Early detection of relapse and evaluation of treatment for mixed chimerism using fluorescence in situ hybridization following allogeneic hematopoietic cell transplant for hematological malignancies

Received: 29 October 1999 / Accepted: 27 March 2000

Abstract In order to detect chimerism, fluorescence in situ hybridization (FISH) and cytogenetic analyses were performed on bone marrow cells from 47 patients with hematological malignancies following allogeneic hematopoietic cell transplant (HCT). The dual-color XY, major Bcr-Abl (M-Bcr-Abl), and specific z-satellite probes were used for sex-mismatched HCT, chronic myeloid leukemia (CML), and myelodysplastic syndrome (MDS) cases with karyotypic abnormalities before HCT, respectively. Donor cells were found using FISH analysis in all 32 cases examined within 2 months following HCT, confirming engraftment. In six cases, however, cytogenetic analysis failed to detect donor cells due to lack of metaphases. Relapse occurred in four of the six cases in which mixed chimerism was detected using FISH analysis after 6 months of HCT. In contrast, after 12 months of HCT, no relapse was found in 24 patients without host cells. For two patients with mixed chimerism, gradual reduction of immunosuppressants or donor lymphocyte infusion resulted in the disappearance of host cells as analyzed using FISH analysis. In three extramedullary relapse cases, however, cytogenetic relapse preceded morphological and FISH relapse. These findings suggest that FISH analysis is more useful for detecting residual host cells after HCT, and the combination of FISH and cytogenetic analyses provide a more detailed evaluation for HCT patients. The results also indicate that monitoring of mixed chimerism using FISH analysis after 6 months of HCT is important for allowing the early detection of hematological relapse.

Key words Fluorescence in situ hybridization · Allogeneic hematopoietic cell transplant · Hematological malignancies · Mixed chimerism · Relapse

Introduction

Hematopoietic cell transplant (HCT) has now been shown to provide a greater benefit in the treatment of hematological malignancies compared with chemotherapy alone [1, 7, 16]. However, primary graft failure and relapse are major obstacles that affect prognosis and survival of patients undergoing HCT. It is important to detect donor cells and residual host cells in order to evaluate graft failure and mixed chimerism, respectively, following HCT. Early diagnosis of either condition permits early treatment and results in improved disease status. Cytogenetic analysis of bone marrow cells is usually used for monitoring chimerism after HCT [12, 21]. However, it requires mitotic cells and, therefore, may not assess the accurate percentage of donor or host cells after sex-mismatched HCT when insufficient metaphases are obtained. Southern blotting analysis requires large amounts of DNA, and its limit of detection of donor or recipient cells is 1–10% of the total cells [4, 8]. Polymerase chain reaction (PCR) analysis using polymorphic DNA markers, such as variable number of tandem repeats, is highly sensitive for detecting the donor or host origin of cells, but does not provide quantitative information [2, 9, 19]. Fluorescence in situ hybridization (FISH) analysis has been reported to be a powerful tool for distinguishing between donor and host cells, especially for sex-mismatched HCT or with specific chromosomal abnormalities [3, 5, 6, 11, 13, 20]. In fact, FISH analysis monitors all cells present in bone marrow, includ-
ing interphase and metaphase cells, and allows both quantitative and qualitative evaluation. Moreover, FISH analysis allows for the rapid screening of large numbers of cells, whereas only small numbers of metaphases are assessable using cytogenetic methods. There are several reports comparing FISH and cytogenetic results with respect to the relationship between mixed chimerism and hematological relapse following HCT [3, 6, 13, 20]. However, whether mixed chimerism found after HCT is predictive of relapse has yet to be determined. Furthermore, it remains to be determined whether hematological relapse after HCT is related to the transplant-associated complications, such as graft-versus-host disease (GVHD) [14]. The present study was undertaken to examine mixed chimerism analyzed using FISH and conventional cytogenetics following allogeneic HCT for hematological malignancies. The results show the usefulness of FISH analysis in evaluating the early detection of relapse and the possibility of a suppressive effect of chronic GVHD on recipient leukemic clones.

### Materials and methods

#### Patients

Forty-seven patients received allogeneic HCT between 1986 and 1998 in our hospital, including ten transplants from an unrelated donor. As shown in Fig. 1, there were 22 chronic myeloid leukemia (CML) patients, all in chronic phase. These patients

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### Fig. 1 Fluorescence in situ hybridization (FISH) and cytogenetic analyses were performed during the clinical course of 47 patients following hematopoietic cell transplant (HCT). ☐ no host cells detected by FISH and cytogenetics; ☰ no host cells detected by FISH, and no metaphase detected by cytogenetics; ☐ mixed chimerism detected by FISH, but no host cells detected by cytogenetics; ☐ cytogenetic relapse, but no host cells detected by FISH; ☐ FISH and cytogenetic relapse; ☐ died/causes of death; ☨ combination chemotherapy; ☦ donor lymphocyte infusion; ☐ reduction of immunosuppressants; ☐ positive for M-BCr-Ab1 probe but negative for XY probe; CMV-IP cytomegalovirus interstitial pneumonitis; TTP thrombotic thrombocytopenic purpura; Ch 8 chromosome 8; Ch 11 chromosome 11.