Table 1. *Cytotoxicity Assays for N19D8 CTL against B-LCL*

B-LCL	Characteristics of B-LCL					Lysis by CTL (%)*
	HLA-A		HLA-B		Sex	
Recipient B-LCL	A*1101	A*2402	B*4403	B*5603	Female	0
First-donor B-LCL	A*1101	A*2402	B*1501	B*5603	Male	36
Second-donor B-LCL	A*2402	A*3303	B*4403	B*5101	Female	0
L11	A*0201	A*1101	B*1501		Female	34
L17	A*2402	A*3303	B*1501	B*4403	Male	35
L79	A*2402	A*3303	B*1501	B*4403	NA	42
JMD	A*0201	A*2901	B*1501	B*4403	NA	44
L16	A*1101	A*2402	B*5401	B*5101	Male	0
L19	A*2402	A*3303	B*0702	B*4403	Male	0
L25	A*1101	A*2402	B*5401	B*5201	Female	0
L59	A*2603	A*3303	B*3501	B*4403	Male	0
L73	A*0201	A*2402	B*0702	B*6701	Female	0
L74	A*1101	A*3303	B*4002	B*5101	Female	0
RAR	A*2901	A*3101	B*2705	B*4403	NA	0

NA indicates not available.
*E:T ratio was 10:1.

were isolated by the limiting dilution cloning from the patient just after the onset of graft rejection; N19D8 was the only clone with cytotoxic activity against the first donor cells. Last, the patient developed graft loss with a transient lymphocyte increase after reaching transient mix chimerism. It is difficult to differentiate immunologic graft rejection from other causes of graft failure in CBT [7]; however, this clinical course is consistent with those of patients who developed immunologic graft rejection following myeloablative or nonmyeloablative BMT [14,20]. Taken together, the results described above support the involvement of immunologic mechanisms such as cytotoxicity of T lymphocyte in the graft rejection.

The following routes of exposure of the patient to HLA-B*1501 can be considered. One possibility is that the patient was exposed to HLA-B*1501 during her pregnancy; her 19-year-old daughter has HLA-B*1501, presumably as an inherited paternal allele. It

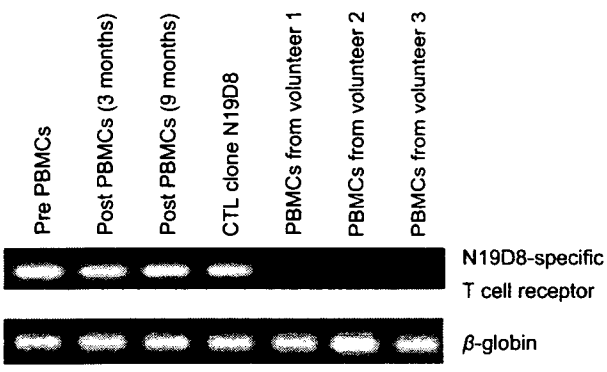

Figure 2. Presence of the N19D8 clone in pretransplant peripheral blood from the patient. Detection of lymphocytes with the N19D8 clone-specific T cell receptor in pretransplant PBMCs. Nested PCR was performed on genomic DNA using a T cell receptor Vβ17-specific primer set for the first PCR. Genomic DNA was prepared from PBMCs obtained from the patient 1 month before the first CBT (pre PBMCs) and 3 or 9 months after the second CBT (post PBMCs). Genomic DNA from the N19D8 clone was used as a positive control. Genomic DNA prepared from PBMCs obtained from unrelated volunteers were used as negative controls. PCR to detect *β-globin* was used as an internal control in each assay.

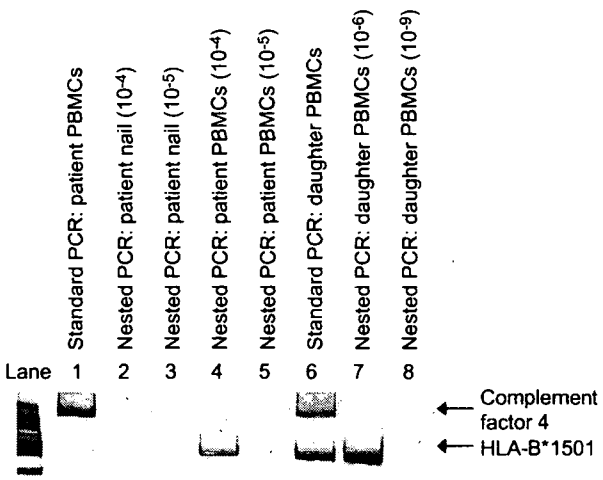

Figure 3. Presence of the N19D8 clone in pretransplant peripheral blood from the patient. The presence of HLA-B*1501 microchimerism in pretransplant PBMCs. Lanes 1 and 6 are standard PCRs specific for HLA-B*1501 on genomic DNA from pretransplant PBMCs from the patient and B*1501-positive PBMCs from the patient's daughter (positive control), respectively. Lanes 2-3, 4-5, and 7-8 are nested PCR specific for HLA-B*1501 on diluted (in parentheses) first PCR products from a posttransplant fingernail sample from the patient (negative control), pretransplant PBMCs from the patient, and PBMCs from the patient's daughter (positive control), respectively. PCR to detect *complement factor 4* was used as an internal control in each assay.

is known that fetal hematopoietic chimerism can persist for decades, maintaining a paternal-specific CTL developed during pregnancy over a long period of time [21,22]. Another possibility is exposure via blood transfusions. The patient had previously received blood transfusions from random donors, and development of HLA-B*1501-specific CTL as a consequence of contamination by HLA-B*1501-positive WBCs in the transfusion products cannot be completely ruled out. Future studies are needed to determine which of these events was related to graft rejection. It is to be noted that the presence of an HLA-B*1501 microchimerism was demonstrated even after the onset of the graft rejection. Although the mechanism is uncertain, the N19D8 CTL clone might not be able to completely eliminate B*1501-positive cells. Further studies focusing on this basic mechanism are warranted.

Interestingly, the patient had the HLA antibodies including those against the second donor HLA, HLA-A*3303, whereas the patient did not have the HLA antibody against HLA-B*1501. This suggested that HLA-antibodies did not have clinical impact on the graft rejection in this patient. However, it is inconsistent with the previous studies in solid organ transplantations or bone marrow transplantations [23,24]. This inconsistency indicates the clinical impact of HLA antibodies is controversial in CBT. Future studies would allow a proper interpretation.

In conclusion, the results of the present study demonstrate a potential role for pretransplant CTL in graft rejection following CBT. Further studies focusing on mismatched HLA-specific CTLs should help to establish an optimal strategy for overcoming graft rejection in CBT.

ACKNOWLEDGMENTS

We are grateful to Chika Wakamatsu, Yuka Nomura, and Ryohei Tanizaki for technical assistance. We are also grateful to Dr. Etsuko Maruya and Dr. Hiroh Saji (HLA Laboratory, NPO, Kyoto, Japan) for detecting microchimerism in this study. This work was supported by grants-in-aid from the Ministry of Health, Labor and Welfare of Japan (to M.M.) and the National Institute of Biomedical Innovation (to T.N.).

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厚生労働科学研究費補助金による研究班報告書

平成17～19年度

平成20年9月発行
発行者：小寺良尚（主任研究者）
事務局：名古屋第一赤十字病院
輸血部　造血細胞移植センター
〒453-8511 名古屋市中村区道下町3丁目35番地
TEL:052－481－5111　FAX:052－483－3647
印　刷：相羽印刷株式会社