

# Identification of Novel Minor Histocompatibility Antigens Responsible for Graft-versus-leukemia (GVL) Effect on Chronic Myeloid Leukemia: Usefulness of Determining The Clonotype of T Cells Associated with GVL Effect after Donor Leukocyte Infusion

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## Abstract

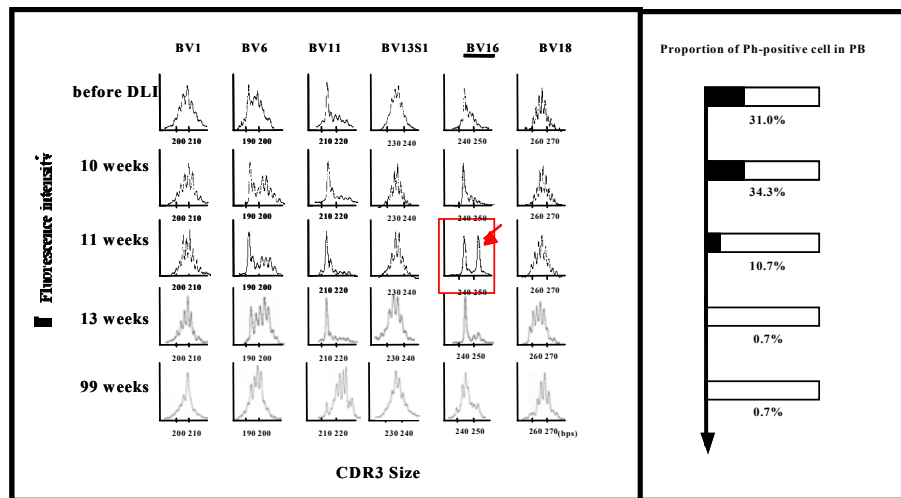
In an attempt to identify novel mHAs that induce GVL effect on chronic myeloid leukemia (CML), we analyzed peripheral blood T cells of 4 CML patients who relapsed after allogeneic stem cell transplantation and received donor leukocyte infusion (DLI), for the presence of antigen-specific T-cell proliferation. When peripheral blood lymphocyte collected from patients every 2-4 weeks after DLI were subjected to complementarity determining region (CDR) 3 size distribution analysis of T-cell receptor  $\beta$  chain, clonal proliferation of a limited number of CD4<sup>+</sup> T cells was detected in all patients 2-4 months after DLI in association with the occurrence of GVL effect. To identify an epitope of the T-cell clone that probably mediates GVL effect, we determined nucleotide sequence of CDR3 of the T cells and screened the database for the presence of T cells with a CDR3 sequence similar to that of the GVL-mediating T cells. In one of 4 patients who showed clonal proliferation of a BV16<sup>+</sup> T cell, a CDR3 motif QDR was shared by a T-cell clone that recognized an 85-99 peptide of myelin basic protein presented by HLA-DRB1\*1501. When the I domain of CD49b, a candidate peptides that could bind to this CDR3 motif in the context of DRB1\*1501, was studied, codon 256 in the I domain of the recipient was ATT (Ile) while that of the donor was GTT (Val). The BV16<sup>+</sup> T cells showed proliferative response to DRB1\*1501 L-cell transfectant pulsed with the recipient type CD49b. Thus, identification of a clonotype of T cells that mediate GVL effect in patients receiving DLI and a search for T-cell clones with a similar clonotype to the GVL-mediating T cells followed by screening of polymorphic peptides that could stimulate the T cells appears to be useful in identifying novel mHAs serving as target antigens of GVL effect.

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Minor histocompatibility antigens (mHAs) restricted to hematopoietic cells are ideal targets of graft-versus-leukemia (GVL) effect although only a few such mHAs have been identified to date. In patients with relapsed chronic myeloid leukemia (CML) responding to donor leukocyte infusion, mHa-driven T-cell proliferation is expected to occur. Determination of the receptor structure (clonotype) of such T cells that probably mediate GVL effect may be useful in deducing mHAs serving as targets of GVL effect. In an attempt to test these

hypotheses, we analyzed peripheral blood T cells of 4 patients relapsed with CML in the chronic phase who were treated with donor leukocyte infusion (DLI) for the presence of antigen-specific T-cell proliferation.

Peripheral blood mononuclear cells (PBMCs) were collected from patients every 2-4 weeks after DLI and were subjected to complementarity determining region (CDR) 3-size distribution analysis of T-cell receptor  $\beta$  chain. Clonal proliferation of a limited number of CD4<sup>+</sup> T cells was detected in all patients 2-4 months after

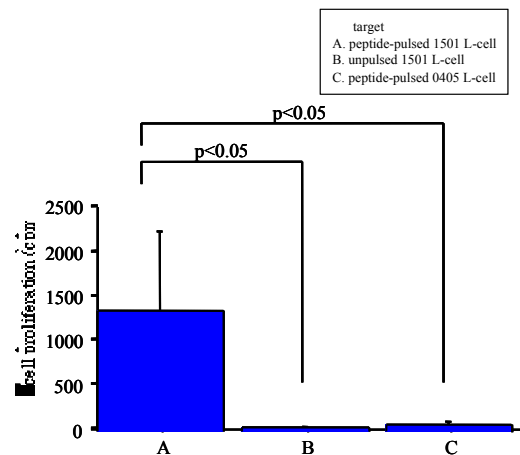


**Figure 1.** Changes in CDR3 size patterns associated with development of GVL effect in a patient relapsed with CML. The arrow indicates a new peak that appeared in association with a decrease in the proportion of Ph<sup>+</sup> cells in the peripheral blood.

DLI in association with the occurrence of GVL effect. Although the emergence of clonal T-cell proliferation in peripheral blood was transient, proliferation of a T cell with the same clonotype as that in peripheral blood persisted in the bone marrow for more than 6 months after patients achieved remission (Figure 1).

Nucleotide sequence of CDR3 of the GVL-mediating T cells was determined using subcloning and sequencing. In one patient shown in Figure 1, a CDR3 sequence of the GVL-mediating BV16<sup>+</sup> T cells was QDR. To determine kinetics of the GVL-mediating T cells after DLI, the number of the T cells was serially estimated by quantifying mRNA of the CDR3 with the QDR motif using real-time PCR. On the contrary to our expectation, the mRNA was detected at a very low level in PBMCs obtained before DLI while it was not amplified from PBMCs of the donor, indicating that the GVL-mediating T cells were originally present in the patients peripheral blood. A copy number of the mRNA in PBMCs remained low until 5 weeks and suddenly increased at 9 weeks after DLI. It declined to 10% of the peak level at 10 weeks while the mRNA copy number in bone marrow mononuclear cells remained high thereafter. These changes in the amount of the GVL-mediating T cells were correlated well with the changes in the CDR3 size pattern demonstrated by the CDR3-size distribution analysis.

To identify an epitope of the T-cell clone with a CDR3 motif of QDR, we screened the database for the presence of T cells with a CDR3 sequence containing QDR and found that the CDR3 motif QDR was shared by a T-cell clone that recognized an 85-99 peptide of myelin basic protein presented by HLA-DRB1\*1501. The structural criteria proposed by Wucherpfening et al. in 1995 (Cell 80:695) enabled us to deduce peptide sequences that could bind to this CDR3 motif in the context of DRB1\*1501. Among more than 3000 candi-



**Figure 2.** Proliferative response of bone marrow T cells from the patient after remission to the host-type CD49b peptide.

date peptides in the SwissProt database, we focused on the I domain of CD49b because this molecule is known to have polymorphisms in several coding regions. mRNA was prepared from the patients CML cells and donors lymphocytes, and cDNA fragments of the CD49b I domain covering the deduced target sequence were amplified using specific primers. Sequencing of the amplified products demonstrated that codon 256 in the I domain of the recipient was ATT (Ile) while that of the donor was GTT (Val). Stimulation of donor-derived PBMCs with autologous dendritic cells that had been pulsed with the recipient type CD49 peptide (CD49b85-99)

induced proliferation of a BV16+ T cell with a CDR3 motif of QDR. This BV16+ T cell showed proliferative response to DRB1\*1501 L-cell transfectant pulsed with the recipient type CD49b85-99 (Figure 2).

Thus, identification of a clonotype of T cells that mediate GVL effect in patients receiving DLI and a

search for T-cell clones with a similar clonotype to the GVL-mediating T cells followed by screening of polymorphic peptides that could stimulate the T cells appears to be useful in identifying novel mHAs that serve as target antigens of GVL effect.