Studies on Transfer of Varicella-Zoster-Virus Specific T-Cell Immunity From Bone Marrow Donor to Recipient

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The transfer of antigen-specific cellular immunity in human bone marrow transplantation (BMT) was studied in 49 donor-recipient pairs, using a varicella-zoster-virus (VZV) specific lymphoproliferative response (LPR) assay. Post-transplant VZV-LPR could be serially measured in 31 specific lymphoproliferative response (LPR) assay. Post-transplant VZV-LPR could be serially measured in 31 long-term surviving recipients. VZV-specific T-cell immunity was detected in the early posttransplant period in 4 of 16 recipients who were, and whose donors were, immune to VZV before BMT, but two of those positive responses diminished in the first 100 days posttransplant. No positive response was detected in the immediate posttransplant period when either only the recipient or the donor was immune to VZV pretransplant. Herpes zoster or chickenpox developed in the recipients depending on a history of pretransplant VZV infection when the VZV-LPR became negative, and recovery from VZV infection was always followed by quick conversion of VZV-LPR. Long-lasting positive VZV-LPR was observed in the two recipients who experienced VZV infection in the immediate posttransplant period and received marrow graft from an immune donor. Our results indicate that a simple or direct transfer of VZV-specific cellular immunity from a marrow donor to a recipient cannot be expected in usual clinical bone marrow transplantation and that there might be a collaboration or recruitment of immune responses involving both donor and recipient that permits the VZV-LPR to remain positive posttransplant.

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The interval between the VZV infection and BMT varied greatly. Most patients had a history several months or years before BMT, whereas two patients had chickenpox or herpes zoster in the immediate pretransplant period, 2 months and 2 weeks, respectively.

**VZV-LPR assay.** Peripheral blood mononuclear cells were separated from heparinized whole blood by Ficoll-Hypaque density gradient centrifugation. The cells were suspended at a concentration of 6 x 10^5/mL in RPMI 1640 supplemented with antibiotics and 20% pooled human AB serum.

All aliquots (0.1 mL) of the cell suspension were incubated in round bottomed 96-well microtiter plates. VZV antigen was added to each well at a final concentration of 1/10 to 1/80, which was determined to give the optimal response by testing each lot against normal control cells. Plates were incubated at 37°C in 5% CO2 for 6 days and then labeled with 1 μCi of 3H-thymidine. Cells were harvested 24 hours later and counted in a scintillation counter. All assays were run in triplicate.

The response was expressed as a stimulation index (SI) calculated as the mean experimental counts per minute (cpm) divided by the mean cpm of cells cultured with medium alone.

**Preparation of VZV antigen.** Varicella virus of Kawaguchi strain was inoculated onto monolayers of human embryonic fibroblasts. After extensive cytopathic change appeared, the infected cells were collected by trypsinization and washed three times with phosphate buffered saline (PBS). Cells were then resuspended in an appropriate volume of PBS, disrupted by sonication and centrifuged at 3,000 x g for 20 minutes. The supernatant was used as VZV antigen.

**RESULTS**

**Clinical infection with VZV.** Forty of 49 patients survived 100 days after BMT. Twenty of 27 long-term survivors who had a history of chickenpox before BMT developed herpes zoster 40 to 233 days post-BMT. Out of 13 long-term survivors without a pretransplant history, 4 developed chickenpox 105 to 350 days post-BMT and 2 received a live attenuated varicella vaccine, 158 and 565 days post-BMT. All patients with herpes zoster were successfully treated with acyclovir. All patients who developed chickenpox did not receive acyclovir and their condition remained mild. No adverse reactions were observed in two vaccinated patients.

**VZV-LPR in normal controls.** The stimulation indices of 72 healthy seropositive controls with a history of chickenpox were higher than 3.0 with two exceptions, whereas those of 21 seronegative individuals without previous VZV infection were lower than 3.0. Therefore, 3.0 was considered to be the cut-off point between positive and negative in this assay (Fig 1).

The magnitude of VZV-LPR did not correlate with anti-VZV titers or to the intervals from VZV infection in normal controls (data not shown).

**VZV-LPR in patients and marrow donors.** Forty-two recipients and 49 donors were tested before transplant conditioning. All seropositive donors and patients with aplastic anemia or genetic diseases who had a history of chickenpox showed positive VZV-LPR, while 5 of 23 leukemic patients with a history and detectable VZV antibodies had stimulation indices lower than 3.0, probably due to chemotherapy-induced immunosuppression.

Thirty-one patients who survived 3 months or longer and were repeatedly tested for VZV-LPR were divided into four groups according to pretransplant immunity to VZV of the recipient and donor: 16 pairs both recipient (R) and donor (D) positive, 7 R(−) D(+) pairs, 2 R(+) D(−) pairs, and 6 R(−) D(−) pairs.

**Responses in R(+) D(+) pairs.** When both the recipient and donor were immune to VZV before BMT, low but detectable responses were measured in the first 100 days in four recipients. However, the positive responses in 2 of 4 diminished rapidly. Herpes zoster developed in 14 of these recipients whose VZV-LPR became less than 3.0. Recovery from zoster was always followed by quick conversion of VZV-LPR, although 4 of 14 patients lacked significant humoral immune responses (Fig 2A).

The responses of two recipients who had a VZV infection in the immediate pretransplant period have been positive for more than 9 months after BMT without apparent development of VZV infection.

**Responses in R(−) D(+) pairs.** When the recipient did not have a history of chickenpox, responses were consistently negative after BMT even if the donor was positive. These recipients acquired VZV-specific immunity by natural varicella infection or varicella immunization (Fig 2B).

**Responses in R(+) D(−) pairs.** When only the recipient was positive before BMT, responses became negative from the first posttransplant test. Herpes zoster developed and conversion of cellular responses was also recognized after recovery of zoster (Fig 2C).

**Responses in R(−) D(−) pairs.** When neither was immune to VZV before BMT, responses were negative throughout. Two patients acquired VZV immunity by natural varicella infection and one by varicella immunization, respectively (Fig 2D).
Fig 2. VZV-specific lymphoproliferative responses in BMT recipients. Patients were divided into four groups according to the pretransplant VZV history of the recipient and donor. (A) Recipient (+) donor (+), N = 16; (B) recipient (-) donor (+), N = 7; (C) recipient (+) donor (-), N = 2; and (D) recipient (-) donor (-), N = 6. Dotted lines, period before posttransplant VZV infection; solid lines, period after VZV infection; closed circles, two recipients in whom VZV-LPR remained positive without VZV infection; shaded area, negative responses (SI < 3.0); Z, herpes zoster; C, chickenpox; V, varicella vaccination.

DISCUSSION

The sensitivity and specificity of our VZV-LPR system enabled the distinction between normal individuals with a history of chickenpox and those without. Responses in immunocompromised patients reflected the VZV specific immunity so that the susceptible period to develop zoster could be predicted.

A direct or simple transfer of VZV specific cellular immunity from an immune marrow donor to a nonimmune recipient can be ruled out because no positive response could be detected after BMT in seven nonimmune recipients who were transplanted from immune donors.

Also, the pretransplant positive responses of immune recipients who were given marrow grafts from nonimmune donors could not be detected posttransplant. Positive post-transplant responses before development of VZV infection were observed in only four recipients who were and whose donors were both immune to VZV before BMT. Those positive responses persisted without VZV infection in two recipients who experienced chickenpox or zoster in the immediate pretransplant period, while the other two positive responses diminished 2 to 4 months after BMT.

The results indicate that there might be a collaboration or recruitment of immune responses involving both donor and recipient that permits the VZV-LPR to remain positive posttransplant. It appears that radioresistant immune cells of the recipient are presenting the first signal to T cells transplanted from the donor to recall the VZV-specific immune memory.

Vafai et al. reported that VZV-specific proteins were detected in circulating mononuclear cells of patients with acute VZV infection or with post-herpetic neuralgia (PHN), but not in those of zoster patients without PHN. Therefore, it is possible to assume that antigen-presenting mononuclear
cells of the immune recipients who experienced VZV infection shortly before BMT can cooperate with T cells transplanted from the immune donor to boost VZV-specific cellular immunity. This kind of donor–recipient cooperation may not occur in most BMT recipients because of the lack of circulating antigen-presenting mononuclear cells that can survive supralethal radiochemotherapy, and herpes zoster or chickenpox will develop as a consequence.

An alternative explanation for the persistence of VZV-specific T-cell immunity in two recipients would be subclinical VZV infection posttransplant. Ljungman et al. reported that a few patients who were seropositive before BMT regained lymphocyte reactivity without clinical disease due to VZV, while the majority of the responses returned to positive only after clinical manifestations of VZV. Because they did not describe the immune status of the donor or intervals between VZV infection and BMT in those whose lymphocyte reactivity became positive without posttransplant VZV infection, their results cannot be directly compared with our two patients whose VZV-LPR remained positive without clinical manifestation of VZV infection. Subclinical infection is less likely in our patients because their responses have been consistently positive throughout the posttransplant period from the first testing.

In contrast to T-cell immunity, B-cell immunity seems to be transferred more often from the donor to the recipient. Several authors observed a rise of specific antibodies to certain antigens and appearance of circulating antibody-secreting B cells in seronegative recipients transplanted from seropositive donors.1,4,9,10 This type of direct transfer does not take place in cellular immunity, as far as VZV antigen is concerned.

Wimperis et al.10 demonstrated that immunization of both donor and recipient produced long-lasting high-titer antibody responses to tetanus toxoid and hepatitis B vaccine. Timing of infection or immunization seems to be important to obtain long-lasting specific immunity after BMT. Appropriate pretransplant immunization of recipient and/or donor may be of clinical benefit and such a study is now under progress in our BMT unit using an attenuated varicella vaccine.

In summary, we demonstrated that VZV-specific cellular immunity could be partially detected in the early posttransplant period if both the donor and recipient were immune to VZV before BMT, and that most of those positive responses diminished in the first 100 days, which resulted in development of herpes zoster. Long-lasting positive VZV-LPR was observed in the two recipients who experienced VZV infection in the immediate pretransplant period and received marrow grafts from the immune donor. Antigen-presenting recipient mononuclear cells might be necessary to cooperate with transplanted donor memory T cells to maintain positive responses without clinical VZV infection. The exact role of antigen presentation and timing to augment the response of donor cells to a specific pathogen in the BMT recipient should be clarified in future studies.

ACKNOWLEDGMENT

We thank Nadia El Borai for preparing the manuscript.

REFERENCES