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COMPATIBILITY TEST OF HEMATOPOIETIC STEM CELL BANK IN JAPAN

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I will introduce an HLA typing system of the Japan Marrow Donor Program（JMDP）and some factors that affect bone marrow transplantation（BMT）outcomes.

The JMDP started donor registration in 1992. About 15,000 people register annually, the total number of donors was more than 210,000 and that of potential donors was 168,000 at the end of March 2003（Fig. 1）. However, this number is only about one-half of our goal of 300,000. The first unrelated bone marrow transplantation through the JMDP was carried out in the year after donor registration was initiated. The number of transplantations is growing yearly, 758 patients had transplantations in 2002. The total number was 4,752 at the end of March 2003（Fig. 2）. This number includes 10 cases transplanted with the help of the Korean Donor Bank. These figures are updated every three months and can be viewed on the JMDP web site.

In 1996, a study group of the Japanese Ministry of Health and Welfare reported that HLA allele matching, particularly HLA-A and -B alleles matching, is important for successful BMT. Figure 3 shows the effect

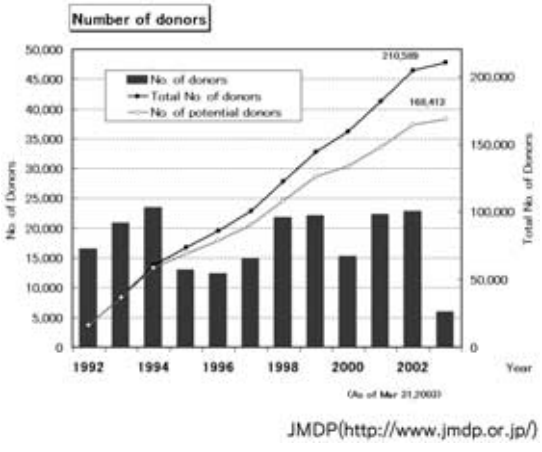

Fig. 1　Donor Registration

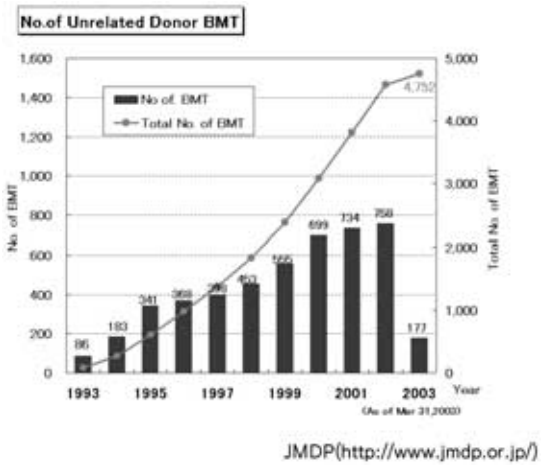

Fig. 2　Unrelated BMT

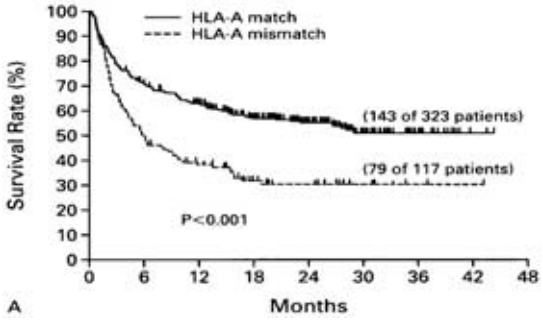

Fig. 3　HLA-matching and BMT outcomes

of HLA-A allele matching on survival rate. It has become clarified that even when HLA is serologically matched, allele level mismatching causes acute GVHD and a decrease in survival rate. Soon after the report, the JMDP started partial DNA typing for HLA class I and class II antigens in addition to serological typing.

Figure 4 shows the procedure of HLA typing starting from donor registration to transplantation. For donor registration, class I antigens are typed serologically and class II antigens are typed by the PCR-microtiter plate hybridization (MPH) method in low-resolution level or serological level. For transplantation, a selected donor's type is determined by high-resolution typing. PCR-MPH plates with a high resolution are used for class II typing. The class I high-resolution typing was designed on the basis of Japanese allele frequency (Fig. 5).

We know that these HLA class I antigens of the Japanese population are encoded by plural relatively highly frequent alleles[1]. Although these alleles are not distinguished serologically, mismatching of these alleles is the main cause of GVHD development and poor outcomes. The major mismatches occurred among A2 alleles and among B40 alleles. Therefore, we determined these alleles by the PCR-MPH method, and confirmed the results by PCR-SSCP (single strand conformation polymorphism) method. This typing system is not applicable to other antigens. Fig-

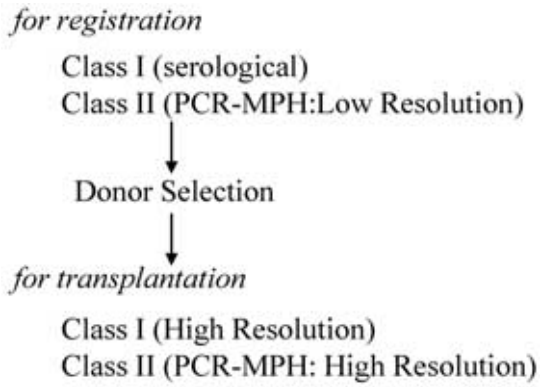

Fig. 4 HLA typing system in Japan

Antigen	Allele	%
A2	A*0201	11
	A*0206	9
	A*0207	3
	A*0210	<1
A26	A*2601	9
	A*2602	2
	A*2603	2
	A*2605	<<1

Antigen	Allele	%
B62	B*1501	8
	B*1507	<1
B75	B*1502	<<1
	B*1511	<1
B39	B*3901	4
	B*3902	<1
	B*3904	<1
B61	B*4002	8
	B*4003	<1
	B*4006	4

Fig. 5 HLA Class I Allele Typing
(PCR-MPH+PCR-SSCP)

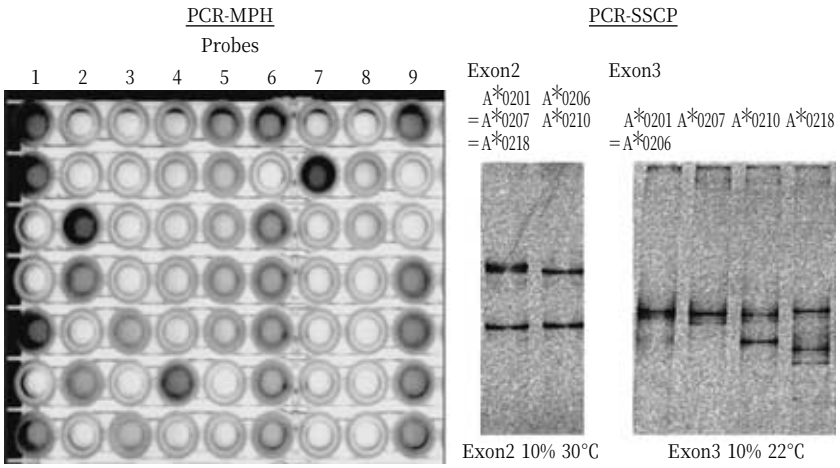

Fig. 6 HLA-A2 Allele Typing

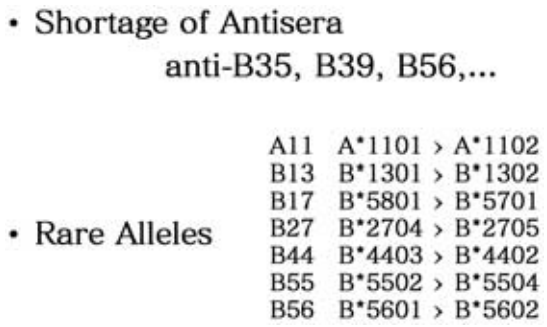

Fig. 7　Issues in HLA typing

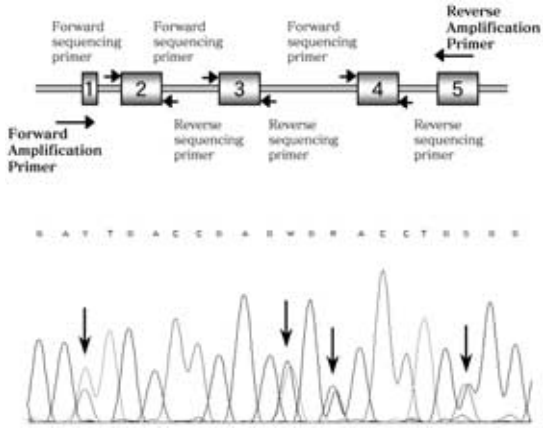

Fig. 8　Sequence-based Typing

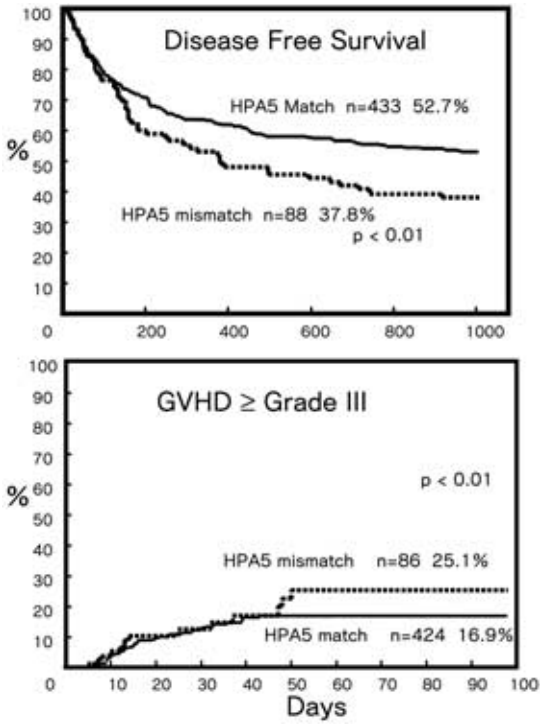

Fig. 9　Effect of HPA-5 mismatching HLA-A, B, DRB1 match

ure 6 shows an example of A2-allele typing. Samples typed serologically as A2 positive are determined of the alleles by the PCR-MPH method. After the typing using a PCR-MPH plate, the PCR products for the PCR-MPH method are further analyzed by the PCR-SSCP method for confirmation. About 99% of Japanese HLA-A and -B alleles can be determined by the combination of serological and the DNA-based typing methods. The present HLA typing system is very efficient, but not yet perfect. One of the problems is the shortage of antisera, particularly monospecific antisera（Fig. 7), as a result of declining birthrate in Japan. About 1% of alleles are not tested by the present system. For example the A11 antigen of the Japanese are encoded by A*1101 and A*1102, although A*1102 has a low frequency. Mismatching of these alleles may cause GVHD development.

New technologies for HLA typing are now available. One of the technologies is multicolored micro luminescent-beads. Each bead binds different DNA probe and the beads are mixed and placed into a single tube. The hybridization of fluorescent-labeled PCR products and the DNA probes is carried out in the tube. HLA types are identified by analyzing the beads ' colors and fluorescent dye using flow cytometry and typing software designed for the luminescent-beads method. This method is for low-resolution or mid-resolution level typing. After the low-resolution typing, sequence-based typing is an alternative for allele typing. Sequence chemistry and typing software for sequence-based typing have been improved significantly in the past few years. Although we require six PCR tubes for cycle sequencing of both strands with three exons, the six sequences are automatically

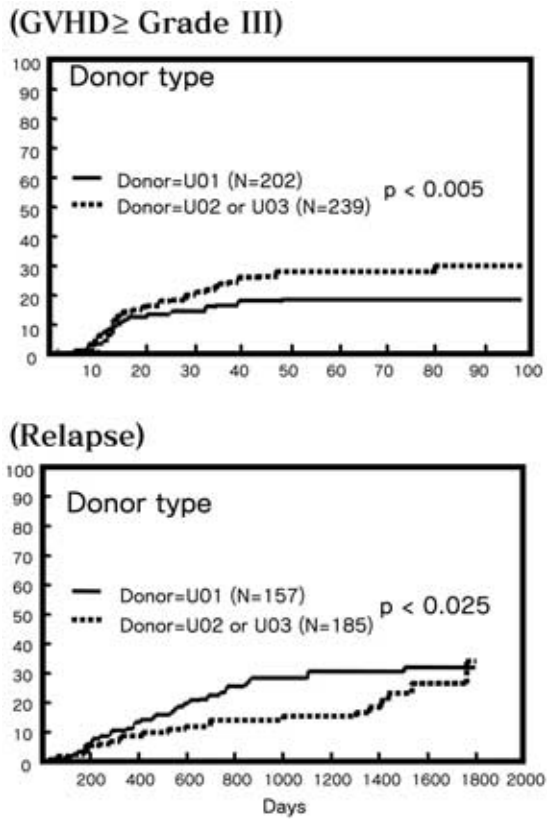

Fig. 10 Effect of *TNFA* polymorphism

connected by the software, which finds out potential alleles from the combination of heterozygous signal peaks (Fig. 8). Some samples of cord blood for transplantation are typed by the combination of the luminescent-beads method and the sequence-based typing.

Increase of HLA matching level apparently improved BMT outcomes. However, some cases of HLA allele-matched transplantation develop acute GVHD, while, in some cases, HLA mismatching is acceptable. It would appear that these phenomena are dependent on minor-antigen mismatching and/or individual difference in the immune response. If the donor's immune system is sensitive, even minor-antigens are recognized and the recipient develops GVHD. If the sensitivity is relatively low, a major antigen mismatch may be overlooked, but the low sensitivity may lead to a relapse.

Human platelet antigen-5 (HPA-5) is a unique antigen associated with BMT outcomes[2]. Figure 9 shows the effects of HPA-5 mismatching on disease-free survival and acute GVHD in HLA allele-matched unrelated BMT. HPA-5 is the only antigen significantly affecting survival rate. It is not confirmed whether HPA-5 is a minor antigen. Because, all minor antigens reported to date induce cytotoxic T cells and affect acute GVHD incidence but not survival rate. A mixed lymphocyte reaction of HPA-5-mismatched combination was positive, but the HPA-5 peptide antigen did not induce cytotoxic T cells. HPA-5 mismatching apparently decreased survival rate but its effect on GVHD incidence was not apparent.

Immune response is controlled by cytokines, and some cytokine gene polymorphisms alter cytokine production. TNF α is an inflammatory cytokine that complicates GVHD. TNF α production is altered by polymorphisms in the upstream region of the TNF α gene, *TNFA*. U02 and U03 are high producer alleles of *TNFA*[3]. BMT cases having these high producer alleles exhibited a high GVHD incidence and a low relapse rate (Fig. 10)[4]. We have preliminary data that suggest other cytokine polymorphisms also affect BMT outcomes.

HLA allele matching, minor antigens, cytokine gene polymorphisms affect BMT success rate. HA-8 is a newly identified minor antigen expressed by various organs. We obtained preliminary data suggesting that HA-8 mismatching decreases survival rate similar to HPA-5 mismatching. Not only *TNFA* polymorphisms, but some other cytokine gene polymorphisms affect GVHD incidence and relapse rate. High-performance and high-resolution HLA typing will be adopted in the near future. I consider that DNA typing of minor antigens and cytokine gene polymorphisms will be informative and helpful in improving BMT outcome.

References

1) Tokunaga, K., Ishikawa, Y., Ogawa, A., Wang, H., Mitsunaga, S., Moriyama, S., Lin, L., Bannai, M., Watanabe, Y., Kashiwase, K., Tanaka, H., Akaza, T., Tadokoro, K., Juji, T.: Sequence-based association analysis of HLA class I and II alleles in Japa-

nese supports conservation of common haplotypes. Immunogenetics, 46：199—205, 1997.
2）Juji, T., Watanabe, Y., Ishikawa, Y., Fujiwara, K., Tonami, H., Tanaka, H., Satake, M., Akaza, T., Tadokoro, K., Kodera, Y., Sasazuki, T., Morishima, Y., Takaku, F.：Human platelet alloantigen（HPA）-5a/b mismatch decreases disease-free survival in unrelated bone marrow transplantation. Tissue Antigens, 54：229—234, 1999.
3）Higuchi, T., Seki, N., Kamizono, S., Yamada, A., Kimura, A., Kato, H., Itoh, K.：Polymorphism of the 5 '-flanking region of the human tumor necrosis factor（TNF）-alpha gene in Japanese. Tissue Antigens, 51：605—612, 1998.
4）Ishikawa, Y., Kashiwase, K., Akaza, T., Morishima, Y., Inoko, H., Sasazuki, T., Kodera, Y., Juji, T.：Polymorphisms in TNFA and TNFR2 affect outcome of unrelated bone marrow transplantation. Bone Marrow Transplant., 29：569—575, 2002.