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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) (α and β) 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 $CD4^+$ cells, and IL-2Rβ 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 10^6/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 10^6 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_2 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 2×10^5 to 5×10^5 cells were incubated with 10 μl of fluorescein isothiocyanate (FITC)-conjugated anti-Leu 3 (CD4) and phycoerythrin (PE)-conjugated anti-CD25 (IL-2Rα) 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 $CD4^+$ 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 $CD4^+$ 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 $CD4^+$ 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 $CD4^+$ cells cultured with VZV antigen was higher than that of CD25-positive $CD4^+$ cells cultured with control antigen in patients off chemotherapy. However, the percentages increased significantly of CD25-positive $CD4^+$ cells and CD45RO-positive cells cul-

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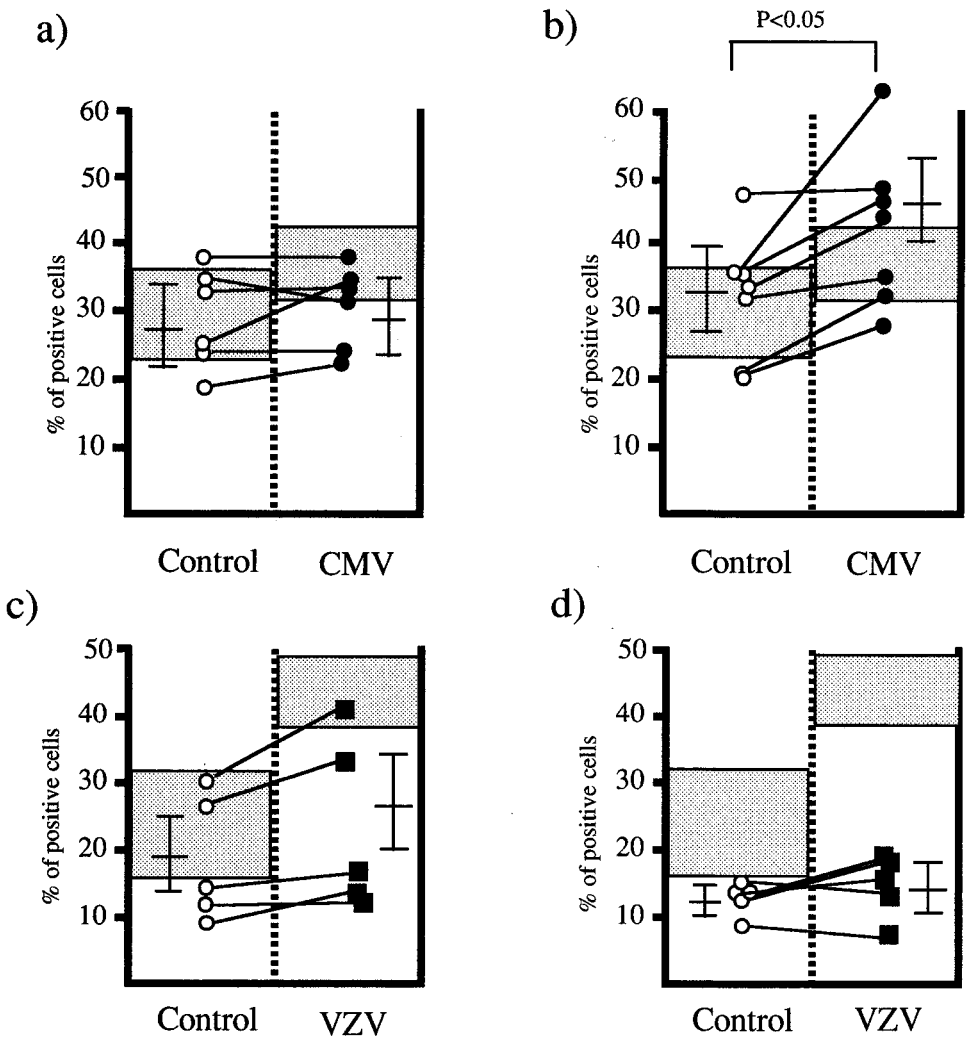

FIG. 1. Percentage of CD25-positive $CD4^+$ cells cultured with CMV, VZV, or control antigen. PBMCs were cultured with CMV (a and b; ●), VZV (c and d; ■), or control (○) antigen for 6 days. The percentage of CD25-positive $CD4^+$ cells was analyzed by flow cytometry. Patients were on chemotherapy (a and c) or off chemotherapy (b and d). Data represent the percentage of CD25-positive $CD4^+$ cells (mean ± 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 $CD4^+$ 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. Increases 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 $CD4^+$ 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-

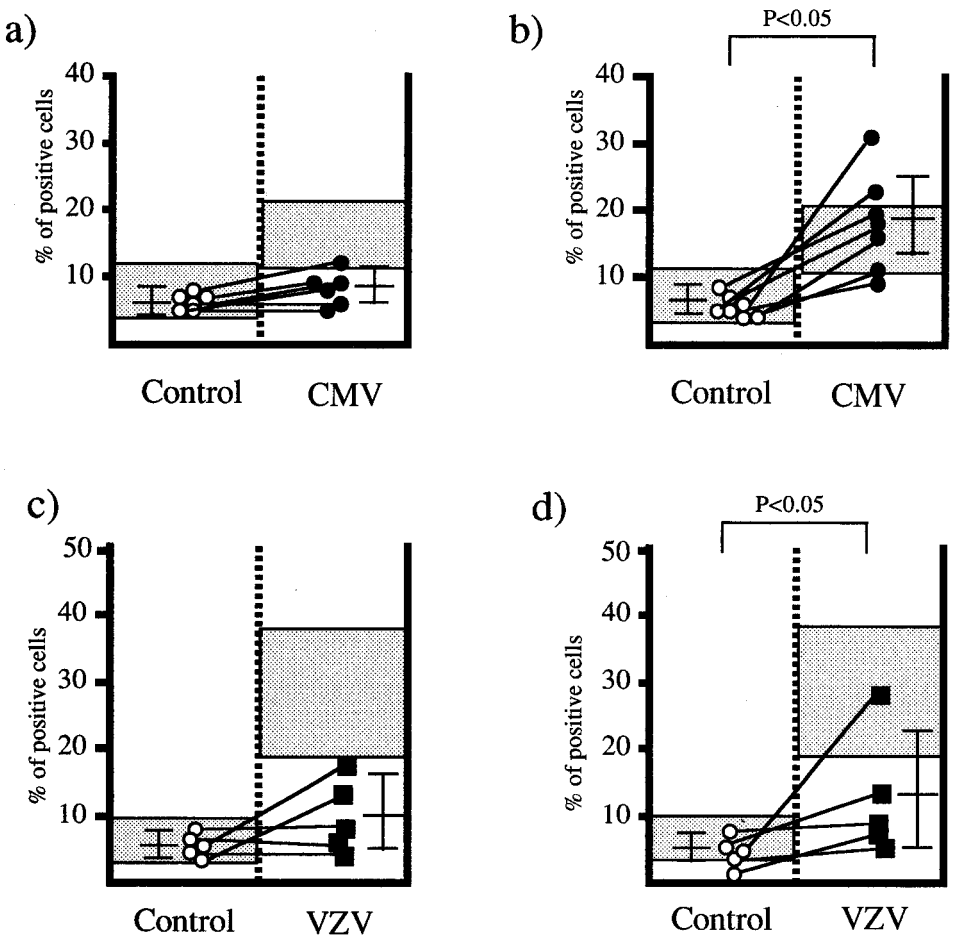

FIG. 2. Percentage of CD45RO-positive cells cultured with CMV, VZV, or control antigen. PBMCs were cultured with CMV (a and b; ●), VZV (c and d; ■), or control (○) antigen for 6 days. The percentage of CD45RO-positive cells was analyzed by flow cytometry. Patients were on chemotherapy (a and c) or off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

ber of $CD4^+$ 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.

REFERENCES

1. **Akbar, A. N., A. Timmis, and G. Janossy.** 1989. Cellular events during memory T-cell activation *in vitro*: the UCHL1 (180,000 MW) determinant is newly synthesized after mitosis. Immunology **66:**213–218.
2. **Araya, S. K., F. Wong-Staal, and R. C. Gallo.** 1984. Dexamethasone-mediated inhibition of human T cell growth factor and γ-interferon messenger RNA. J. Immunol. **133:**273–276.
3. **Bakkeren, J. A. J. M., G. A. M. de Van, and E. D. A. M. Schretlen.** 1972. Phytohemagglutinin stimulation of peripheral lymphocytes in children with acute lymphoblastic leukemia during remission. Scand. J. Haematol. **9:**36–42.
4. **Borella, L., and R. G. Webster.** 1971. The immunosuppressive effects of long-term combination chemotherapy in children with acute leukemia in remission. Cancer Res. **31:**420–426.
5. **Bowden, R. A., L. M. Day, D. E. Amos, and J. D. Meyers.** 1987. Natural cytotoxic activity against cytomegalovirus-infected target cells following marrow transplantation. Transplantation **44:**504–508.
6. **Buchbinder, S. P., M. H. Kats, N. A. Hessel, J. Y. Liu, P. M. O'Mally, R. Underwood, and S. D. Holmberg.** 1992. Herpes zoster and human immunodeficiency virus infection. J. Infect. Dis. **166:**1153–1156.
7. **Cox, F., and W. T. Hughes.** 1975. Cytomegaloviremia in children with acute lymphocytic leukemia. J. Pediatr. **87:**190–194.
8. **Feldman, S., W. T. Hughes, and C. B. Daniel.** 1975. Varicella in children with cancer: seventy-seven cases. Pediatrics **56:**388–397.
9. **Gillar, R. H., R. A. Bowden, M. J. Levin, L. J. Walker, D. G. Tubergen, and A. R. Hayward.** 1986. Reduced cellular immunity to varicella zoster virus during treatment for acute lymphoblastic leukemia of childhood: in vitro studies of possible mechanisms. J. Clin. Immunol. **6:**472–480.
10. **Grills, S., G. R. Crabtree, and K. A. Smith.** 1979. Glucocorticoid-induced inhibition on T cell growth factor production: the effect on mitogen-induced lymphocyte proliferation. J. Immunol. **123:**1624–1628.
11. **Henson, D., S. E. Siegel, and D. A. Fucillo.** 1972. Cytomegalovirus infections during acute childhood leukemia. J. Infect. Dis. **126:**469–482.
12. **Horst, H.-J., and H.-J. Flad.** 1987. Corticosteroid-interleukin 2 interactions: inhibition of binding of interleukin 2 to interleukin 2 receptors. Clin. Exp. Immunol. **68:**156–161.
13. **Hughes, W. T., S. Feldman, and F. Cox.** 1974. Infectious diseases in children with cancer. Pediatr. Clin. N. Am. **21:**583–615.
14. **Ito, M., T. Nakano, T. Kamiya, K. Kitamura, Y. Komada, T. Ihara, H. Kamiya, and M. Sakurai.** 1992. Activation of lymphocytes by varicella-zoster virus (VZV): expression of interleukin-2 receptors on lymphocytes cultured with VZV antigen. J. Infect. Dis. **165:**158–161.
15. **Kaplan, M. P., K. Lysz, S. A. Rosenberg, and J. C. Rosenberg.** 1983. Suppression of interleukin-2 production by methylprednisolone. Transplant. Proc. **15:**407–410.
16. **Kettering, J. D., N. J. Schmidt, D. Galls, and E. H. Lennette.** 1977. Anti-

complement immunofluorescent test for antibodies to human cytomegalovirus. J. Clin. Microbiol. **6:**627–632.
17. **Klatt, E. C., and D. Shibata.** 1988. Cytomegalovirus infection in the acquired immunodeficiency syndrome: clinical and autopsy findings. Arch. Pathol. Lab. Med. **112:**540–543.
18. **Meyers, J. D., N. Flournoy, and E. D. Thomas.** 1980. Cytomegalovirus infection and specific cell-mediated immunity after bone marrow transplant. J. Infect. Dis. **142:**816–824.
19. **Meyers, J. D., N. Flournoy, and E. D. Thomas.** 1986. Risk factors for cytomegalovirus infection after human marrow transplantation. J. Infect. Dis. **153:**478–488.
20. **Nakano, T., M. Ito, T. Mizuno, K. Mizutani, T. Ihara, H. Kamiya, and M. Sakurai.** 1993. Increase of interleukin 2 receptor and CD45RO antigen on lymphocytes cultured with human cytomegalovirus. Cell. Immunol. **147:**73–80.
21. **Reed, J. C., A. H. Abidi, J. D. Alpers, R. G. Hoover, R. J. Robb, and P. C. Nowell.** 1986. Effect of cyclosporin A and dexamethasone on interleukin 2 receptor gene expression. J. Immunol. **137:**150–154.
22. **Reusser, P., S. R. Riddell, J. D. Meyers, and P. D. Greenberg.** 1991. Cytotoxic T-lymphocyte response to cytomegalovirus after human allogeneic bone marrow transplantation: pattern of recovery and correlation with cytomegalovirus infection and disease. Blood **78:**1373–1380.
23. **Schuchter, L. M., J. R. Wingard, S. Piantadosi, W. H. Burns, G. W. Santos, and R. Sanal.** 1989. Herpes zoster infection after autologous bone marrow transplantation. Blood **74:**1424–1427.
24. **Terry, L. A., M. H. Brown, and P. C. L. Beverly.** 1988. The monoclonal antibody, UCHL1, recognizes a 180,000 MW component of the human leucocyte-common antigen, CD45. Immunology **64:**331–336.
25. **Waldmann, T. A.** 1986. The structure, function, and expression of interleukin 2 receptors on normal and malignant lymphocytes. Science **232:**727–732.
26. **Wallace, D. L., and C. L. Beverley.** 1990. Phenotypic changes associated with activation of $CD45RA^+$ and $CD45RO^+$ T cells. Immunology **69:**460–467.
27. **Williams, V., A. Gershon, and P. Brunnell.** 1974. Serologic response to varicella-zoster virus membrane antigens measured by indirect immunofluorescence. J. Infect. Dis. **134:**669–672.
28. **Zaia, J. A., P. L. Leary, and M. J. Levin.** 1978. Specificity of the blastogenic response of human mononuclear cells to herpesvirus antigens. Infect. Immun. **220:**646–651.