QUANTISATION OF MIXED CHIMERISM IN BLOOD AND BONE MARROW

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Bone marrow transplantation (BMT) in haemato-oncological patients is a recent area of applied DNA analysis. The determination and quantisation of donor cells in samples of blood or bone marrow in allogenic BMT verifies engraftment and detects relapses, especially if there is no specific marker for the leukaemic disease. Quantisation of components in tissues of different genetic origin might also be valuable in mixed lymphoid chimerism (MC) after liver transplantation or in zygosity studies of twins.
We developed a method to quantify MC in BMT patients by density analysis of single bands employing Southern blotting. In detail, we extracted DNA from blood or bone marrow by hypotonic lysis of red blood cells, high salt removal of proteins and ethanol precipitation (2). After digestion of purified DNA by a restriction enzyme (Hinf-I, Hae-III, Taq-I), we separated the DNA fragments by agarose gel electrophoresis, transferred them to a nylon membrane and hybridized with the multilocus probe MZ13. The DNA bands were analysed by automated densitometry (Image Master System, Pharmacia) and the percentage of host cells was calculated by polynomic regression of standard curves, employing common bands of host and donor origin as internal standard. In total, 14 of 36 patients, transplanted with homologous bone marrow for haematological malignancies, developed hemopoietic MC. In 5 patients, MC developed within 4 weeks after BMT, did never exceed 20% host cells and these patients did not relapse during the observation period (5 months to 2 years). 1 patient died of a very early relapse (10 days after BMT) and 7 patients developed "late MC" (> 4 weeks) or MC with > 20% host cells followed by a relapse ($p = 0.0269$). We conclude from these data, that stable "early MC" (< 4 weeks) after BMT does not predict a relapse, whereas "late MC" or MC with > 20% host cell DNA is highly predictive for a leukaemic relapse.

Introduction

Mixed chimerism is the **persistence of recipient blood cells** together with donor cells after **allogenic bone marrow transplantation** (BMT). The reappearance of recipient blood cells after the aplastic period in BMT can either be caused by the coexistence of **benign recipient cells**, resulting in a so-called **stable mixed chimerism**, or by **relapsing malignant recipient blood cells**. Some authors reported, that the occurrence of the mixed chimerism is correlated with a decreased incidence of an acute graft-versus-host-reaction and possibly correlated with a decreased graft-versus-leukaemia-effect (7). Whether a mixed chimerism, appearing early after allogenic bone marrow transplantation is influencing a later relapse, has been discussed controversially (6). The differentiation of a relapse from a stable mixed chimerism using DNA-techniques is still a problem, because often there are no leukaemia specific DNA-sequences available, with the exception of some cases of chronic myelogenic leukaemia (CML) (4, 5). We show in this study, that the **early occurrence of a stable mixed chimerism is not associated with a higher incidence of a relapse**. Taking into account the date of the reappearance and also the percentage of the recipient cells in the patient's blood, it is thus possible to **distinguish between relapse and stable mixed chimerism** using this method.

Materials and methods

Patients: 36 patients, 4 treated for a CML, 3 for a MDS, 9 for an ALL, 1 for a NHL, 11 for AML, 5 for a SAA, 2 for a MLD and 1 for a Hurler disease were trans-planted between 1990 and 1992 with unpurged bone marrow. Blood and bone marrow samples were analyzed 1 to 3 years after transplantation. Bone marrow biopsies were taken to distinguish between benign mixed chimerism and relapse.

RFLP-Analysis: To evaluate the percentage of donor and recipient haematopoiesis, genetic fingerprints were prepared from peripheral blood and bone marrow of the patient and of the donor before and after transplantation using the minisatellite DNA-probe MZ 1.3 (1, 3). Genomic DNA was prepared after red blood cell-lysis using proteinase K and non-ionic detergent (SDS), removal of proteins with saturated NaCl and DNA-precipitation with pure ethanol. HINF 1 (TAQ 1, HAE 3) was used for restriction enzyme digestion. After gel electrophoresis DNA-fragments were transferred to a positively charged nylon membrane and immobilized, DNA-fragments were hybridized to the digoxigenated DNA-probe MZ 1.3 (2). Hybridized probe was detected by a digoxigenin specific antibody, labelled with alkaline phosphatase and colorimetric staining with BCIP and NBT.

Quantisation of nuclear donor- and recipient cell compartments: Standard curves were established from dilution series of DNA of different size from densitometric scanning of the data and polynomic regression. To measure the recipient nuclear cell compartment, donor and recipient specific bands were evaluated. The ratio between donor- and recipient cell compartment serves as an additional internal standard.

Results

Sensitivity of the method: Recipient specific bands were detectable in mixed dilution experiments down to ratios of 2% recipient-DNA and 98% donor-DNA.

Applications of the method: In each of the 36 cases the RFLP band patterns of the donor and the recipient were sufficiently different (> 2 bands different in size) to distinguish their genotypes (4). This quantitative technique needs the setup of a standard curve only once and enables the user to evaluate fingerprints on different membranes without introducing additional internal standards. We tested the reproducibility of standard curves and sample data by testing the intra-class-correlation-factor. This factor is 0.98 for standard curves considering fragments of sizes between 9.42 kb and 6.56 kb and 0.66 for fragments of sizes between 23.13 kb and 9.42 kb.

The influence of an early mixed chimerism on a later relapse: 6 patients with malignant diseases of the haematopoietic system showed mixed chimerism within the first 4 weeks after transplantation, 1 of them developed an early relapse 10 days after BMT whereas 5 did not show any sign of a relapse during the surveillance period of 1-3 years. We can conclude, that **the appearance of a mixed chimerism is not correlated with an increased incidence of a later relapse**, compared with those patients, who show a complete donor band pattern within the first 6 weeks after transplantation ($p = 1.0$).

Predictive value of late onset of mixed chimerism for a relapse: 7 of 36 patients developed a relapse after BMT. 6 of these relapsed patients developed their recurrent disease later than 4 weeks after transplantation and each patient, who developed a relapse, showed host derived hemopoietic cells in his peripheral blood as unstable MC with increasing fraction of host cells. Analysis of the data proved, that **MC with an onset later than 4 weeks after transplantation is highly predictive for a relapse** ($p = 0.017$).

	stable mixed chimerism	relapse
MC < 4 weeks after BMT	4	1[1]
MC < 20% host DNA	3	0
MC > 4 weeks after BMT	1[2]	3
MC > 40% host DNA	0	4

[1]Patient relapsed 2 weeks after BMT, [2]not tested until 4 months after BMT

Correlation of the amount of patient's DNA in MC with the occurrence of relapse: Quantitative determination of host and donor haematopoiesis in 4 out of 6 patients with stable MC always yielded < 20% patient's haemopoiesis. In contrast, 4 of 7 patients, who relapsed, showed more than 20% patient's DNA shortly before their relapse became clinically evident. Thus **MC with more than 20% host DNA is highly correlated with relapse** of the leukaemic disease ($p = 0.008$).

Conclusion

Confirmation of engraftment of HLA-identical allogenic bone marrow after transplantation by detection of donor DNA polymorphism and the differentiation of stable or transient MC from a relapse of the leukaemic disease is important for BMT patients, because the incidence of MC after allogenic bone marrow transplantation is rather high. Patients transplanted with T cell depleted bone marrow developed a stable mixed chimerism in around 25% (7). The detection of host derived DNA often is the only early evidence for reoccurring leukaemic clones. The technique of quantitative RFLP turned out as a **simple and fast method** to distinguish between benign MC and the relapse of leukaemic clones as well as to **confirm the engraftment of the donor stem cells**. The results of this study show also, that **MC occurring early after BMT is not correlated with the later occurrence of a relapse**. Although stable MC could induce immunological tolerance and subsequently lower graft-versus-host (GvH) reaction, a decreased graft-versus-leukaemia (GvL) effect from immunological reasons obviously does not play an important role in the frequency of a later relapse of leukaemic disease. It may be even useful, to establish a stable MC in patients undergoing an allogenic bone marrow transplantation in order to diminish acute graft-versus-host-disease (GvHD).

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