Human Cytomegalovirus Neutralizing Antibody Response in Japanese Children with Bone Marrow Transplantation

YOSHIFUMI OHIZUMI, HIROSHI SUZUKI, YOSHIO NUMAZAKI, MASUE IMAIZUMI*, YOSHITSUGU KOISUMI*, HOSHIRO SUZUKI*, KEIYA TADA*, MASAYOSHI MINEGISHI†, SHIGERU TSUCHIYA† and TASUKE KONNO†

Clinical Research Division, Sendai National Hospital, Sendai, 983, *Department of Pediatrics, Tohoku University School of Medicine, Sendai 980–77 and †Department of Pediatric Oncology, Institute of Development, Aging and Cancer, Tohoku University, Sendai 980–77

OHIZUMI, Y., SUZUKI, H., NUMAZAKI, Y., IMAIZUMI, M., KOISUMI, Y., SUZUKI, H., TADA, K., MINEGISHI, M., TSUCHIYA, S. and KONNO, T. Human Cytomegalovirus Neutralizing Antibody Response in Japanese Children with Bone Marrow Transplantation. Tohoku J. Exp. Med., 1994, 174 (1), 11–17 — Thirty-two children with bone barrow transplantation (BMT) received intravenous injections of gammaglobulin (IVIG) with a high titer of neutralizing (NT) antibody against human cytomegalovirus (HCMV) (200 mg/kg/week) from 1 week before to 4 months after transplantation. NT antibody titers before BMT and the highest levels in serial determinations conducted after BMT were compared for each patient. They were classified into three groups according to the antibody response: primary HCMV infection as group I, endogenous reactivation or external reinfection as group II, and indeterminable cases as group III. Two (6.3%) out of 32 patients examined had BMT-associated primary HCMV infections, but did not show any clinical symptoms. Significant changes in clinical parameters were also lacking in all the other 30 patients, independent of whether they shed viruses into the urine, or demonstrated on antibody boost. It was concluded from the group variation that the antibody response was indeed due to the engraftment of BMT, rather than to a direct effect of treatment with IVIG. Our results further indicate that passive immunization with HCMV antibody does not prevent infection, but confers some protection against symptomatic disease. —— cytomegalovirus; bone marrow transplantation; neutralizing antibody; intravenous gammaglobulin

Human cytomegalovirus (HCMV) infection has been documented to be associated with interstitial pneumonitis, which is the most common infectious cause of death after allogenic bone marrow transplantation (BMT) (Zaia 1990). While significant advances have been made in prevention of HCMV disease (Zaia and Schmidt 1992) and humoral immunity is considered to be of importance in

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protection from progressive infection, especially in the immunocompromised host (Rasmussen et al. 1991), a protective role for antibodies against HCMV is still a matter of some controversy (Miller et al. 1986; Grob et al. 1987; Zaia and Schmidt 1992). In particularly the significance of neutralizing (NT) antibody in protective immunity against HCMV infection and disease remains incompletely defined (Chou 1989). In the present study, for the purpose of clarifying the relationship between recipient pretransplant serology and antibody response after transplantation, serum NT antibodies were assayed in pediatric patients undergoing BMT.

**MATERIALS AND METHODS**

**Patients and clinical specimens**

Thirty-two pediatric patients who underwent allogeneic BMT during the five-year period from 1988 to 1992 and were admitted to the Department of Pediatrics of Tohoku University School of Medicine or the Department of Pediatric Oncology of the Institute of Development, Aging and Cancer, Tohoku University were included in this study. Their clinical diagnoses are shown in Table 1. Age of the patients ranged from 4 months to 17 years (medium, 9 years and 11 months). Twenty-three were male and nine female. Serum specimens as well as urine samples were serially collected from individual patients before and after BMT during hospitalization. All patients received intravenous injections of gammaglobulin (IVIG) with a high titer of NT antibody against HCMV (200 mg/kg/week) from 1 week before to 4 months after BMT.

**Virus isolation**

Urine specimens were inoculated for virus isolation onto human embryonic fibroblast monolayers as previously described (Numazaki et al. 1970).

**NT antibody assay**

NT antibody titers were examined by plaque reduction assay as described.

<table>
<thead>
<tr>
<th>Clinical diagnoses</th>
<th>No. of recipients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute lymphocytic leukemia</td>
<td>11</td>
</tr>
<tr>
<td>Acute nonlymphocytic leukemia</td>
<td>6</td>
</tr>
<tr>
<td>Aplastic anemia</td>
<td>5</td>
</tr>
<tr>
<td>Chronic myelocytic leukemia</td>
<td>4</td>
</tr>
<tr>
<td>Mucolipidosis</td>
<td>2</td>
</tr>
<tr>
<td>Congenital neutropenia</td>
<td>1</td>
</tr>
<tr>
<td>Wilms' tumor</td>
<td>1</td>
</tr>
<tr>
<td>Acute undifferentiated leukemia</td>
<td>1</td>
</tr>
<tr>
<td>Severe combined immunodeficiency</td>
<td>1</td>
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</table>
previously (Tanaka and Numazaki 1979). Briefly, 0.05 ml samples of serially diluted serum were mixed with an equal volume of virus suspension (approximately 400 pfu of Davis strain/0.05 ml) in the presence of guinea pig complement (C') (Kyokoku, Tokyo). After incubation for 1 hr, the mixtures were inoculated into 24-well culture plates (Nunc, Denmark) containing monolayers of human diploid fibroblasts, and overlayed with Eagle's minimum essential medium containing 5% fetal calf serum, 1.5% methyl cellulose, and antibiotics. After incubation for 10 days, the plates were counted after staining. NT antibody titers were expressed as the final dilution producing 60% plaque reduction.

**Definitions**

Seropositivity for HCMV was defined as a NT titer of >1:4 and interpreted as indicating prior exposure. A four-fold or more increase in titer of antibodies to HCMV was designated as a significant antibody boost after HCMV infection or reactivation.

**RESULTS**

**NT antibody responses to HCMV**

The serum NT antibody titers before BMT and the highest levels attained in the post-transplantation serial determinations were compared as the NT antibody response for each individual patient. As shown in Fig. 1, the patients examined could be classified into three groups according to the antibody responses. The mean antibody titers in each group are shown in Table 2. Group I included two patients who had no detectable NT antibody before BMT but a significant increase after BMT, suggesting primary HCMV infection (Fig. 2). In both patients the antibody rose rapidly 1 month to 2 months post BMT without persistent viruria or symptomatic infection. It is noteworthy that out of the 32 patients examined only two (6.2%) were seronegative for HCMV before BMT.

There was a significant difference in titer between group II and group III. Group II included 18 patients who had low NT antibody titers before BMT and a significant increase after transplantation, suggesting reactivation of endogenous virus or reinfection by a donor HCMV strain. Group III included 12 patients

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**Table 2. Comparison of mean neutralizing antibody titers among groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>Neutralizing antibody titera</th>
<th>Before</th>
<th>After</th>
</tr>
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<tbody>
<tr>
<td>I</td>
<td>&lt;4</td>
<td></td>
<td>&gt;812±300</td>
</tr>
<tr>
<td>II</td>
<td>50 ± 46**</td>
<td></td>
<td>607±506</td>
</tr>
<tr>
<td>III</td>
<td>&gt;533±439**</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a reciprocal titer.

**p < 0.01.
who had high NT antibodies before BMT and no significant antibody rise after BMT, with no possibility of determining whether they were reinfected with HCMV. Twenty-three of the patients did not show any clinical symptoms of HCMV infection. Only 4 cases died due to cardiac failure, leukemia relapse, or graft versus host disease.

**Urinary HCMV excretion**

As shown in Table 3, the frequencies of HCMV viruria in individual groups were not significantly different, although values for group II tended to be much higher than in group III. The frequency of HCMV isolation from urine varied with patient: both extremes of only once or continuously for 20 months starting from 1 month to 3 months after BMT were observed.

**DISCUSSION**

Patients who were seropositive for HCMV before BMT accounted for 93.8% in our series as defined by an NT antibody titer. This high seropositive rate is in accord with seroepidemiologic data for Japanese pregnant women (Hirota et al. 1992) and implies that the majority of Japanese transplant donors are also
Classification of the patients into three groups according to the NT antibody response allowed determination of primary HCMV infection in group I, and endogenous reactivation or external reinfection in group II. These findings are important in ruling out a role for IVIG since they all received the same treatment from one week before BMT to 4 months after BMT. NT antibody titers increased significantly in subjects who had relatively low NT antibody titers before BMT in groups I and II, as opposed to those with higher titers before BMT in group III, as in the previous report of seropositive pregnant women (Tanaka et al. 1991).

**Fig. 2.** Serial variations in serum NT antibody against HCMV in 2 patients in group I.

**Table 3. Urinary CMV excretion rates among groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>Viruria</th>
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<tbody>
<tr>
<td></td>
<td>Positive (%)</td>
</tr>
<tr>
<td>I</td>
<td>1 (50.0)</td>
</tr>
<tr>
<td>II   *</td>
<td>10 (55.6)</td>
</tr>
<tr>
<td>III  *</td>
<td>3 (25.0)</td>
</tr>
</tbody>
</table>

\[ * x^2 = 1.6 \]

Classification of the patients into three groups according to the NT antibody response allowed determination of primary HCMV infection in group I, and endogenous reactivation or external reinfection in group II. These findings are important in ruling out a role for IVIG since they all received the same treatment from one week before BMT to 4 months after BMT. NT antibody titers increased significantly in subjects who had relatively low NT antibody titers before BMT in groups I and II, as opposed to those with higher titers before BMT in group III, as in the previous report of seropositive pregnant women (Tanaka et al. 1991).
Generally the rise occurred 1.5 months-4.5 months after BMT, despite the passive immunoprophylaxis treatment. It is therefore concluded that the observed antibody responses were due to the engraftment of BMT, rather than a direct effect of the IVIG treatment.

The frequencies of HCMV viruria in the three groups did not significantly differ, and no clinical symptoms of HCMV infection were found. The role of NT antibody in protective immunity against HCMV infection and disease remains incompletely defined. Evidence has accumulated that viremia and viruria can occur despite moderate to high titers of NT antibody, even when assayed against the specific infecting strain (Chou 1989). On the other hand, HCMV antibodies (presumably including the NT antibody) do exert demonstrable ameliorating effects on symptomatic HCMV disease (Chou 1989). In view of these studies and our results, the titer of HCMV antibody present before transplantation can be regarded as not influencing the HCMV infection rate. It is generally accepted that although a humoral immune response including NT antibodies does not protect from infection, they do offer defense against severe disease (Snydman et al. 1987; Snydman 1990).

The present findings are of interest in terms of the possibility of passive immunoprophylaxis with immune serum globulin or intravenous gammaglobulin against HCMV. Recently, it was shown that between 40% and 70% of the neutralizing capacity of human sera is directed against gp 58/116 (Britt et al. 1990). In connection with this, it would be of interest to evaluate the efficacy of human monoclonal antibodies to the HCMV glycoprotein 58/116 with antiviral activity (Meyer et al. 1990; Tomiyama et al. 1990; Ohizumi et al. 1992).

References


