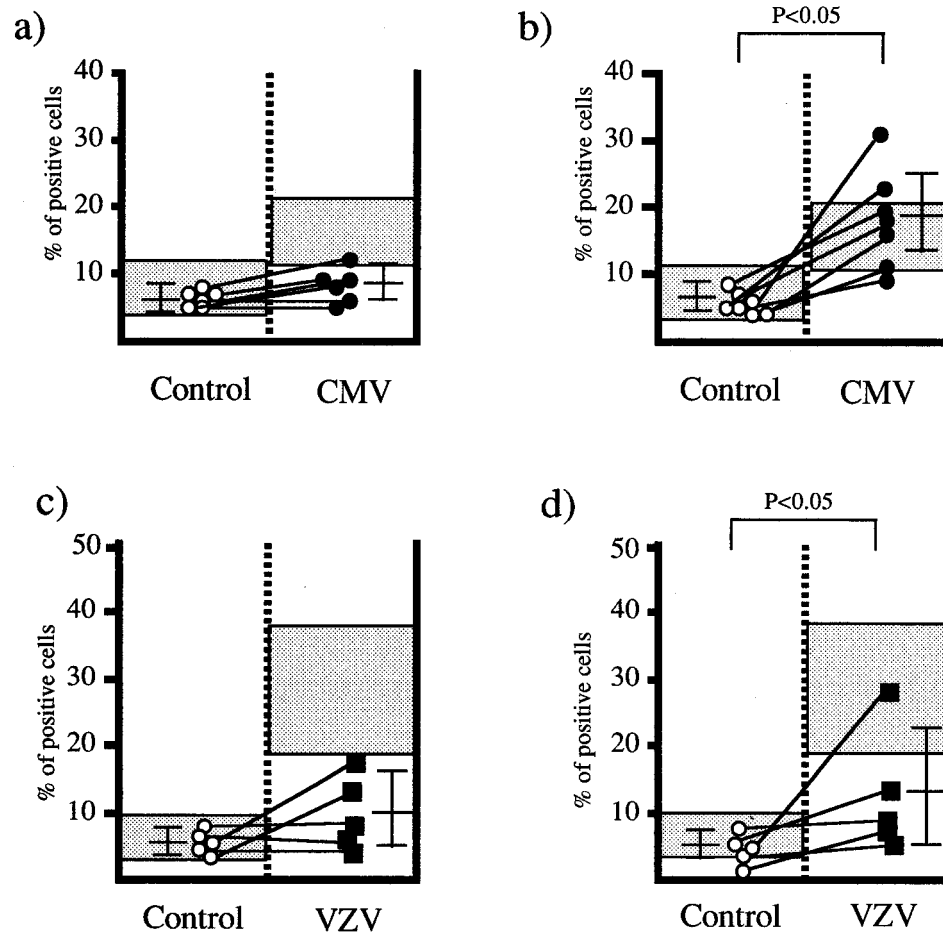


FIG. 2. Percentage of CD45RO-positive cells cultured with CMV, VZV, or control antigen. PBMCs were cultured with CMV (a and b; ●), VZV (c and d; ■), or control (○) antigen for 6 days. The percentage of CD45RO-positive cells was analyzed by flow cytometry. Patients were on chemotherapy (a and c) or off chemotherapy (b and d). Data represent the percentage of CD45RO-positive cells (mean  $\pm$  standard error). □, values in healthy children.  $P < 0.05$  compared with control antigen (paired  $t$  test).

ber of CD4<sup>+</sup> and CD45RO-positive cells (data not shown). It has been reported that anticancer agents inhibit IL-2 production (10, 15), IL-2R gene expression (2, 21), and IL-2R expression on the cell surface (12).

It is suggested that the defects in the immune response to CMV and VZV antigens in patients with ALL on chemotherapy are in the expression of CD25 and in the switching of CD45RA cells to CD45RO cells. These abnormalities may increase the risk of reactivation of CMV and VZV. We could not confirm whether or not that defect is due to a blockage or a lack of signal transduction. Further study is needed to clarify the precise mechanism of these immunological abnormalities.

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