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"Minisatellite" DNA probes detect engraftment and/or chimerism in recipients of HLA-matched bone marrow transplants

Luc Xerri[1], Dominique Maraninchi[2], Marie-Françoise Bertheas[3], Marie-Hélène Gaspard[2], Claude Mawas[1], and Françoise Birg[1]

[1] Unité 119 de l'Institut National de la Santé et de la Recherche Médicale and
[2] Unité de Transplantation Médullaire, Institut Paoli-Calmettes, 27, Boulevard Lei Roure, F-13009 Marseille, France
[3] Laboratoire de Cytogénétique et d'Hématologie, CHR de Saint Etienne Nord, F-42277 Saint-Priest-en-Jarez

Abstract. A study was carried out to determine whether the minisatellite DNA probes described by Jeffreys and coworkers could be used routinely to analyze engraftment, hematopoietic chimerism, and relapse in recipients of bone marrow transplants. The probes were informative for all of the recipient/donor pairs analyzed. Their limit of sensitivity was determined in reconstruction experiments and was found to vary from 2%, in the best cases, to 10%. We were able to confirm that engraftment and hematopoietic chimerism can, indeed, be sought routinely using this simple molecular approach. Only one set of probes is required for all patients and, unlike cytogenetic analysis, this analysis can be used whether or not blood or marrow cells are dividing.

Key words: Minisatellite DNA - Southern blotting - Hematopoietic chimerism.

Bone marrow transplantation (BMT) has become the therapy of choice for treating a number of hematopoietic disorders and malignancies [13]. It appears critical to establish the origin of hematopoietic and lymphoid cells post-BMT, and the pattern of engraftment is of special interest in the case of recipients of T-cell-depleted grafts. Indeed, if T-cell depletion has been found to reduce both the incidence and the severity of graft versus host disease (GVHD) [12], it has, at the same time, led to an increase in both graft failure and relapse [1, 10]. Documentation of mixed chimerism has proved useful in defining the pitfalls of this approach [2-4].

Offprint requests to: F. Birg

Analysis of restriction fragment length polymorphism (RFLP) with cloned DNA probes is a powerful method for distinguishing cells of patient and donor origin. Several groups have reported analyses of hematopoietic chimerism post-BMT [3-5, 8, 11, 15]. The applicability of this analysis is, however, restricted by the availability of informative polymorphism between donor and recipient. Thus, several probes are usually required for a given donor/recipient pair, making this analysis too sophisticated for routine clinical survey.

Jeffreys and coworkers [6] have recently cloned DNA segments called "minisatellite" (ms) DNA which, upon hybridization to restriction enzyme-digested DNA, produce a set of labeled fragments characteristic of a given individual and allow one to distinguish between siblings [7]. We report here that these probes, already shown to be useful in the identification of bone marrow engraftment in one patient [14], can be used routinely to detect engraftment, partial chimerism, and leukemic relapse.

Materials and methods

Blood samples were obtained from graft recipients and donors. Thirteen recipients of allogeneic bone marrow transplants were included in our study. Five of them had received T-cell-depleted bone marrow transplants and eight had received nondepleted grafts. These same patients were the subjects of a parallel cytogenetic survey [2]. High molecular weight DNA was prepared from nucleated blood cells using standard methods [9]. Two ms DNA probes - subclones 6.3 and 15.1.11.4 - were kindly provided by Dr. A. J. Jeffreys [6]. Single-stranded DNA was prepared [9] and labeled in vitro as described [6].

A total of 10 μg DNA aliquots were digested with either Hae III or Hinf I, following the manufacturer's recommendations. This was then submitted to electrophoresis, transferred to nitrocellulose, and hybridized with 1×10^5 cpm/ml denatured probe as described [9].

Results

Sensitivity of the minisatellite probes

The sensitivity of these probes in detecting one DNA sample in a mixture was estimated in reconstruction experiments where two samples from different individuals DNA were mixed in given ratios. The sensitivity varied from 2% to 10% (Fig. 1) according, on the one hand, to the enzyme used and, on the other hand, to the DNA samples analyzed. The presence of informative bands giving strong hybridization signals allowed us to obtain a better sensitivity.

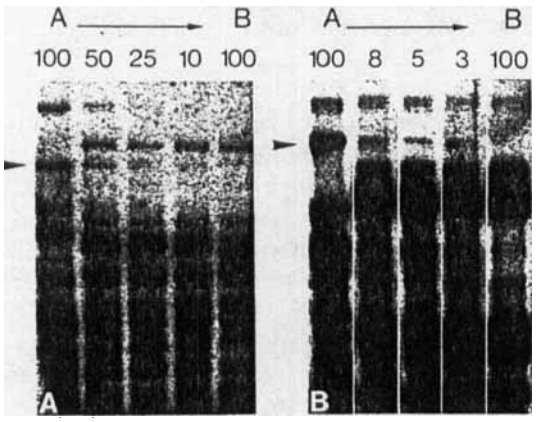

Fig. 1 A, B. Sensitivity of the minisatellite DNA probe in detecting two different DNA samples (total of 10 μg aliquots). **A, B** Different samples mixed in the ratios indicated and then submitted to restriction enzyme digestion and Southern blotting. *Arrows* indicate the informative DNA bands

Analysis of bone marrow graft recipients

Figure 2 shows representative analysis of graft recipients using the ms probes. The DNA of pretransplantation recipients (lanes R) and of the matched donors (lanes D) show distinct band patterns in all the cases, further demonstrating the power of the ms probes in this type of analysis [14]. Comparisons of the DNA patterns of recipients pre- and post-BMT (Fig. 2, lanes R and G, respectively) to the patterns of donors indicate donor engraftment in all of the recipients of nondepleted grafts as shown for patients AL31 and AL73. Two of the five recipients of T-cell-depleted grafts were total chimeras at the time of analysis, as shown for patient AL68. Two other patients (AL57 and AL70), who were in cytogenetic and clinical relapse at the time of analysis, had identical DNA band patterns before and after transplantation (data not shown). One patient (AL76) was a partial chimera.

Discussion

The goal of this study was to evaluate the feasibility of using RFLP as an alternative to cytogenetic analysis [2] in order to detect hematopoietic chimerism in recipients of bone marrow transplants. For routine clinical survey, such an analysis should be sensitive, informative, reproducible, and easy to perform. Minisatellite DNA probes [6], which produce a specific "fingerprint" for each individual [7], were therefore chosen for this study. They indeed turned out to

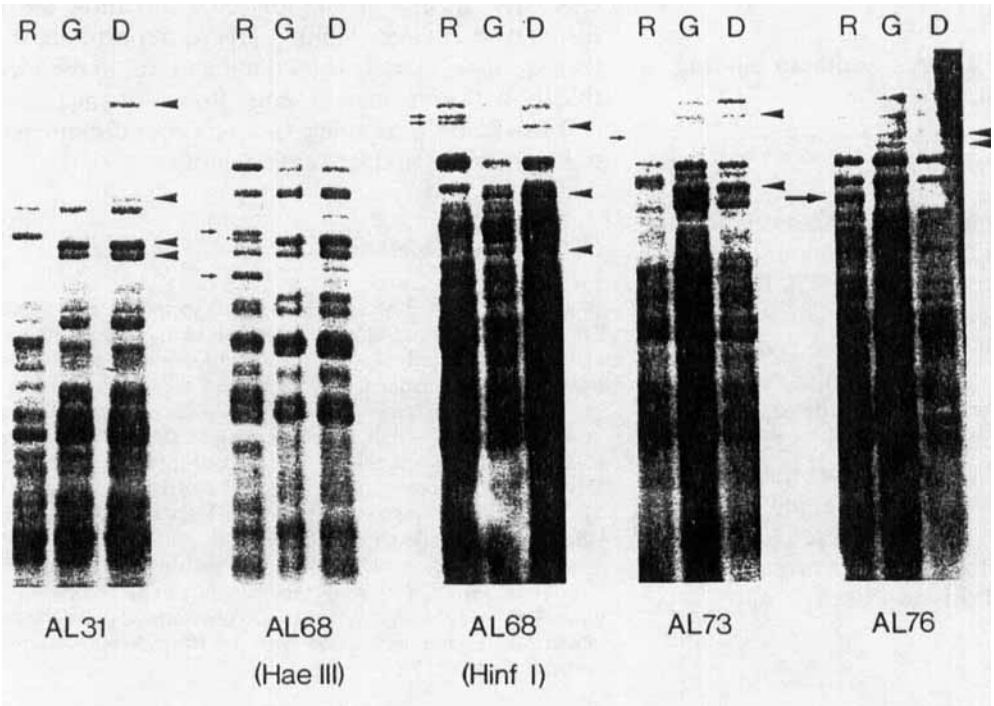

Fig. 2. Analysis of DNA samples from bone marrow transplant recipients with the minisatellite probe. Patients are indicated by their unique patient number (UPN) [2]. The different sets of *arrows* indicate the positions of informative DNA bands in recipient DNA *(R)* and in donor DNA *(D)*. For patient AL76, the *arrow* on the left side of the panel indicates the position of the residual recipient DNA band in the post-graft DNA *(G)* sample

be informative for all of the recipient/donor pairs analyzed.

The level of sensitivity attained with ms probes was determined in reconstruction experiments; a level of 10% was always obtained, and 2% was reached in the best cases. The level of sensitivity depended in part on the DNA samples themselves: a better sensitivity was obtained when intense, high molecular weight bands were present. A higher level of detection (around 0.1%) can, in fact, be obtained with a probe specific for repeated sequences on the Y chromosome [4] (and our unpublished results), but this probe is obviously informative only in the case of sex-mismatched grafts.

The sensitivity of molecular techniques is, thus, in the same range or even better (Y-specific probe) than that of cytogenetic analysis (at best 1% when at least 100 mitoses can be examined [2]). RFLP analysis with ms probes has the advantage of always being informative, whereas cytogenetic analysis relies on the presence of chromosomal markers and is, thus, only informative in about 80% of the cases. Another advantage stems from the nature of the population analyzed: all the nucleated blood cells are taken into account by Southern blot analysis, whereas only stimulated lymphocytes or bone marrow precursors undergoing mitosis are analyzed by cytogenetic methods.

In conclusion, molecular hybridization with ms probes appears to be a fast, sensitive, and reliable method for monitoring engraftment, hematopoietic chimerism, and leukemic relapse. It allows one not only to analyze all the cells of a given population, whether or not they are in mitosis, but also to analyze purified cell subsets from blood or marrow samples. However, the sensitivity of the method would not allow one to use it to search for residual disease.

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