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Behavior of Genetic Markers in Recipients After Bone Marrow Transplantation and Problems in Forensic Medicine

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ABSTRACT: The authors report studies on four pairs of donors and recipients in bone marrow transplantation (BMT). A broad range of gene markers at 41 gene loci, including 11 red blood cell markers, 5 human lymphocyte antigen (HLA) types, 12 serum protein markers, 5 red cell enzyme markers, and 8 salivary markers were evaluated before and after BMT over 2 months. As a result, 9 out of 41 gene loci of genetic markers in recipients were transformed into the donor type. BMT between family members may lead to transformation of gene markers, but within a pattern compatible with family inheritance patterns, and no genetic paradox will be found in later surveys of familial genetic relationships. However, in a personal identification system in forensic medicine using genetic markers as an index, the appearance of a phenotype incompatible with a blood relationship is possible after BMT with a non-blood-relative donor. This result is similar to the inheritance pattern observed after artificial insemination by a donor's semen (AID), a more complete out-of-family cross.

KEYWORDS: pathology and biology, genetic typing, bone marrow transplantation, forensic medicine, changes in genetic markers

One key to the success of bone marrow transplantation is the inhibition of graft-versus-host disease (GVHD), and it is most desirable to select a donor with nearly complete human lymphocyte antigen (HLA) compatibility. Recently, HLA compatibility has been somewhat overemphasized, even to the point of ignoring other important marker genes, including the ABO system, an essential consideration for blood transfusion [*1*]. A study of the genetic markers of the bone marrow recipient after bone marrow transplantation (BMT) reveals an alteration of the genetic markers to the donor type.

Blume et al. [*2*] have discussed the transformation in the MN, Rh, Kidd, and gamma-globulin marker (Gm) systems as representative of the genetic marker transformation from the recipient to the donor type. Dijk et al. [*3*], Wolpl et al. [*4*], and Ikemoto et al.

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[5] reported the transformation of genetic markers in red cells, serum protein, and red cell enzymes accompanying BMT. Mori et al. [6] studied the red cell antigens of a recipient with an ABO incompatibility. Despite being unable to detect a type-transferring enzyme, these authors found ABO antigens of the donor type. The antibodies to the donor type also declined rapidly. Yam et al. [7] concluded that deoxyribonucleic acid (DNA) restriction fragment length polymorphism (RFLP) are powerful and practical genetic markers in bone marrow transplantation studies and that further studies of mixed hematopoietic chimerism are warranted. Kitano et al. [8] reported observations of the behavior of red cell membrane antigens of donors and recipients as an index for early recognition of transplanted bone marrow engraftment.

As a result, in BMT with incompatible MN and Kidd system phenotypes, the recipient red cell types transformed into the donor type after transplantation. According to antigen analysis, using a monoclonal antibody and flow cytometry, 99.2% of the erythroblasts from bone marrow obtained 21 days after BMT were transformed to the donor type (N type) from the recipient type (MN type). At 70 days after BMT, 98.0% of the peripheral blood cells were Type N.

Case Reports

The present studies are on 4 pairs of donors and recipients of BMT. Experimental blood and saliva samples were obtained from the blood transfusion service of the Jichi Medical School Hospital and from Tottori Central Hospital. A broad range of gene markers at 41 loci, including red blood cell markers (ABO, MNSs, Lewis, P, Rh, Duffy, Kidd, Lutheran, Kell-Cellano, Diego, and Xg), HLA systems (HLA-A, -B, -C, -DR, and -DQ), serum protein markers (Gc, Tf, Hp, Gm, Km, PLG, Bf, C2, C6, C7, C8-1, and FXIII-B), red cell enzyme markers [esterase D (EsD), acid phosphatase (AcP), glyoxalase (GLO), phosphogluconate dehydrogenase (6PGD), and phosphoglucomutase (PGM)], and salivary markers (Se, Pa, Pb, Pr, PmF, Db, PIF, Amy_1) were evaluated before and after BMT over 2 months. Blood grouping and HLA typing were used [9]. The serum protein and red cell enzyme types were analyzed according to experimental methods described by Tamaki and Nishimukai, [10]; salivary typing was determined by methods previously described [11]; and flow cytometry techniques were carried out using methods described by Hashimoto et al. [12].

A summary of the transformation in genetic markers before and after BMT in recipients with different phenotypes from the donor follows. The red cell antigens transformed type O to type A and type O to type B (the pre-BMT recipient type to the post-BMT donor type), anti-A and anti-B in blood type O to anti-B in blood type A and anti-A in blood type B, MN/ss to M/ss, MN/ss to N/ss, CcDEe to CCDee, $P_1(+)$ to $P_1(-)$, Jk(a+b+) to Jk(a+b−), and Jk(a+b+) to Jk(a−b+) (Table 1 and Fig. 1). The red cell enzyme types transformed as follows: EsD 2−1 to EsD 1-1, PGM 2-1 to PGM 1-1, and AcP A to AcP AB (Table 1 and Fig. 2). Some genetic markers which did not transform when the donor phenotypes differed from the recipient phenotypes include the Lewis, Hp, Tf, FXIII-B, Se, Pa, Pr, Db, and PmF markers (Table 2 and Fig. 3). Other genetic markers, including the Duffy, Lutheran, Kell-Cellano, Xg, HLA, Gc, Gm, Km, Bf, PLG, GLO, 6PGD, Pb, PIF, Amy_1, and various complementing systems could not be evaluated because the donor and recipient phenotypes were identical.

Discussion

Many transformations in genetic markers which accompany BMT illustrate the relationship between gene products and the bone marrow. Many antigens from red cell membrane substances of the glycolipid and glycophorin systems are produced primarily

TABLE 1—*Transformation of genetic markers in four recipients after bone marrow transplantation.*

	Types of Recipients of Bone Marrow Transplantation	
Donor Types	Before	After
A	O	A
B	O	B
anti-B	anti-A,B	anti-B
anti-A	anti-A,-B	anti-A
M/ss	MN/ss	M/ss
N/ss	MN/ss	N/ss
CCDee	CcDEe	CCDee
$P_1(-)$	$P_1(+)$	$P_1(-)$
Jk(a+b−)	Jk(a+b+)	Jk(a+b−)
Jk(a−b+)	Jk(a+b+)	Jk(a−b+)
EsD 1-1	EsD 2-1	EsD 1-1
PGM 1-1	PGM 2-1	PGM 1-1
AcP AB	AcP A	AcP AB

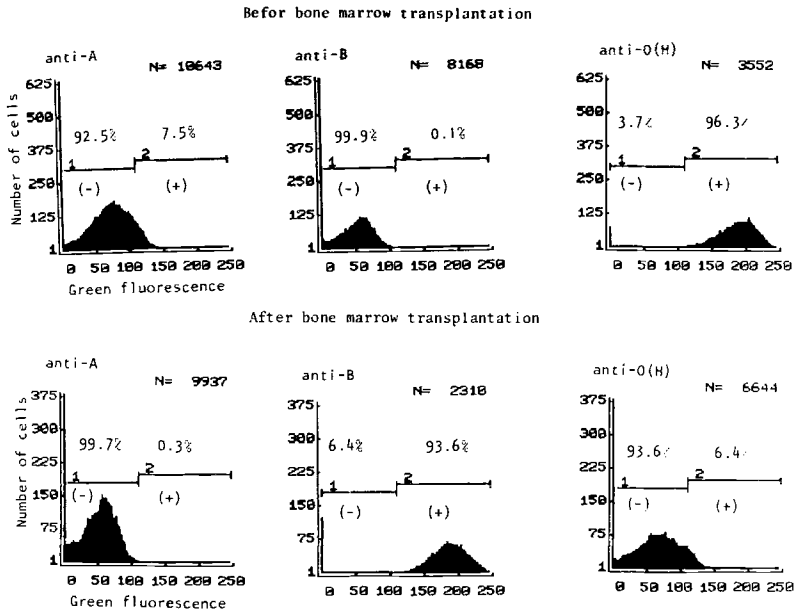

FIG. 1—*ABO phenotypes of peripheral red cells separated with flow cytometry using monoclonal anti-A, anti-B, and anti-O(H). The ordinate shows the number of analyzed cells and the abscissa shows the fluorescence intensity of the cells. The antibody reactive cells were divided into two populations, reactive (+) and unreactive (−). The red cell antigens transformed type O to type B (the pre-BMT recipient type to the post-BMT donor type).*

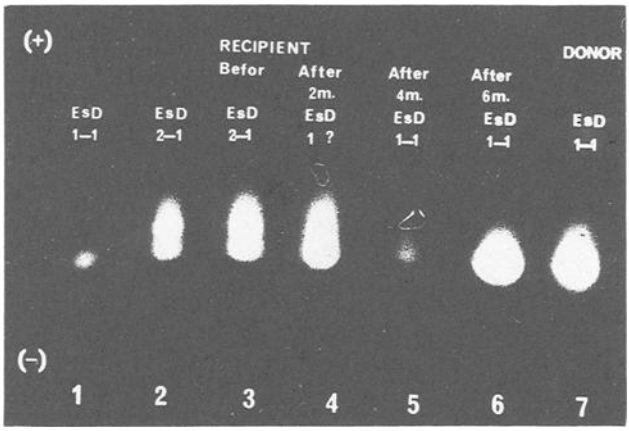

FIG. 2—*Transformation of the EsD system by bone marrow transplantation. The red cell enzyme EsD system transformed type EsD 2-1 to type EsD 1-1:* (1 and 2) *control types EsD 1-1 and 2-1;* (3) *recipient before BMT;* (4, 5, and 6) *recipient after BMT;* (7) *EsD 1-1 type of donor.*

in the bone marrow. Production of the Lewis antigen, blood-group-related substances secreted in saliva, a portion of the serum proteins, and enzyme gene markers does not appear to take place in the bone marrow.

Changes in the red blood cell agglutinin appear to be related to antibody production under gene control and are presumably produced by the transformed Gm system (immunoglobulin allotype) rather than being the result of serum antibody absorption by antigens, which leaves only nonself antibodies [2]. The transformation of the red cell enzyme genetic markers (EsD, PGM, AcP) provides a useful clue for therapy for enzyme defects.

BMT between family members may lead to transformation in the gene markers, but within a pattern compatible with family inheritance patterns, and no genetic paradox will be found in later surveys of familial genetic relationships. However, in a personal identification system in forensic medicine using genetic markers as an index, the appearance of a phenotype incompatible with a blood relationship is possible after BMT from a

TABLE 2—*Genetic markers in four recipients not transformed into donor types after bone marrow transplantation.*

	Types of Recipients of Bone Marrow Transplantation	
Donor Types	Before	After
Le(a+b−)	Le(a−b+)	Le(a−b+)
Se	Se	Se
Hp 2-1	Hp 2-2	Hp 2-2
FXIII-B 2-3	FXIII-B 3	FXIII-B 3
Pa(+)	Pa(−)	Pa(−)
Pr 2-1	Pr 1-1	Pr 1-1
Db(+)	Db(−)	Db(−)
PmF(+)	PmF(−)	PmF(−)

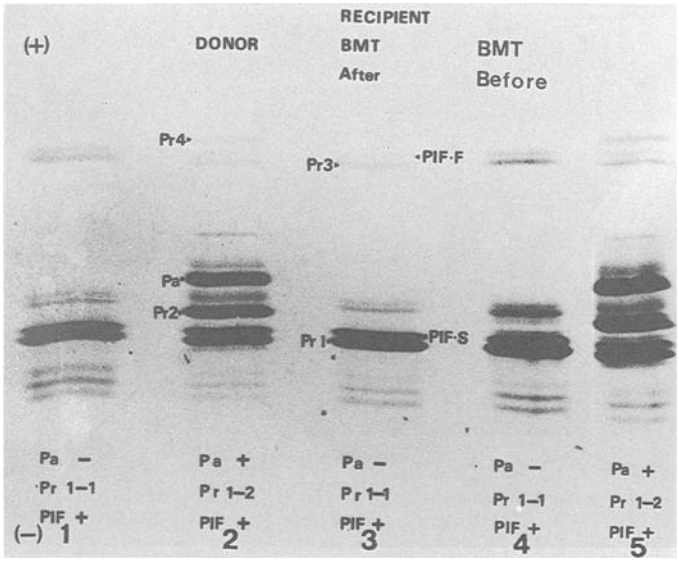

FIG. 3—*Parotid salivary polymorphisms in recipient and donor with bone marrow transplantation. Markers were not transformed when the donor types differed from the recipient types in the Pa, Pr, and PIF systems:* (1) *control types Pa(−), Pr 1-1, and PIF(+);* (2) *donor types Pa(+), Pr 1-2, and PIF(+);* (3 and 4) *recipient types Pa(−), Pr 1-1, and PIF(+);* (5) *control types Pa(+), Pr 1-2, and PIF(+).*

donor who is not a blood relative. This result is similar to the inheritance pattern observed after artificial insemination by a donor, a more complete out-of-family cross.

As development of immune suppressants such as cyclosporins improves, BMT will be performed more frequently based upon HLA histocompatibility alone. Care will be required to track the transformation in personal identification and paternity testing based on genetic markers.

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