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Relationship between pre-existing anti-varicella zoster virus (VZV) antibody and clinical VZV reactivation in hematopoietic stem cell transplantation recipients

Running title: pre-existing anti-VZV and VZV reactivation after HSCT

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Abstract

Reactivation of latent varicella zoster virus (VZV), presenting as localized zoster or as disseminated infection, is a common and potentially serious complication in hematopoietic stem cell transplantation (HSCT) recipients. We retrospectively studied anti-VZV IgG titers by the immune adherence hemagglutination method after HSCT and VZV DNA by real-time PCR during clinical VZV reactivation using cryopreserved serum samples. No significant difference was found between anti-VZV titers in 13 patients with VZV infection (localized zoster in 11 patients and disseminated zoster in 2 patients) and those in 13 subjects without VZV infection at each time point after HSCT. Pre-existing anti-VZV titers of disseminated zoster cases tended to be lower than those of localized zoster cases (P=0.10). Serum VZV-DNA copy numbers at onset of disseminated zoster cases tended to be higher than those of localized zoster cases (P=0.09). A strong inverse correlation was found between pre-existing anti-VZV titer and serum VZV-DNA at onset (r=-0.90, P=0.006). In HSCT recipients, pre-existing antibody does not prevent development of VZV reactivation but may contribute to decreased viral load at onset, resulting in a mild clinical course.

Introduction

Reactivation of varicella-zoster virus (VZV) is a common event in patients undergoing hematopoietic stem cell trasnplantation (HSCT) (1,4,6,9). In post HSCT recipients, VZV reactivation can occur frequently as localized zoster and sometimes as disseminated cutaneous lesions resembling varicella with or without visceral involvement, which results in a high mortality rate (1,4,6,9). Previous studies revealed that VZV-specific memory T cells play a crucial role in suppressing reactivation (1,8). Although all recipients experience a T cell immune-deficient period after HSCT, clinical severity of VZV infection after HSCT varies from self-limiting cases to fatal disseminated cases. The risk factors that determine clinical severity have not been elucidated. Despite current use of varicella zoster immunoglobulin (VZIG), the role of humoral immunity against VZV infection has been poorly evaluated. The aim of this study was to determine the relationship between sustained anti-VZV antibody titers and clinical manifestation of VZV reactivation in HSCT recipients.

Patients and Methods

Patients. Patients who had undergone HSCT in our department during the period from February 1995 to March 2004 were enrolled as subjects of this study. Thirteen patients with VZV infection after HSCT were enrolled. Eleven patients (allo, 7; auto, 4) suffered localized zoster. Two patients (both allo) suffered disseminated zoster with visceral involvement (1 with acute abdomen and 1 with interstitial pneumonia). Thirteen patients who had been followed for at least 3 years after HSCT without clinical VZV infection were studied as control subjects. Patients' characteristics are shown in TABLE 1. The backgrounds of the two groups, including age, male/female ratio, autologous/allogeneic

HSCT, TBI regimen and GVHD, are almost the same. In an allo-HSCT setting, acyclovir was administered orally at a dose of 1,000 mg/day from day -7 to day 35 to prevent HSV infection. Ten grams of immunoglobulin was administered intravenously on day 0 and every other week until day 100 for prophylaxis of opportunistic viral infection in patients who had undergone allo-HSCT. In an auto-HSCT setting, acyclovir was administered orally at a dose of 1,000 mg/day from day -7 to engraftment, and 2.5 grams of immunoglobulin was administered on days –7, 0, 7 and 14. We retrospectively studied the transition of anti-VZV-IgG titer by the immune adherence hemagglutination (IAHA) method (before and at 3, 6, 12 and 24 months after HSCT) and VZV DNA by real-time PCR during clinical VZV infection (onset and paired serum) using cryopreserved serum samples.

Diagnosis of clinical VZV infection. VZV infection was defined by the appearance of typical cutaneous vesicular lesions or the detection of VZV antigen. Localized zoster was defined as the presence of vesicular lesions in a dermatomal distribution. Disseminated zoster was defined as a generalized vesicular eruption that is identical to that of varicella. Visceral dissemination was defined as clinical evidence of internal organ involvement in the absence of other identified pathogens that might have accounted for the clinical syndrome.

IAHA assay. IAHA analysis was performed as described previously (6).

Real-time PCR assay. VZV DNA extraction from serum was performed by using a QIAamp DNA Blood Mini Kit (QIAGEN GmbH, Hilden, Germany). The primer pair (VZV28-F: 5'-CAGATTATCCGACATGCAGTCAA-3', VZV28-R: 5'-ACCGGCAAGTCGCCAAT-3') and the probe (VZV28-T: 5'-CAACGTCGCTTAACG-3') for real-time PCR were designed in the DNA polymerase gene (gene 28) of VZV using Primer Express software (Perkin-Elmer, Norwalk, CT). The reaction mixtures (50 μl) contained 2x TaqMan Universal PCR Master Mix (Perkin-Elmer, Norwalk, CT), 15 μl of DNA, the primers

(each 900 nM) and the TaqMan MGB probe (200 nM). After 2 min at 50℃, the AmpliTaq Gold DNA polymerase was activated at 95℃ for 10 min, followed by 40 cycles at 95℃ for 15 s and at 60℃ for 1 min. All reactions were carried out six times with a 7500 ABI Prism sequence detector (Applied Biosystems, Foster City, CA). A real-time PCR standard, pCR2.1/VZV28 plasmid, was constructed by cloning a PCR product containing the VZV gene 28 region to the TA cloning vector pCR2.1 (Invitrogen, Carlsbad, CA). The pCR2.1/VZV28 plasmid was diluted to $5x10^7$, $5x10^6$, $5x10^5$, $5x10^4$, $5x10^3$, $5x10^2$ and $5x10^1$ copies in 15 μl, and they were used to generate a standard curve.

Statistical analysis. Comparisons between different groups of patients or clinical data were made using the Fisher's exact test for categorical data and the t test or Mann-Whitney U test for numerical variables. Correlation between two values was analyzed by Pearson's correlation test. VZV DNA was analyzed after log transformation. The lower limit of the assay was used in the case of negative PCR results.

Results

VZV infection occurred within 1 year after HSCT in 11 of the 13 patients. The onset of VZV infection varied day 56 to day 595 (median, day 155) after HSCT. All VZV infections occurred after cessation of prophylaxis with acyclovir. In 6 of the 10 allo-HSCT cases, VZV infection occurred during use of an immunosuppressant. All patients were treated with acyclovir. There were no deaths from VZV infection. All patients were seropositive for VZV before HSCT (FIG. 1A). None of the patients had a known history of VZV exposure before VZV infection. All VZV infections were considered to be reactivation of innate VZV. One patient with VZV reactivation, most severe case, had become seronegative before the development of disseminated zoster and interstitial pneumonia.

VZV reactivation in the other 12 cases occurred during the persistent existence of anti-VZV. There was no statistically significant difference between anti-VZV titers in the 13 patients with VZV reactivation and those in the 13 controls at each time point after HSCT (FIG 1A). The last results before clinical VZV infection (pre-existing anti-VZV) varied widely from <x2 to >x256 (median, x32). Pre-existing anti-VZV titers of disseminated zoster cases tended to be lower than those of localized zoster cases (P=0.10). Acceleration of the titer (IAHA: >x4) after VZV infection was not observed in any of the 8 recipients with VZV infection within the first 6 months after HSCT but was observed in 3 of the 5 recipients with VZV infection after 6 months (P=0.04) (FIG. 1B). Seven available samples that had been obtained from 2 patients with disseminated zoster and 5 patients with localized zoster at onset of VZV infection (stored on day 1 of skin eruption before administration of acyclovir) were examined by real-time PCR. VZV DNA was detected in all disseminated zoster cases and in 3 of the 5 localized zoster cases, whereas VZV was undetectable in all paired sera (2 to 9 weeks after onset; data not shown). VZV DNA copy numbers at onset of disseminated zoster cases tended to be higher than those of localized zoster cases (P=0.09). Pre-existing anti-VZV titer and serum VZV DNA were inversely correlated (r=-0.90, P=0.006) (FIG. 1C).

Discussion

Reactivation of latent VZV, presenting as localized zoster or as disseminated infection, is a common and potentially serious complication in HSCT recipients. Previous studies revealed that 23% to 60% of patients can be expected to develop VZV infection after HSCT (1,4,6,9). Analyses of risk factors such as allogeneic versus autologous transplant, graft-versus-host disease (GVHD), underlying disease, and pre-BMT irradiation have not

revealed definitive associations (1,4). In spite of the single viral reactivation, clinical manifestation and severity of VZV reactivations vary widely, from localized cases to fatal disseminated cases. Although the importance of cell-mediated immunity in VZV infection has been recognized (1,8), there has been little investigation of the role of humoral immunity.

Passive immunization in the form of VZIG has been clinically used mainly for prophylaxis after exposure and sometimes for treatment of VZV infection. The Center for Disease Control guideline recommends immediate VZIG administration after VZV exposure for HSCT recipients (3). Anti-VZV can interfere with the initial phases of VZV replication and reduce attack rate (10). Even if infection occurs, the severity of the cutaneous disease and the risk for dissemination are modified by VZIG prophylaxis, indicating that specific antibodies present at the beginning of the incubation period can restrict the pathogenic potential of the virus (2). In contrast, passive antibodies administrated after the onset of illness do not alter the severity of varicella (2).

In the case of primary VZV infection, no one has pre-existing anti-VZV and the disease is always disseminated. In this situation, early detection of high anti-VZV titers does not predict milder infection (2). In contrast, in the case of VZV reactivation, loss of specific cell-mediated immunity is observed in all cases, but titers of pre-existing anti-VZV vary. Mazur et al. reported that the absence or low level of circulating innate anti-VZV was a significant risk factor for dissemination of virus in herpes zoster (7). Based on these findings, we speculated a protective role of pre-existing anti-VZV titer against VZV reactivation after HSCT.

Although pre-existing anti-VZV could not prevent development of VZV reactivation in our series, a strong inverse correlation was found between pre-existing anti-VZV and serum

VZV-DNA at onset of VZV infection. Our data suggest that a low level of pre-existing anti-VZV results in a severe and disseminated clinical course in HSCT recipients. We speculated that circulating anti-VZV cannot prevent cell-to-cell spread of VZV, which leads to development of VZV infection, but that it has a neutralizing effect against viremia, which determines severity of VZV disease.

Our results suggest that monitoring anti-VZV titer has little diagnostic value, especially within 6 months after HSCT, because an increase in anti-VZV titer (IAHA: >4x) in paired sera after VZV infection was not observed in this period. In 2 of the 3 cases with active humoral immune response, anti-VZV was already increased at clinical onset. Anti-VZV titer at clinical onset was not correlated with clinical severity and this may be the reason why previous studies failed to show a role of humoral immunity by analyzing anti-VZV at clinical onset. Active humoral immune response was dependent not on severity of clinical presentation (localized or disseminated disease) but on timing of onset after HSCT. This observation is consistent with a previous finding that reconstitution of cytotoxic T lymphocytes (CTL) was also dependent on time after HSCT (1).

In conclusion, pre-existing antibody does not prevent the development of VZV reactivation but may contribute to decreased viral load at onset, resulting in a mild clinical course in HSCT recipients. These findings should be confirmed in a larger series along with evaluation of cellular immunity.

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References

1. Arvin, A. M. 2000. Varicella-zoster virus: pathogenesis, immunity, and clinical management in hematopoietic cell transplant recipients. Biol Blood Marrow Transplant. 6:219-230.
2. Arvin, A. M. 2001. Chapter 79 Varicella-Zoster Virus, pp.2731-2767. Fields VIROLOGY 4th Edition. Lippincott Williams & Wilkins.
3. Centers for Disease Control and Prevention. 2004. CDC guidelines for preventing opportunistic infections among hematopoietic stem cell transplantat recipients. MMWR Morb Mortal Wkly Rep. 49:RR-10.
4. Han, C. S., W. Miller, R. Haake, and D. Weisdorf. 1994. Varicella zoster infection after bone marrow transplantation: incidence, risk factors and complications. Bone Marrow Transplantation. 13:277-283.
5. Kalter, Z. G., S. Steinberg, and A. A. Gershon. 1997. Immune adherence hemagglutination: further observations on demonstration of antibody to varicella-zoster virus. J Infect Dis. 135:1010-1013.
6. Leung, T. F., K. W. Chik, C. K. Li, M. M. K. Shing, P. K. S. Chan, V. Lee, and P. M. P. Yuen. 2000. Incidence, risk factors and outcome of varicella-zoster virus infection in children after haematopoietic stem cell transplantation. Bone Marrow Transplantation. 25:167-172.
7. Mazur, M. H., R. J. Whitley, and R. Dolin. 1979. Serum antibody levels as risk factors in the dissemination of herpes zoster. Arch Intern Med. 139:1341-1345.
8. Takahashi, M. 2004. Effectiveness of live varicella vaccine. Expert Opin Biol Ther. 4:199-216.
9. Wacker, P, O. Hartmann, E. Benhamou, E. Salloum, and J. lemerle. 1989.

Varicella-zoster virus infections after autologous bone marrow transplantation in children. Bone Marrow Transplantation. 4:191-194.

10. Zaia J. A. M. J. Levin, S. R. Preblud, et al. 1983. Evalutation of varicella-zoster immune globulin: Protection of immunosuppressed children after household exposure to varicella. J Infect Dis. 149:737.

Figure legends

TABLE 1. Patients' characteristics

FIG. 1A. Transition of anti-VZV titer after HSCT (control: ▲, localized zoster: ●, disseminated zoster:○). In cases of VZV infection, only the results before VZV onset were plotted.

FIG. 1B. Transition of anti-VZV before, at onset of and after VZV infection in 13 cases of VZV reactivation (localized zoster:●, disseminated zoster:○). VZV infection within the first 6 months after HSCT (continuous line); VZV infection at more than 6 months after HSCT (dashed line)

FIG. 1C. Correlation of pre-existing anti-VZV titer and serum VZV-DNA (localized zoster: ●, disseminated zoster: ○). Pre-existing anti-VZV titer and serum VZV-DNA were inversely correlated (determined by Pearson's correlation test; r=-0.90, P=0.006)

Table 1. Patients' characteristics

	VZV infection (+)	VZV infection (-)	P value
Patient number	13	13	
Male/Female	7/6	7/6	-
Age (yrs)	21-65 (median, 35)	16-53 (median, 36)	0.23
Auto/Allo HSCT	3/10	1/12	0.30
TBI regimen	+ 9; - 4	+ 9; - 4	-
aGVHD(0-I/II-IV)	3/7 in 10 allo HSCT	8/4 in 12 allo HSCT	0.10
cGVHD	+ 8; - 2 in 10 allo HSCT	+ 6; - 6 in 12 allo HSCT	0.16

Figure 1A Onozawa M

Figure 1B Onozawa M

Figure 1C Onozawa M