Subtyping of Erythrocyte Phosphoglucomutase-1 as a Genetic Marker for Bone-Marrow Engraftment and Hematopoietic Chimerism after Allogeneic Bone-Marrow Transplantation in a Patient with Acute Lymphoblastic Leukemia

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We report an effective follow-up of the establishment of bone-marrow function after an allogeneic bone-marrow transplantation in a patient with acute lymphoblastic leukemia, by means of a suitable genetic marker, phosphoglucomutase-1 (EC 5.4.2.2) isoenzyme. A patient with acute lymphoblastic leukemia received allogeneic bone-marrow graft from a sibling who was of the same sex and blood group, HLA-identical, and mixed-lymphocyte-culture nonreactive. To monitor the bone-marrow engraftment and the type and degree of chimerism established, we used a genetic marker, the phosphoglucomutase-1 isoenzyme system, to reveal the difference between the bone-marrow host and donor. We did phosphoglucomutase-1 isoenzyme subtyping of the host's and donor's erythrocytes before transplantation, and isoenzyme phenotyping of the host's erythrocytes during a year after transplantation. Establishment of bone-marrow graft function, a period of temporary mixed chimerism with a population of both host's and donor's erythrocytes, a period of the exclusive presence of donor's erythrocytes, and the resumed appearance of host's erythrocytes after eight months, with no signs of relapse of leukemia, were all observed by analysis of phenotypes. These isoenzymes served as a significant and practical genetic marker, which could be successfully used in studies on bone-marrow transplantation.

Additional Keyphrases: isoenzymes • isoelectric focusing

Transplantation of allogeneic bone marrow in patients with acute leukemia is preceded by intensive radiochemotherapy, destroying both the affected and healthy hematopoietic tissues of the patient. The transplanted bone-marrow cells colonize the destroyed hematopoietic tissue of the host and produce hematopoietic cells that genetically match those of the bone-marrow donor. The bone-marrow host will thus become a life-long chimera. Both clinically and scientifically, it is of utmost importance to determine, as early and reliably as possible, whether the graft is functioning, the type and degree of chimerism established, and any relapse of disease. Techniques for these determinations include genetic-marker analyses; cytogenetic analyses; determinations of blood groups, immunoglobulin types, and blood-cell isoenzymes; and DNA restriction fragment length polymorphism analyses (1-3).

When allogeneic bone-marrow transplantation is performed between HLA histocompatible relatives (most frequently between siblings), the number of effective genetic markers in particular cases is reduced. The choice of genetic marker and the sensitivity of the analytical method will contribute considerably to the information obtained about the graft's functioning and the degree of chimerism. The degree and type of chimerism are important because they allow assessment of graft function, identification of the origin of hematopoietic cells, and determination of the host's and donor's cell population ratio. The problem of tolerance and coordination between immunocompetent cells of the host and donor, imminent to this biological phenomenon, has not yet been fully theoretically elucidated. In some patients the appearance of patient's cells after bone-marrow transplantation is related to the underlying disease relapse; in others it indicates that a certain balance—i.e., stable mixed chimerism with persistent remissions—has been established (3). It is therefore necessary to carefully examine each case of chimerism by using markers with marked polymorphism and sensitive methodology.

In the case of the patient described, we chose to use the phosphoglucomutase-1 isoenzyme system, a key genetic marker, because the host and the donor were phenotypically different in this system. Cytogenetic analyses could not be performed because of the same sex of the subjects, and the analysis of chromosomes did not reveal any specific differences.

Phosphoglucomutase-1 (PGM; EC 5.4.2.2, formerly EC 2.7.5.1) isoenzymes are bound to a polymorphism locus on chromosome 1 (4-7). Isolelectric focusing on polyacrylamide gel can separate 10 phenotypes of this enzyme, making the system a very suitable genetic marker because of its high phenotypic discriminating power in a population. The discriminating power for our population, found to be 0.75, was calculated on the basis of data on the frequency of PGM1 phenotypes in the population. The probability for two siblings to be distinguishable, calculated as described by Knowlton et al. (8), was 0.62.

Case Report

In February 1987, a 22-year-old man was admitted to the Center of Bone Marrow Transplantation, Clinical Hospital Center, Zagreb University School of Medicine, with the diagnosis of acute lymphoblastic leukemia in the first remission. After radiochemotherapy, he underwent transplantation of bone marrow from his brother, who had

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identical HLA-A, B, and DR phenotypes and the same blood group (A, Rh+). Cyclosporine was given to prevent graft rejection. On day 11 after the bone-marrow transplantation, sternal biopsy revealed the elements of hematopoiesis of all the three series of cells; reticulocytes and stab cells appeared in the peripheral blood on day 13. Additional therapy consisted mostly of platelets, except that on day 45 after the transplantation, the patient received a single dose of 400 mL of packed and irradiated erythrocytes. He received no additional blood preparations until his discharge in May 1987. The patient exhibited a mild form of graft-vs-host disease, grade II.

He visited the Center for control checkups at four- to six-week intervals. On control checkups, hematologic variables were determined in peripheral blood, immune state tests carried out in vivo and in vitro, and leukocyte markers (antigens of differentiation) analyzed. For the last time, bone-marrow biopsy was performed two months after transplantation and revealed a complete remission of disease. Further follow-up of the patient's state, with other findings normal, indicated bone-marrow biopsy. At the last checkup (January 1988), laboratory findings were within normal limits, and his overall condition was good.

Materials and Methods

Peripheral blood from the bone-marrow host and donor was collected with EDTA as anticoagulant (1 mg per milliliter of blood). The erythrocytes were promptly washed three times with isotonic saline and lyzed by freezing at −20 °C. Phosphoglucomutase-1 isoenzyme phenotyping was successively done and the samples stored at −20 °C. Comparative analysis was performed after all samples had been collected. Immediately before analysis, the samples were thawed, diluted with two volumes of distilled water, mixed thoroughly, and centrifuged (1900 × g, 10 min, 4 °C). The supernate was used for the PGM1 isoenzyme analysis.

For isoelectric focusing we used an LKB Multiphor chamber and polyacrylamide gel plates (LKB 1804-121), pH 5.0–6.5, applying maximal voltage of 2000 V, power of 20 W, and current of 15 mA for 150 min. To stain PGM1 isoenzymes, we applied formazan in an agarose overlayer (5).

We subtyped PGM1 isoenzymes in the erythrocytes from the bone-marrow host and donor before the transplantation. The host's phenotype PGM1 pattern was monitored during the following 12 months, at one, two, three, five, eight, 10, and 12 months.

Results

Before the bone-marrow transplantation, phenotyping of PGM1 isoenzymes in erythrocytes by isoelectric focusing indicated that the host had PGM1, 2+2− phenotype, whereas the bone-marrow donor had PGM1, 1+1− phenotype (Figure 1). Figure 2 shows the changes in PGM1 phenotype in the bone-marrow recipient after transplantation. One month after transplantation, isoenzymatic bands were present for both the host's and the donor's phenotypes.

Hematologic recovery of the patient was followed up on the basis of hematologic variables for peripheral blood, which were within normal limits. A bone-marrow biopsy done two months after the transplantation showed graft function to be normal: all three hematopoietic series were present and there were <5% blasts, a cytollic indicator of the disease's remission.

Discussion

Although there are several potential genetic markers that may serve in ascertaining the genetic origin of hematopoietic cells, only a few can be used with any given transplant patient (2,3). The choice of an appropriate genetic marker depends on the difference between the bone-marrow donor's and host's genotypes, i.e., on the number of particular genetic marker phenotypes allowing better individual differentiation. It is also important that the methods applied be adequately sensitive to enhance the reliability of the procedure.
In this study, the genetic marker phosphoglucomutase-1 isoenzyme system was used for follow-up of the establishment of the bone-marrow graft function and for assessment of the form and degree of hematopoietic chimerism. The bone-marrow host and the donor, brothers who both were blood group A and Rh+, differed by their PGM₁ system, which was considered appropriate. In earlier studies (1, 2), a less-sensitive method of PGM₁ isoenzyme typing on starch gel, yielding only three phenotypes, was used. In contrast, we used a method of isoelectric focusing (5) that separated the PGM₁ isoenzymes into 10 phenotypes. Thus applied, the PGM₁ isoenzymatic system becomes a potent genetic marker, adequately discriminative and methodologically sensitive to allow even minor oscillations in the type of chimerism to be successfully followed up.

For the PGM₁ analysis we used erythrocyte lysates, which was quite feasible, because during the post-transplantation period the patient received a single infusion of erythrocytes only (on day 45, 400 mL of packed and irradiated erythrocytes).

After a transitory mixed hematopoietic chimerism during the first 60 days after the transplantation, in the third month the patient's hematopoiesis was found to belong genetically to the bone-marrow donor's type. Such a condition persisted until the eighth month after the transplantation when the initial signs of reactivation of the host's hematopoietic tissue were observed. The state of mixed chimerism remained unchanged until the twelfth month, i.e., until the time of the last analysis. During that period of time, the patient was in a state of remission, with satisfactory laboratory findings.

The importance of the type and degree of hematopoietic chimerism for the duration of remission or the occurrence of disease relapse has not yet been fully elucidated, thus representing an interesting field for future clinical and scientific research. The method of PGM₁ isoenzyme subtyping may find its place in this field of research as an efficient and useful marker in the studies on bone-marrow transplantation.

References