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Chapter 4

Human Leucocyte Antigen Matching Strategy

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Additional information is available at the end of the chapter

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1. Introduction

The HLA system includes a complex array of genes located on chromosome number 6 and their molecular products that are involved in immune regulation and cellular differentiation. Human leukocyte antigen (HLA) molecules are expressed on almost all nucleated cells, and they are the major molecules that initiate graft rejection. There are three classical loci at HLA class I: HLA-A, -B, and -Cw, and five loci at class II: HLA-DR, -DQ, -DP, -DM, and -DO. HLA loci are the most genetically variable gene loci in human. Two hundred and twenty four loci of HLA complex have been identified so far. Among these, 128 are functional loci that encode proteins, and 39.8% of HLA genes are related to the immune system, particularly those belong to class II loci. Almost all these genes display immune-related functions. Approximately 100 HLA genes loci have been cloned and named and at least 18 of them have alleles. Since these loci have various amounts of alleles and each allele encodes a corresponding HLA antigen, the HLA complex has the most abundant genetic polymorphism in the human immune system.

Systemic investigations of the alleles in HLA loci began in 1987. There were just over 10 identified alleles at that time. The allele numbers in HLA-I and HLA-II loci were increased to 100 and 50 respectively in 1989. The allele number of HLA-I and HLA-II reached 1028 in 2000. As of July 2013, the total allele number of HLA loci has reached 9719. HLA-A, HLA-B and HLA-C loci have 2365, 3015 and 1848 alleles respectively. DRA site has 7 and DRB site has 1456 alleles. DQA1 and DQB1 sites have 51 and 416 alleles respectively; DPB1 sites have 37 and 190 alleles, respectively (Fig 1). Theoretically, it is very difficult to find an unrelated donor with a perfectly matched HLA genotype (at the allele level) in the general population.

The polymorphism of HLA makes it difficult to find a match between unrelated donor and recipient in the allo-transplantation. Currently, the most commonly used HLA typing in organ transplantations around the world is based on HLA-A, B, C and DR genes. There are up to 8600 alleles in these genes corresponding to more than 100 specific antigens. With the increas-

ing number of patients who need hematopoietic stem cell transplantation, the lack of appropriate donors has become a significant challenge. Therefore, there is an urgent need to develop novel scientific, practical, and feasible HLA typing methods in the field of organ and hematopoietic stem cell transplantation.

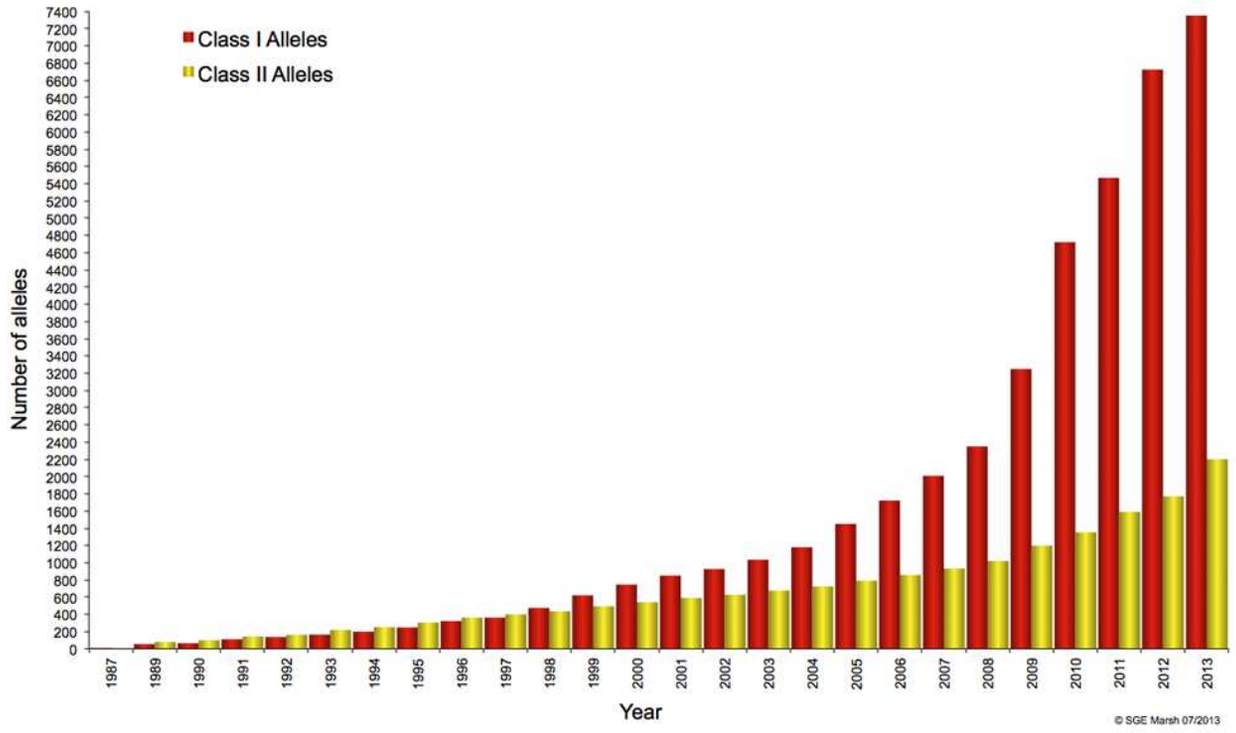

Figure 1. Increasing number of HLA alleles from 1987 to July 2013

2. The influence of HLA compatibility on organ and hematopoietic stem cell transplantation

The influence of HLA compatibility on organ transplant survival was analyzed in more than 150,000 recipients transplanted from 1987 to 1997 at transplant centers participating in the Collaborative Transplant Study. A statistically highly significant effect of HLA matching on graft and patient survival rates was found in the analysis of kidney transplants ($P < 0.0001$). Ten years after transplantation, the graft survival rate of first cadaver kidney transplants with a complete mismatch (6 HLA-A+B+DR mismatches) was 17% lower than that of grafts with no mismatch. During the first post-transplant year, the class II HLA-DR locus had a stronger impact than the class I HLA-A and HLA-B loci. During subsequent years, however, the influence on graft survival of the three loci was found to be equivalent and additive. For optimal graft outcome, compatibility at all three HLA loci is, therefore, desirable. The excellent correlation of HLA matching observed in recipients of cadaver kidneys with very short

ischemic preservation (0-6 hours) or recipients of kidneys from living unrelated donors contradicts reports that short ischemia can eliminate the influence of matching.

Although HLA has a significant effect on graft outcome regardless of the state of presensitization, the matching effect is potentiated in patients with highly reactive preformed lymphocytotoxic antibodies. Among first cadaver transplant recipients with an antibody reactivity against > 50% of the test panel, the difference in graft survival at 5 years between patients with 0 or 6 mismatches reached 30%. A collaborative project, in which molecular DNA typing methods were employed, showed that the correction of serological HLA typing errors by more accurate DNA typing results in a significantly improved HLA matching effect. Moreover, matching for the class II locus HLA-DP, a locus that can be typed reliably only by DNA methods, showed a significant effect in cadaver kidney retransplants, especially in the presence of preformed lymphocytotoxic antibodies. The analysis of heart transplants showed a highly significant impact of HLA compatibility on graft outcome ($P < 0.0001$). This result is of particular interest because donor hearts are not allocated according to the HLA match. A biasing influence of donor organ allocation (i.e. a preferential allocation of good matches to good risk recipients) can, therefore, be excluded. In liver transplantation, neither matching for HLA class I nor HLA class II could be shown to influence transplant outcome.

The first successful human bone marrow transplantation between identical twins in 1957 has provided a new approach for the treatment of leukemia and other hematologic malignancies. After the successful hematopoietic stem cell transplantation between unrelated donor and recipient with matched HLA, a bone marrow donor registry was established in 1988 (National Marrow Donor Program, NMDP) in the USA. Later on, a public cord blood bank was established. According to the World Marrow Donor Association (WMDA), as of July 2012, the association has 68 bone marrow banks in 49 countries and regions. It also has 46 cord blood banks in 30 countries and regions. The registered bone marrow and umbilical cord blood donors have exceeded 20 million. Meanwhile, the technology of HLA typing has been transformed from simple serotyping to more accurate genotyping. Although there are hundreds of reports regarding the effect of HLA matching degree on the efficacy of hematopoietic stem cell transplantation, these results are not consistent due to the differences in sample size, disease type and stage, and HLA typing. In addition, the interpretation of HLA genotyping results and their biological significance is becoming increasingly complicated. It is challenging for the clinicians outside of the HLA field to select an unrelated donor with the best-matched HLA. To meet this challenge, WMDA, NMDP of the USA and European Federation of Immunogenetics (EFI) have provided guidelines for HLA typing.

3. The history of HLA typing strategy evolution

The technology for HLA typing has evolved from the serological level to the cellular level, to the molecular level. Serotyping was the mainstream method for HLA type and has played a critical role in organ transplantations before 1990s. However, most HLA antisera are polyclonal and often have cross-reactions, making it difficult to distinguish antigens with subtle structural

differences, and leading to misidentifications. Furthermore, many factors, such as a prolonged transportation time of the blood sample and excessive amount of immature cells, may affect the result of serotyping and cellular typing.

The development of polymerase chain reaction (PCR) and its application in biomedical sciences has made the HLA typing at the DNA level possible. Therefore, using molecular methods to type HLA at the DNA level has gradually replaced serotyping and cellular typing. Commonly used DNA based HLA typing methods include PCR based sequence specific primers (PCR-SSP), and PCR based restriction fragment length polymorphism (PCR-RFLP), single-strand conformation polymorphism (PCR-SSCP), sequence-specific oligonucleotide (PCR-SSO) and single nucleotide polymorphism (PCR-SNP).

In recent years, there have been emerged many advanced techniques applied into HLA typing, such as microarray, reference strand mediated conformation (RSCA), Pyrosequencing™, flow cytometry and DNA sequencing. In the early 1990s, new permissible mismatching strategies based on HLA epitope and/or similarity between donor-recipient pairs were also established and employed in clinical application.

4. Serological typing and cellular typing

4.1. Serological typing

4.1.1. Principle of microlymphocyto toxicity test technology

As early as 1956, Gorer et al. created a complement-dependent cytotoxicity assay for detecting alloantibodies in mice. In 1964, at the University of California, Los Angeles (UCLA), the Terasaki group introduced the microlymphocytotoxicity testing technique (microcytotoxicity assay) to human leukocyte antigen (HLA) typing studies after making several improvements and scaling down the procedures of HLA serological testing. Because the method was simple, reliable, and precise, yielding reproducible results, it was widely adopted for serological study of HLA and became an international standard technology recognized by the United States National Institute of Health (NIH). HLA cytotoxic antibodies are IgG and IgM isotypes. In the presence of complements, these antibodies are capable of binding with their corresponding antigens on the surface of lymphocytes and inducing holes on the membrane. There is no such effect if the lymphocytes do not carry the corresponding antigens. The principle for this reaction is shown in Figure 2. Dead lymphocytes with damaged membrane can be observed in a number of ways, the simplest of which staining with eosin or trypan blue. Dead cells are stained and appear expanded due to incorporation of the dye; live cells are not stained. Generally, the extent of the antigen-antibody reaction is determined on the basis of the percentage of dead cells. NIH criteria are shown in Table 1.

Subsequently, an improved one-step method was developed in which antibodies, lymphocytes, and complement are successively added and then stained and fixed. Results were observed under a microscope. An operational diagram is shown in Figure 3.

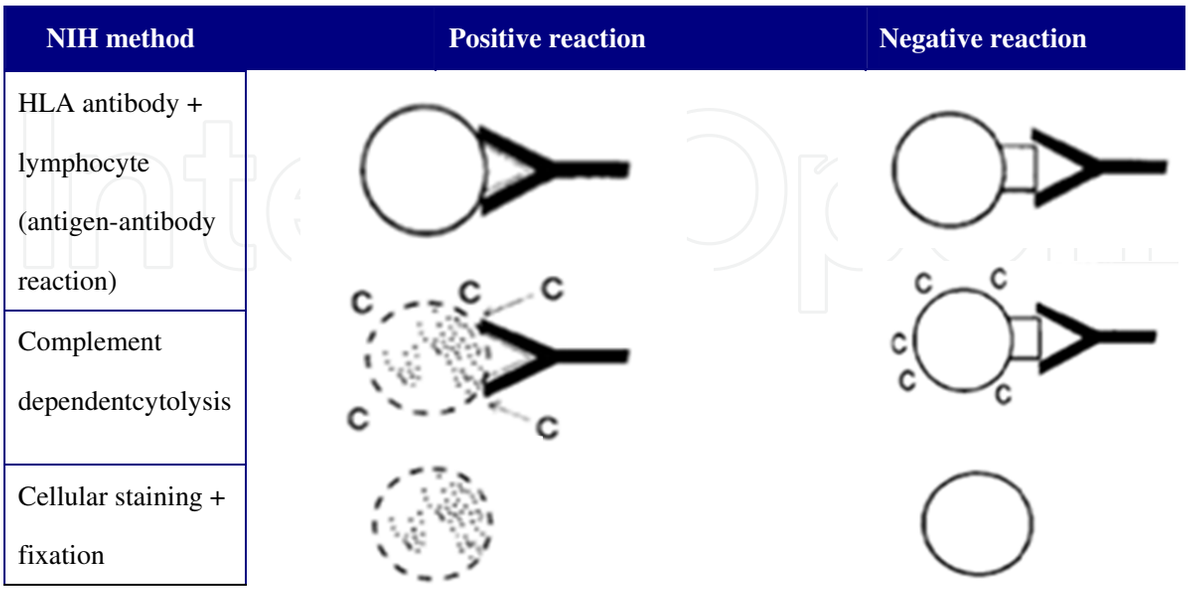

Figure 2. Microlymphocytotoxicity test principle diagram

Score	Mortality rate	Definition
1	0-10	Negative
2	11-20	Negative
4	21-50	Weak positive
6	51-80	positive
8	81-100	Strong positive
0		Not determinable

Table 1. NIH scoring criteria

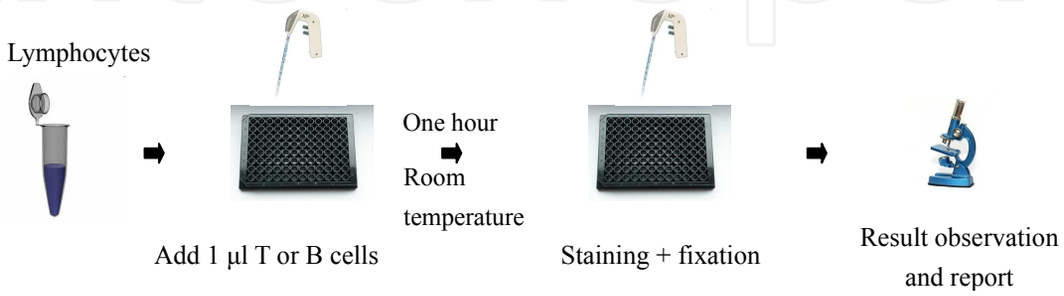

Figure 3. Schematic diagram of the one-step microlymphocytotoxicity test

4.1.2. Technique for HLA monoclonal antibody typing

Microlymphocytotoxicity tests have significantly promoted HLA research in basic and clinical applications since their introduction into serotyping. Some claim that serological typing techniques are the basis of HLA research because they are such important tools. In modern research, serological typing methods are still the main methods in HLA-I antigen typing. However, as advancing HLA research has placed increasing demands on typing techniques, shortcomings of serotyping methods have been identified, and these have been difficult to overcome. (1) The standard antiserum in serological methods is mainly from human serum or placenta. Because the rate of positive HLA antibody in sera is generally low, in particular, for some rare antibodies, it can only be obtained through collection and screening of a large number of serum samples. The technology for screening antisera is complex, difficult, and resource and labor intensive. (2) There are significant numbers of strong cross-reactions that can occur between serological tests; it is difficult to distinguish the sample antigen from the subtype antigen. (3) A high variability between serum batches significantly affects the quality of HLA typing reagents. (4) Serological match plates must be transported and preserved at low temperatures (−80°C), constraints that are inconvenient for clinical applications.

In order to solve such problems in serological typing, Terasaki et al. in the late 1980s began to develop HLA monoclonal antibodies to replace the standard antiserum. Formal production of HLA monoclonal antibody matching reagents plates began in 1992, and its availability has significantly improved the quality of HLA matching reagents. A comparison of the main technical indicators of monoclonal antibody match plates with serological match plate is shown in Table 2.

Main indexes	Serum plate	Monoclonal antibody plate
Identification of antigen determinants	specific and non-specific	specific
Specific	univalent and polyvalent	univalent
Titer	low (<1:8)	high (>20000)
Dosage	limited	almost unlimited
Anti-complement activity	may exist	non
Quality	stable	un-stable
Transportation	Dry ice low temperature	room temperature
Storage	-80°C	-20°C

Table 2. Comparison of key indicators of HLA monoclonal antibody typing plates with serological typing plates

4.2. Cellular typing

4.2.1. Homozygous typing cell

A homozygous typing cell (HTC) is homozygous for the A antigen, A/A. In the assay, HTCs are used as stimulator cells. Cells to be examined contain an unknown antigen, X/X, and are responding cells. In the reaction, a one way mixture lymphocyte culture (MLC) consists of

HTC (A/A) stimulator cells and the responder cells (X/X) being examined. An MLC reaction indicates that the responder cells could recognize the stimulator cells's A antigens, and that the responder cells being examined do not have A antigens. Absence of the MLC reaction indicates that the cells being examined have A antigens. The examined cells could be either A heterozygous (A/X) or A homozygous (A/A). In a repeated test, cells can be determined to have the same antigen only when the cells are examined with a negative HTC cell reaction. This procedure is also known as negative typing. This technique is rarely used because of the difficulty in identifying HTC individuals by this method.

4.2.2. Primed lymphocyte typing technique

In 1975 SheChy and others established a primed lymphocyte typing method (PLT) based on characteristics of a secondary response. It can specifically identify primed cells and it is also known as positive typing. In the initial MLC of responding cells A and stimulation cells B, after 9–12 days of culture, responding cells A proliferated into lymphoblastoid cells, after which they reverted to small lymphocytes. These inactive small lymphocytes were actually sensitized memory cells, also known as primed lymphocytes (PLs). When PLs and former stimulator cells were tested in a second MLC, there were very high responses within 20–24 hours. The stimulator cells are called primed cells (priming) in this process. According to this principle, experimental results of PLT depend on both priming and responding cells. Therefore, when conducting PLT, PL grouped cells must be carefully selected. More than one type of PL should be used in the identification of a PLT antigen. Preparation and sources of pretreatment cells are complex and difficult, so this method is rarely used.

4.2.3. Mixed lymphocyte culture

In 1964, Bain and Bach et al. found that lymphocytes from two unrelated individuals could stimulate each other in a mixed culture in a suitable in vitro environment. A lymphocyte could be activated and converted into a mother cell, resulting in the proliferation. After further research, other groups confirmed that mixed lymphocyte culture (MLC) is a good in vitro model for studying the cellular immune response, especially transplantation immunity. MLC technology was once widely used in organ transplantation and hematopoietic stem cell transplant matching. Because the technology is complex, has long testing cycles, involves radioactive elements, and has other drawbacks, it is gradually being replaced by the more convenient and higher-resolution genotyping methods. I will not go into the details of that method.

5. PCR based typing strategies

The technology for HLA typing has evolved from the serological level to the cellular level, to the molecular level. Serotyping was the mainstream method for HLA type and has played a critical role in organ transplantations before 1990s. However, most HLA antisera are polyclonal and often have cross-reactions, making it difficult to distinguish antigens with subtle structural

differences, and leading to misidentifications. Furthermore, many factors, such as a prolonged transportation time of the blood sample and excessive amount of immature cells, may affect the result of serotyping and cellular typing. These are the limitations of traditional HLA typing methods. The development of polymerase chain reaction (PCR) and its application in biomedical sciences have made the HLA typing the DNA level possible. Therefore, using molecular methods to type HLA at the DNA level has been gradually replacing serotyping and cellular typing. Commonly used DNA based HLA typing methods include PCR with sequence specific primers (PCR-SSP), and PCR detection of restriction fragment length polymorphism (PCR-RFLP), single-strand conformation polymorphism (PCR-SSCP), sequence-specific oligonucleotide (PCR-SSO) and single nucleotide polymorphism (PCR-SNP).

5.1. PCR-SSP (PCR with sequence specific primers)

To identify point mutations in a DNA molecule, Newton invented the amplification refractory mutation system (ARMS) for in vitro DNA amplification. The technique requires an allele sequence specific 3′ primer for the PCR amplification. Otherwise the PCR reaction will not be effective. This is because the Taq DNA polymerase used in the PCR reaction has 5′ to 3′ polymerase activity and 5′ to 3′ exonuclease activity but 3′ to 5′ exonuclease activity. Therefore, the enzyme cannot repair the single mismatched nucleotide in the 3′ primer. In order to amplify the allele with a specific sequence, the primer with the corresponding sequence is designed. The conditions for PCR reaction are strictly controlled so that the amplification of the fragment with its sequence perfectly matching to the primer is much more effective than the sequence with one or more mismatched nucleotide. One mismatched nucleotide between the 3′ primer and the template is sufficient to prevent the amplification. The PCR product is further analyzed by electrophoresis to determine whether the amplicon corresponds to the anticipated primer-specific product. Since the DNA sequence of HLA class I and class II genes are known, PCR primers can be designed based on the specific sequence of each allele for PCR-SSP genotyping.

The encoding allele sequences of various HLA antigens can be amplified with sequence specific primers. By controlling the conditions of PCR reaction, a specific primer can only amplify its corresponding allele, not other alleles. Therefore, whether there is a PCR product can be used to determine the presence or absence of a specific allele. The specificity of PCR product can be further determined by agarose gel electrophoresis. Fig 4 shows the principle of PCR-SSP.

In the first step of PCR reaction, double-stranded DNA is denatured into single-stranded DNA. In the second step, specific primers anneal to the template DNA. In the third step, double stranded DNA is generated by TaqDNA polymerase by incorporating 4 types of dNTP into the newly synthesized DNA strand. After 30 cycles of amplification, the target gene is increased to 10^8 fold.

The main advantage of this method is that it is simple and fast, and the result is easy to interpret. The heterozygosity can be easily detected as well. Therefore, PCR-SSP is the currently most used method for HLA typing. There are several FDA approved high-resolution and low-resolution detection kits available for HLA class I and class II typing. Many clinical laboratories in China have been using this method for accurate pre-transplantation HLA typing. The

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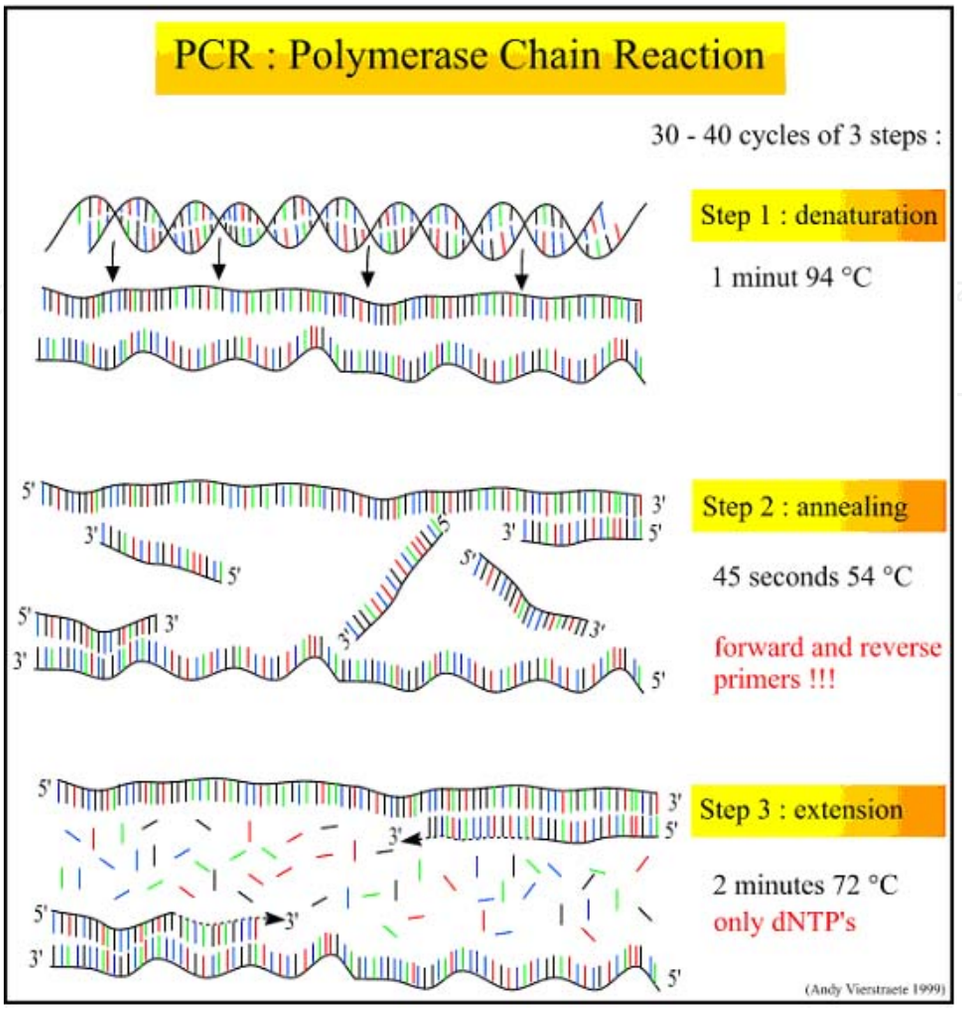


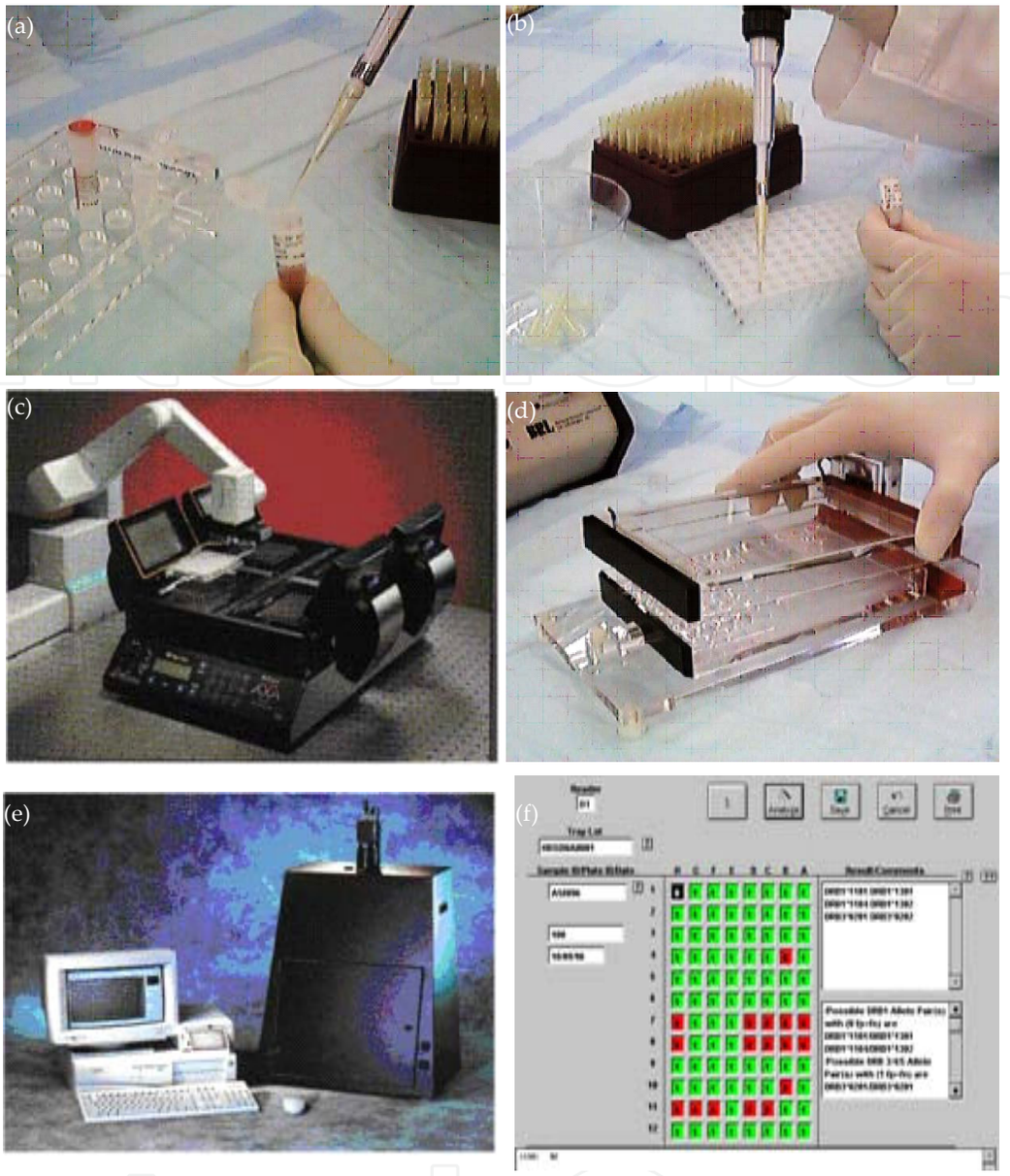
Figure 4. The diagram of PCR reaction

procedure of PCR-SSP is shown in Fig 5. One disadvantage of this method is that it requires multiple primers in order to amply all relevant alleles.

5.2. PCR-RFLP (restriction fragment length polymorphism)

Restriction endonucleases have unique recognition sites. Using computer software, restriction endonucleases that can recognize HLA sequence polymorphism are chosen to digest the PCR product. Because of sequence difference among the alleles, enzyme digestion will yield DNA fragments with unique patterns of length, which can be distinguished by electrophoresis.

Compared to serotyping, PCR-RFLP method is specific, simple and rapid and does not require probes. It can accurately detect single nucleotide difference and two linked polymorphic sites. The disadvantage of this method is that if the enzyme cannot completely digest the PCR product, the DNA fragments with similar lengths may be difficult to distinguish after electrophoresis. In addition, alleles need to have endonuclease recognition sites. Furthermore, PCR-RFLP cannot distinguish certain HLA heterozygosities. It requires multiple endonucleases for



(a) DNA polymerase and DNA sample are added to the tube containing PCR reaction buffer and dNTP. (b) 10 μl of mixture of DNA and D-mix is added to the SSP kit. The negative control does not have this mixture. (c) PCR amplification, (d) electrophoresis (2-4 min) (e) Automatic gel imaging system, (f) Analysis of the result by software

Figure 5. The process of PCR-SSP

those alleles with high polymorphism such as HLA-DRB1, and may produce complicated restriction maps. For these reasons, this method is rarely used for HLA typing nowadays.

5.3. PCR-SSCP (single-strand conformation polymorphism)

Orita *et al* in Japan have found that single-stranded DNA fragment has complex spatial conformation. The three-dimensional structure is generated by the intramolecular interactions among the base pairs. Changing of one nucleotide will affect the spatial conformation of the

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DNA strand, more or less. Single stranded DNA molecules have their unique size exclusion characters in polyacrylamide gels due to their molecular weights and three-dimensional structures. Therefore, they can be separated by non-denaturing polyacrylamide gel electrophoresis (PAGE). This method is sensitive enough to distinguish molecules with subtle structural differences, and is called single-stranded conformation polymorphism (SSCP). The authors later applied SSCP in the detection of mutations in PCR products and developed PCR-SSCP technique, which has further improved the sensitivity and simplicity for mutation detection.

This method is simple, rapid, sensitive, requiring no special equipment and suitable for clinical applications. However, this method can only detect mutations. The location and the type of the mutation need to be determined by sequencing. In addition, the conditions of electrophoresis need to be tightly controlled. Furthermore, point mutations in certain locations may have no effect or little effect on the DNA conformation. Therefore, different DNA molecules may not be able to separate by PAGE due to these reasons and other factors. Nevertheless, this method has a relatively high detection sensitivity compared with other methods. It can detect mutations in unknown locations in the DNA molecule. Takao has demonstrated that SSCP can detect 90% single nucleotide mutations in a DNA fragment smaller than 300bp. He believes that most of known single nucleotide mutations can be detected by this method. Mutant DNA molecules can be separated and purified by PAGE due to the different migration rates, and the mutation can be eventually identified by DNA sequencing.

In SSCP analysis, the separation of single stranded DNA by non-denaturing PAGE is not just based on their molecular weights and electric charges, but also on the retention force caused by their spatial conformations. Therefore, the migration rate of a DNA fragment does not reflect its molecular size. Since the wild type and mutant DNA molecules may migrate very closely and be difficult to be distinguished, it is generally required for DNA molecules to migrate for more than 16-18 cm in the gel. Mobility is calibrated using reference DNA as an internal control. Because of these reasons, this method cannot clearly determine the HLA genotype.

5.4. PCR-SSO (sequence specific oligonucleotide)

In PCR-SSO, specific probes are synthesized according to the sequence in the HLA polymorphic region. The target DNA fragment is amplified *in vitro* first. Then a specific probe will be hybridized to the PCR product under certain conditions based on base pair complementarity. The hybridized product can be detected by radioactive or non-radioactive signals. There are two types of SSO method, direct hybridization and reverse hybridization. In the direct hybridization, the PCR product is fixed on the membrane while in the reverse hybridization, the probe is fixed on membrane. Figure 6 is the diagram of PCR-SSO.

In 1986, Saiki *et al* were the first to report the analysis of DQA1 polymorphism using PCR and 4 ASO probes. Michelson has typed the DR loci by serotyping and PCR-SSO in 268 specimens. The success rate of serotyping is 91.0% while the success rate of PCR-SSO is 97.0%. Overall, PCR-SSO has a high success rate, a wide source of reagents, a high specificity and resolution. It can detect the difference of one nucleotide. In addition, PCR-SSO can be used for a large

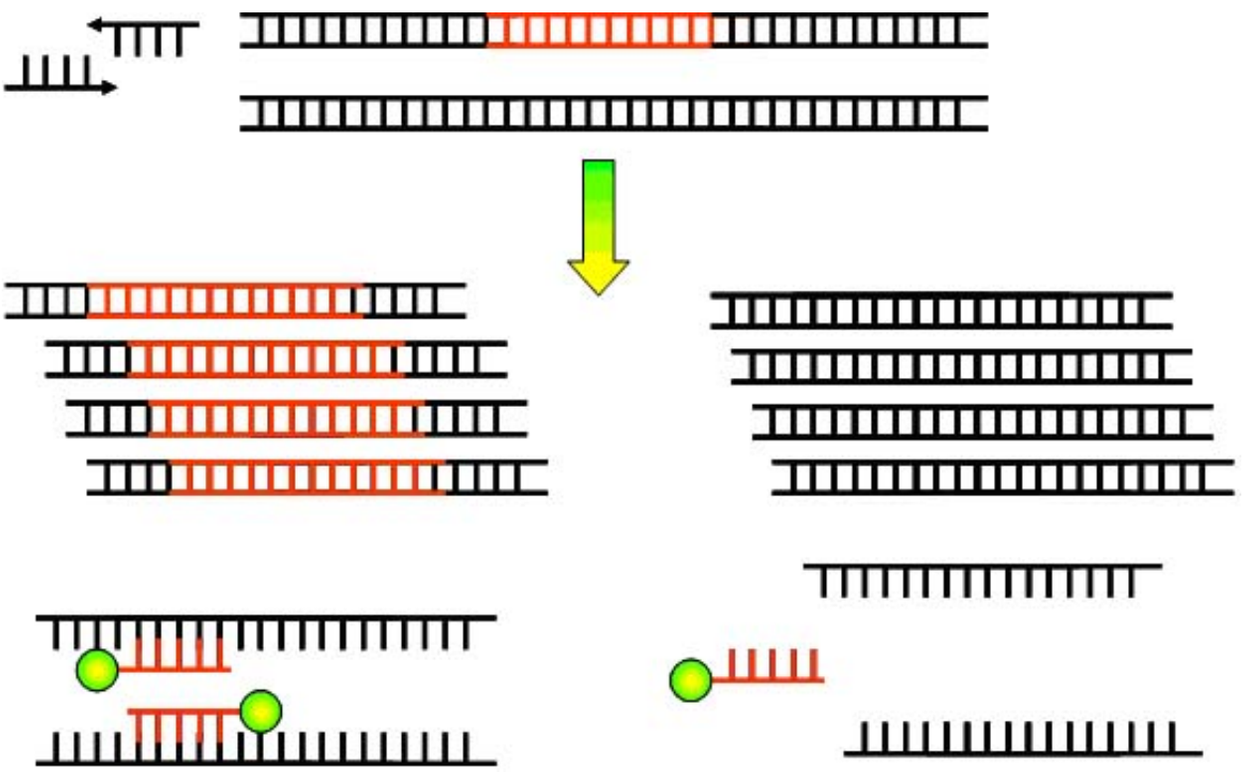

Figure 6. The diagram of PCR-SSO process

number of samples with accurate and reliable results. However, this method is time consuming. It often takes a few days and needs a large amount of probes. In addition, it is difficult to detect heterozygous alleles, particularly those of the complicated HLA-DRB1 genes.

Overall, PCR-SSO is an accurate HLA genotyping method, and can identify all known HLA alleles for accurate analysis of HLA polymorphism. HLA is a super gene family and new alleles are continuously been identified. SSO probes can only be designed based on the sequences of known alleles. Although PCR-SSO may discover new HLA polymorphism through its hybridization pattern, dot-hybridization often leads to false positives. In addition, when an allele is identified in the sample, it is difficult to determine whether the allele is homozygous or heterozygous. Therefore, the HLA allele frequency and haplotype frequency cannot be precisely determined by this method.

5.5. PCR-SNP (single nucleotide polymorphism)

Single nucleotide polymorphism (SNP) is the inheritable and stable biallelic single nucleotide difference. In the human genome, every 1000 base pairs have one to 10 SNPs. SNP may have some regulatory functions in gene expression and protein activity. High SNP density has been found in HLA class I genes with one SNP in every 400bp, setting the basis for high-throughput MHC-SNP analysis. Compared with other methods, SNP is less time consuming and with a low cost. Gou *et al* have developed a simple and effective oligonucleotide microarray to detect SNPs in the coding sequence of HLA-B locus. Based on the known polymorphism in the exon

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2 and 3 of HLA-B genes, 137 specific probes were designed. In a double-blind experiment, these probes were used in the PCR-SNP analysis of 100 specimens from unrelated individuals. The result showed that this method could explicitly identify all SNPs in the HLA-B locus. Bu Ying *et al* have established a rapid, efficient and cost effective SNP detection method using a single tube.

In this method, 4 primers are used for the PCR amplification. Two primers are used to amplify the DNA fragment containing the SNP region, and the other two primers are SNP specific. The primer extension error is significantly reduced when 4 primers simultaneously carry out the PCR reaction, thereby the accuracy of SNP analysis is greatly improved. With the development of third-generation genetic markers, it is expected to find a series of single nucleotide polymorphisms in the HLA complex, and generate high-density SNP maps. In order to develop SNP technology into a simple and effective HLA typing method, production of high-density SNP maps in the HLA regions and development of HLA-SNP genotyping kits have been proposed in the 13th IHWC conference.

6. Reference-strand-mediated conformation analysis (RSCA)

Arguello *et al* invented the double-stranded conformation analysis (DSCA) technique in 1998 for the detection and analysis of gene mutations and complex polymorphic loci. Based on this technique, reference strand mediated conformation analysis (RSCA) has been developed. This is a major technical breakthrough in HLA typing. This technique combines sequencing and conformational analysis to overcome the limitations of the methods that just employ DNA sequencing or conformational analysis. The principle of RSCA is that fluorescent labeled reference strand is hybridized with the amplified product of a specific gene to form stable double stranded DNA with unique conformation. After non-denaturing polyacrylamide gel electrophoresis or capillary electrophoresis, HLA alleles can be detected by laser scanning and computer software based analysis. Figure 7 is the diagram of RSCA.

Compared with PCR-SSP, the most commonly used method of HLA genotyping, RSCA has the following advantages: (1) high resolution and sensitivity. RSCA is based on the differential migration rate of fluorescent-labeled double stranded DNA during the electrophoresis. Alleles with different sequences will produce DNA duplexes with different spatial structures after hybridization with their fluorescent labeled probes. Two alleles with one nucleotide difference will cause a change in the spatial structure of hybridized duplex, resulting in an altered migration rate in electrophoresis. Therefore, RSCA can distinguish the alleles with a single nucleotide difference. For example, HLA*0207 and A*0209 alleles only differ one nucleotide at the site 268 of exon 2 and 3. In this site, A*0207 has a G while A*0207 has an A. Likewise, HLA-A*0224 and A*0226 only differ one nucleotide. These alleles all can be distinguished by RSCA. (2) high reproducibility. In RSCA, each lane in the non-denature polyacrylamide gel has markers and each gel has a DNA ladder. Therefore, the alteration caused by different gels or lanes can be eliminated. (3) new allele or mutation identification. RSCA is based on the electrophoretic mobility difference caused by different spatial structure of the duplexes after

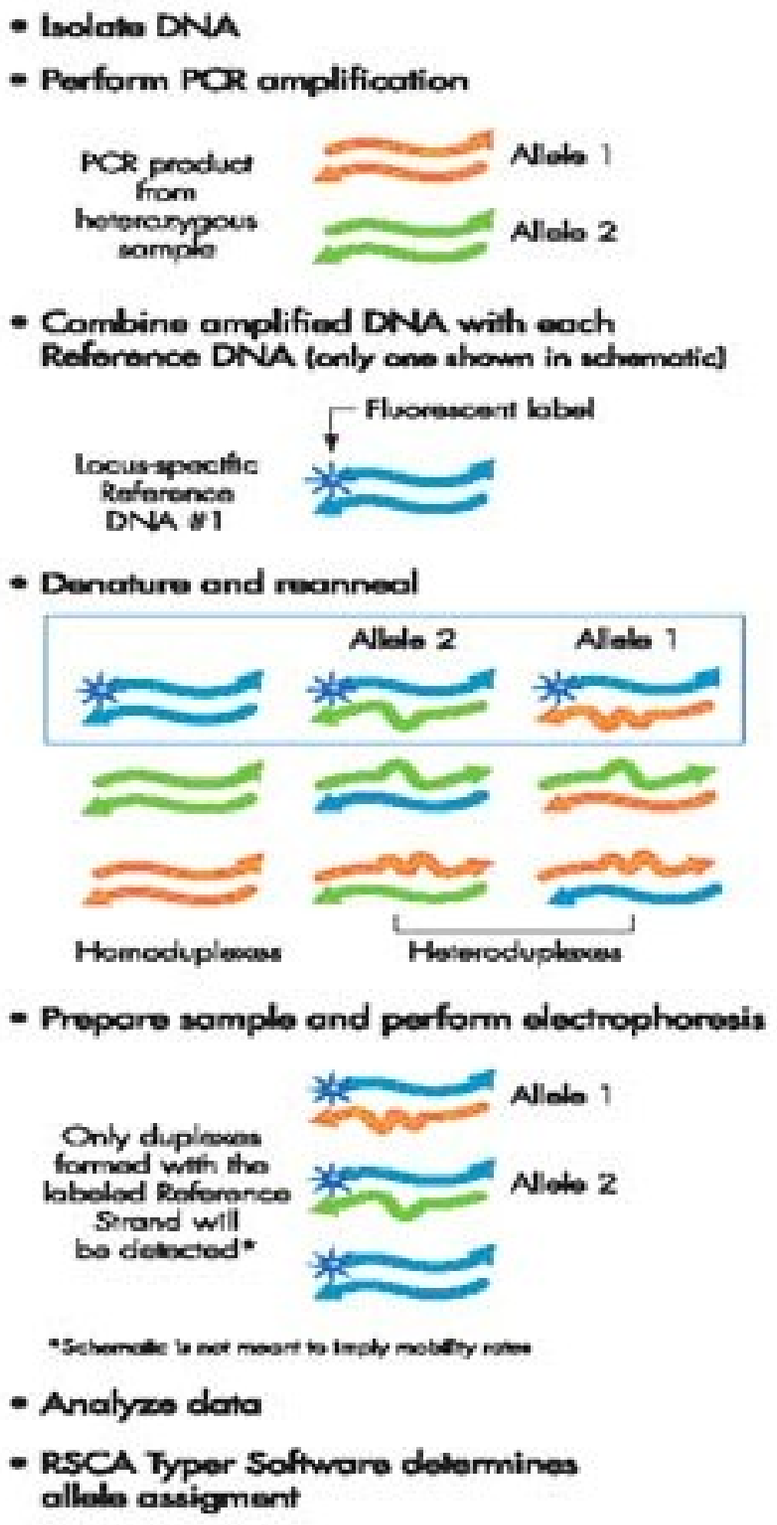

Figure 7. The diagram of RSCA

allele-FLR hybridization. New alleles or mutations will have electrophoretic mobility different from that of known alleles. (4) RSCA can be applied at a large scale with a low cost.

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The disadvantages of RSCA are (1) time-consuming for a single sample; (2) requiring high quality samples; PCR-SSP requires 10-100ng/ml of DNA, which can be obtained with a regular DNA purification kit from patients even with a low amount of white blood cells. However, RSCA requires 50-100ng/ml of DNA. It may require an increased amount of blood sample for patients with low levels of white blood cell in order to obtain sufficient DNA; and (3) insufficient database.

7. Pyrosequencing™ — A high-resolution method for HLA typing

Pyrosequencing is a new HLA genotyping technology based on real time sequencing during DNA amplification. The reaction system contains 4 enzymes (DNA polymerase, ATP sulfurylase, luciferase and apyrase), substrate (APS: adenosine 5′ phosphosulfate), fluorescein (luciferin), primers and the single stranded DNA template. After one type of dNTP (dATP, dTTP, dCTP and dGTP) is added to the reaction system, it will be incorporated into the newly synthesized chain if it is complementary to the nucleotide on the template. Incorporation of dNTP will generate the same molar amount of pyrophosphate (PPi). ATP sulfurylase converts APS and PPi into ATP, which provides energy for luciferase to oxidate luciferin and emit light. The amount of light signal is proportional to the amount of ATP. The optical signal is detected by a CCD (charge couple device) camera and generates a peak in the pyrogram. The principle of Pyrosequencing is shown in Fig 8.

The height of each signal's peak is proportional to the number of incorporated nucleotide. Unincorporated dNTPs and excessive ATP are converted to dNDPs, which are further converted to dNMPs by apyrase. The optical signal is quenched and the system is regenerated for the next reaction. The next dNTP can be added to the system to start the next reaction after the unincorporated dNTPs and excessive ATP are removed. The reaction cycle continues until the complementary DNA strand is synthesized. Under the room temperature, it takes 3-4 seconds from polymerization to light detection. In this system, 1 pmol of DNA will generate $6x10^{11}$ pmol of ATP, which in turn yields $6x10^{9}$ pmol of photon with a wavelength of 560nm. The signal can be easily detected by a CCD camera. For the analysis of DNA with unknown sequence by Pyrosequencing, a cyclic nucleotide dispensation order (NDO) is used. dATP, dGTP, dTTP and dCTP are sequentially added to the reaction. After one nucleotide is incorporated, the other three will be degraded by the apyrase. For the DNA with known sequence, non-cyclic NDO can be used and will yield a predicted pyrogram. The sequence of the complementary DNA strand can be determined based on the NDO and peak value in the pyrogram.

Since nucleotides are differentially incorporated, Pyrosequencing can produce high-resolution results. Typing HLA-DRB1*04, 07 and DRB4* in the donor's DRB genes by Pyrosequencing not only yields the same result as using the SSOP typing kit, but also produces the result with a higher resolution. Compared with SSP, SSOP, direct or reverse hybridization, Pyrosequencing can be used to solve ambiguous allele combinations of HLA-DQ and HLA-A/B in a short time. The types of HLA-DQB1 and HLA-DRB alleles have been accurately determined by Pyrosequencing.

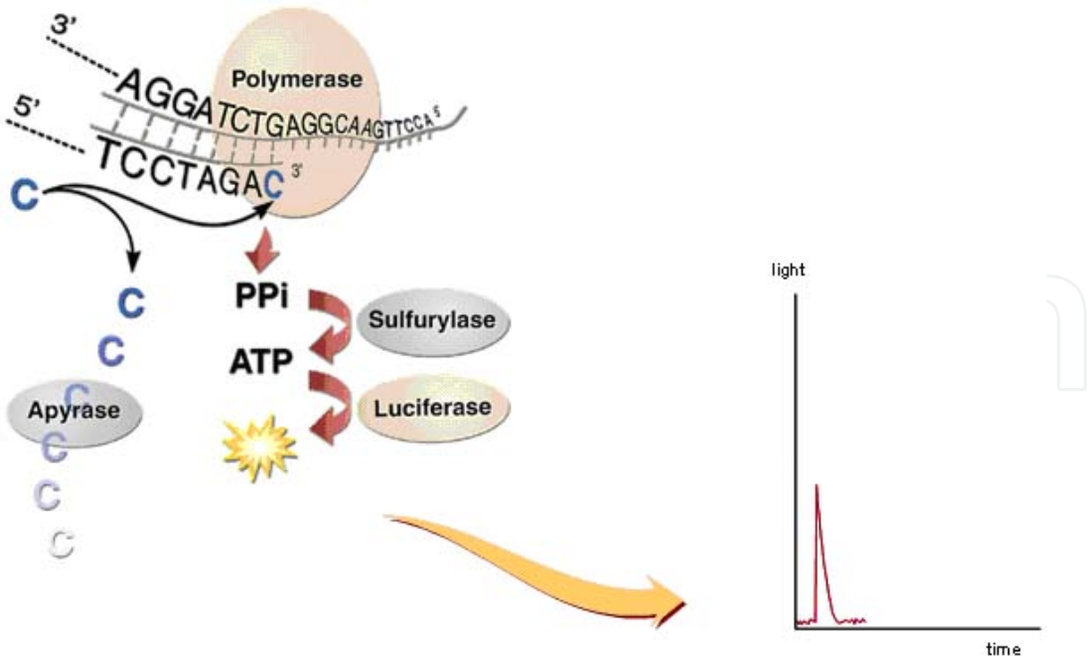

Figure 8. The principle of Pyrosequencing

An inherent problem with this technology is the de novo sequencing of polymorphic region in heterozygous DNA, although polymorphism can be detected in most cases. When the nucleotide in the polymorphic region is altered, synchronized extension can be achieved by the addition of the substituted nucleotides. If there is a deletion or insertion in the polymorphic region, and the deleted or inserted nucleotide is the same as the adjacent nucleotide on the template, the sequence after the polymorphic region will be synchronized. However, if the deleted or inserted nucleotide is different from the adjacent nucleotide on the template, the sequence reaction can be out of phase, making the subsequent sequence analysis difficult. Another issue with this technology is the difficulty in determining the number of incorporated nucleotides at the homopolymeric region. The light signal will become nonlinear after the incorporation of more than 5-6 identical nucleotides. Studies on the polymerization efficiency of the homopolymeric region have shown that it is possible to incorporate less than 10 identical nucleotides in the presence of apyrase. However, it needs specific software algorithm of signal integration to determine the precise number of incorporated nucleotides. For re-sequencing, the nucleotide is added twice to ensure complete polymerization in the homopolymeric region. Another limitation of this technology is the length of the sequencing.

8. Application of flow cytometry in HLA typing

Flow cytometry has failed to become a main method for HLA typing since it was applied to the field of immunology for the first time in 1977. This is mainly due to the large number of specific probes required for HLA typing. The flow analyzer LABScan100 that combines the

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flow cytometry and reverse SSO technology has a trend to replace three conventional methods, SSO, SSP and SBT (sequence-based typing, direct sequencing), in HLA typing.

On a suspension platform, multiple types of color-coded beads conjugated with SSO probes specifically bind to the single stranded DNA. Each type of bead has its unique spectral characteristics due to the different amount of fluorescent dye conjugated to the beads. When beads pass through a flow cytometer, the difference in the light scattering pattern from various angles can distinguish HLA genotypes.

Currently, LabType TM SSO is a relatively more mature technique compared with others in HLA typing. Its unique advantage is that thousands of molecules can be simultaneously analyzed in a matter of seconds. Therefore, this technique can be used for a large-scale analysis. Overall, this technique has following main advantages. (1) It has increased accuracy due to the automated detection system. (2) The workload and reagent consumption are reduced. One reaction tube can have 100 different SSO probes, thus greatly reducing the workload and reagent consumption. (3) It produces rapid and objective results. The ambiguous results can be avoided with Specialty Probe Technology TM (SP Technology). (4) Unlike regular flow cytometry that requires fresh samples, this technique can examine the sample at any time upon request or retrospectively. DNA samples can be analyzed right after extraction or stored at –20oC for more than 1 year without affecting the results. (5) The technique can analyze multiple HLA loci with low, medium and high resolutions. (6) It can be used in laboratories with large or small sample size. More than 100 probes can be put in one test tube for one sample or in a 96-well plate for 96 samples. The analysis of 96 samples takes less than 90 min after amplification. (7) The pollution to the environment and potential harm to the staff are reduced because electrophoresis is not required in this method.

9. Gene chip or DNA microarray

In gene chip or DNA microarray, large amount of probe molecules (usually $6x10^4$ molecules/cm^2) are attached to a solid surface. Labeled DNA samples are hybridized to the probes. The amount and sequence information of the target can be determined by the intensity of the hybridization signal. Gene chip or DNA microarray technology was first developed by Affymetrix in the USA, and has been improved significantly within a few years. The technology is based on the principle of reverse dot hybridization. Thousands of oligonucleotide probes representing different genes are spotted on a solid surface by a robot. These probes will bind to radioactive isotope or fluorescent dye labeled DNA or cDNA through complementary sequences. After autoradiography or fluorescence detection, signals are processed and analyzed by computer software. The intensity and distribution of hybridization signal reflect the expression level of the gene in the sample. The operation process of microarray is shown in Fig 9. Balazs *et al* spotted amplified DNA samples on silicon chips and compared the microarray results with PCR-SSO results in 768 specimens. It has been found that microarray has a high sensitivity and specificity. The consistent rate of genotyping results from microarray and PCR-SSO is 99.9%.

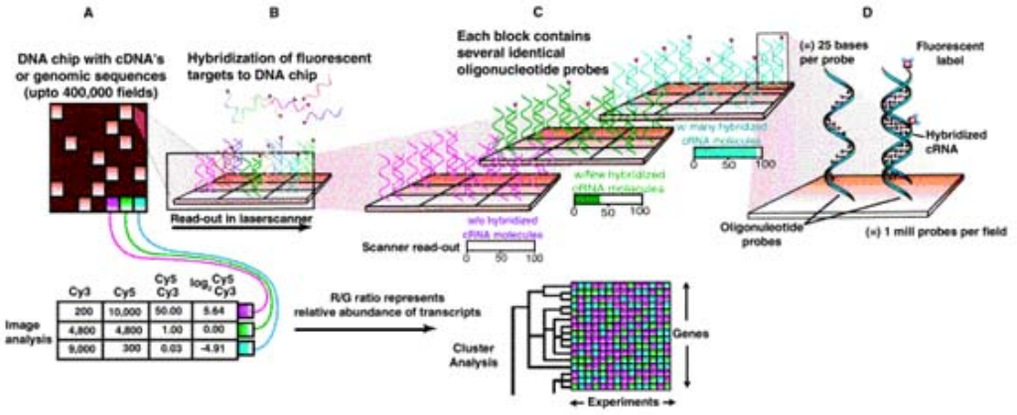

Figure 9. The procedure of gene chip/microarray analysis

Compared with existing genotyping methods, gene chip or microarray has the following advantages. (1) high intensity; The dot intensity on a chip can be higher than $6x10^4/cm^2$. Therefore, probes to thousands of HLA-A, B, C, DR, DQ and DP sequences can be spotted on a tiny chip of several square centimeters to obtain the information of individual HLA genes simultaneously. (2) high resolution; It can obtain information at the allele level. (3) simple operation; The results are generated by fluorescence scanning instead of gel electrophoresis, which greatly simplifies the procedure and shortens operation time. (4) high sensitivity; Signals are amplified twice with first PCR amplification of the template DNA and second amplification of fluorescence signal. Therefore, the sensitivity is greatly improved. (5) high accuracy; The intensity of the fluorescent signal generated by the perfect pairing of the probe and the sample is 5 to 35 times higher than the signal generated by the probe and the sample with one or two mismatched nucleotide. Accurate detection of fluorescent signal intensity is the basis of the detection specificity. Studies have shown that the consistency between microarray and Sanger sequencing in the detection of mutations and polymorphism is 99.9%. (6) high efficiency; The whole process is highly automatic, which saves manpower and time for data analysis. Genotyping of genes such as HLA-A, B, DR and DQ in multiple samples can be done with one PCR reaction and hybridization on one chip. (7) high level of standardization; Using a variety of multi-point synchronized hybridization and automated analysis, the human error is minimized to ensure the specificity and objectivity. (8) low cost. Since the chip fabrication and signal detection are all automatic, only small amount of probes and samples are required. One chip can be used for the analysis of samples from multiple individuals, which further reduces the cost. The biggest drawback of microarray analysis is its expensive equipment, which prevents it from becoming widely used. Only institutions with a large program can afford the equipment.

10. DNA sequencing technology

For the analysis of gene structure, sequencing is the most direct and accurate method. In this case, the DNA fragment is amplified by PCR and followed by sequencing. The basic process

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of this method is shown in Figure 10. Since the entire nucleotide sequence of the amplified fragment is obtained, this is the most reliable and through genotyping method. It can not only identify the sequence and genotype, but also lead to the discovery of new genotypes. Currently, the newly identified HLA alleles can only be verified by sequencing. It has been reported that if the HLA type cannot be determined by serotyping or the results from PCR-SSP and PCR-SSOP are inconsistent, sequence-based typing (SBT) often can yield accurate and reliable results with a high resolution. Hurley *et al* have typed HLA alleles by PCR-STB in 1775 bone marrow transplant patients and unrelated donors in NMDP, USA. The study has found that the degree of HLA allele mismatching between the recipient and donor of bone marrow transplantation is much higher than previously thought after examining the antigen matching results of HLA-A, HLA-B and HLA-DR.

The advantage of SBT over PCR-SSP and PCR-SSOP is its ability to analyze the entire gene sequence including the non-polymorphic region. SBT can be used not only for DNA sequencing but also for cDNA sequencing to determine gene expression. With increasing popularity of DNA sequencing technology, the PCR-SBT method has gained much attention for genotyping. PCR-SBT has advantages over other typing methods in terms of accuracy, efficiency and the degree of automation. Specialized software and solid phase sequencing kits with automatic loading are available for HLA typing. In addition, the cost of DNA sequencing has been greatly reduced. Therefore, PCR-SBT is an ideal method for HLA typing in researches. With the further decrease in the cost of automatic sequencing, this genotyping method will be widely used.

Currently, PCR-SBT is the gold standard of HLA typing. This method has several advantages. (1) It can accurately determine gene type in the exon 8 by a high-resolution sequencing, sufficient to meet the need in researches and clinics. (2) It can analyze more than 15,000 samples every month with high throughput detection. (3) Automated SOP and advanced data management system can reduce human error. (4) It has high quality assurance. Ten percent blind samples are used repeatedly as internal quality control and 100% accuracy is achieved for 10 consecutive times using UCLA external quality assurance samples. The results are confirmed by SSP. (5). It may lead to the discovery of new alleles. (6) HLA genotype can be updated by re-analyzing the sequence after the HLA database is updated.

11. HLA matchmaker

In organ transplantation, the degree of matching is generally determined by counting the number of mismatched HLA-A, B, DR antigens of the donor. It is well known that the zero-antigen mismatches have the highest success rates but why do so many mismatched transplants do so well? The answer to this question may be related to the fact that antibodies produced against HLA mismatches are significant risk factors for transplant failure. An important consideration is that HLA antigens have multiple epitopes that can be recognized by specific antibodies. The original description of the epitope repertoire was based on serological cross-reactivity between HLA antigens and antibody specificities against so-called

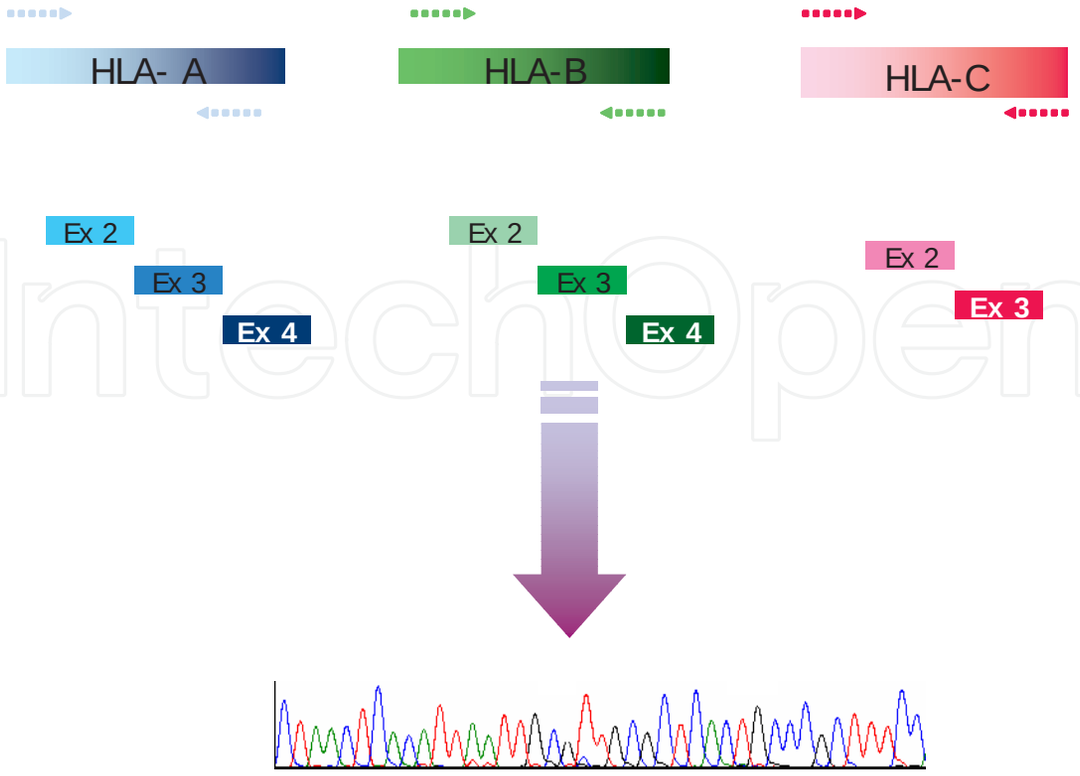

Figure 10. The diagram of DNA sequencing

private and public determinants. Elucidation of three-dimensional molecular structures and amino acid sequence differences between HLA antigens has made it possible to define the structural basis of HLA epitopes. The general concept is that HLA epitopes are determined by polymorphic amino acid residues on the molecular surface. Three-dimensional modeling of HLA antigens has revealed many clusters of polymorphic residues. In spite of this highly complex polymorphism it has become possible to determine HLA compatibility at the structural level.

HLA MATCHMAKER is a computer algorithm that assesses human leukocyte antigen (HLA) compatibility at a structural level by intralocus and interlocus comparisons of polymorphic amino acid sequences of HLA molecules. In its first version, each HLA antigen was seen as a chain of short, lineal sequences of polymorphic amino acids in an antibody-binding position (triplets); these triplets are considered the key elements of epitopes able to induce specific antibody production. The most recent version—Eplets HLA Matchmaker—introduces the concept of sequences of polymorphic amino acids in discontinuous positions that create on the surface of the HLA molecule conformational epitopes. The eplet version provides a broader repertoire of structural defined HLA epitopes and may provide a more accurate evaluation of the HLA compatibility.

HLA Matchmaker is based on the following principles. First, each HLA antigen is represented by different chains of epitopes structurally defined as potential immunogenic particles capable of inducing specific antibody production. Second, patients cannot produce antibodies against

epitopes present on their own HLA molecules. Initially, the program was developed to increase the chances of finding acceptable donors for hypersensitized patients. Subsequently, Duquesnoy et al demonstrated that it might also be useful in predicting the risk of graft loss according to the number of HLA-I mismatch triplets. This was proved in sensitized and nonsensitized patients. Haririan et al also showed that this triplet compatibility could give information about renal graft outcome in African-American patients. Nevertheless, Laux et al based on their own studies questioned the predictive role of triplet compatibility in graft survival. Other authors have also questioned the consistency of the epitopes in which this algorithm is based, pointing out that they might not be the unique epitopes inducing antibody formation. HLA Matchmaker has also been evaluated for clinical use in the selection of donors in pediatric renal desensitized receptors and HLA allosensitized thrombocytopenic patients. It has also been applied in unrelated bone marrow transplantation, lacking definitive proof of its benefit in patient survival.

12. HistoCheck

When there is no genotypically identical sibling and there are several alternative potential donors that all have a mismatch at an HLA class I or II locus, the allogenicity of mismatches may be estimated using the Sequence Similarity Matching concept described by our working group. In this concept the amino acid differences between HLA alleles are evaluated and rated with regard to position within the molecule (peptide binding, contact with the T-cell receptor) and with regard to functional similarity of amino acids within proteins. This procedure led to a dissimilarity score (allogenicity index) whereby high values represent high dissimilarity. When there are several mismatched donors, dissimilarity scores may be calculated for any of them, and the donor with the least may be preferred.

The importance of HLA-DPB1 matching for the outcome of allogeneic hematologic stem cell (HSC) transplantation is controversial. Previous findings identified HLA-DPB1 alleles as targets of cytoxic T cells mediating in vivo rejection of an HSC allograft. These HLA-DPB1 alleles encode T-Cell epitopes shared by a subset of HLA-DPB1 alleles that determine non-permissive mismatches for HSC transplantation. Retrospective evaluation of transplantations showed that the presence of non-permissive HLA-DPB1 mismatches was correlated with significantly increased hazards of acute grade II to IV graft-versus-host disease and transplantation-related mortality but not relapse as compared with the permissive group. Based on these findings, an algorithm for prediction of non-permissive HLA-DPB1 mismatches was developed (details in http://www.mh-hannover.de/institute/transfusion/histocheck/).

13. Conclusion

Currently, PCR-SSP genotyping is a commonly used method for HLA typing in clinical laboratories worldwide. Like SSP method, PCR-SSP method depends on specific primers for

genotyping. Although the process is simple and rapid, high-resolution genotyping requires a large number of sequence specific primers, which leads to a high cost and prolonged operation time. Similarly, SSO technique is based on the sequence-specific oligonucleotide probes. High-resolution genotyping by SSO significantly increases the cost and complexity. Therefore, it is rarely used for HLA typing today. PCR-SNP is a simple and fast method with a high resolution. PCR-SNP is expected to become more popular in HLA typing as the technology continues to improve. Although RSCA and Pyrosequencing can achieve high-resolution results, their applications in HLA typing will be gradually eliminated as the technology of gene chip and sequencing continues to improve and the cost continues to decrease. HLA-chip genotyping is still largely dependent on the known sequence. It cannot identify new alleles with unknown sequence. At this moment, PCR-SBT technology has significant advantages over other HLA typing methods in terms of accuracy, efficiency and automation. There are specialized software and automatically loaded sequencing reagents for HLA typing by PCR-SBT. In addition, the operation cost has been greatly reduced. In conclusion, PCR-SBT technology with HLA-chip is the best method for HLA typing in research. With the reduction in the cost of automated nucleic acid sequencing, this genotyping method will be widely used in the field of basic research as well as in clinical transplantation.

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References

[1] Erlich HA, Opelz G, Hansen J: HLA DNA typing and transplantation. Immunity 2001, 14:347–356

[2] Methilinos J. HLA testing: the state of the art of genomic methods in 1996. Nephrol Dial Transplant 1996;11:2129-34.

[3] Opelz G, Methilinos J, Scherer S, et al. Survival of DNA-DR typed and matched cadaver kidney transplants. Lancet 1991;338:461-3.

[4] Otten HG, Tilanus MG, et al. Serology versus PCR-SSP in typing for HLA-DR and DQ: a practical evaluation. Tissue Antigens 1995;45:36-40.

[5] Bozon MV, Delgado JC, Selvakuman A, et al. Error rate for HLA-B antigen assignment by serology: implications for proficiency testing and utilization of DNA-based typing methods. Tissue Antigen 1997;50:387-94.

[6] Schrender GM, Hurley CK, March SG, et al. The HLA Dictionary: a summary of HLA-A, B, C DRB1 13, 14, 15, DQB1 alleles and their association with serologically defined HLA-A-B-C DR and DQ antigens. Tissue Antigens 1999;54:409-37.

[7] Opelz G, Wujciak T, Döhler B, Scherer S, Mytilineos J. HLA compatibility and organ transplant survival. Collaborative Transplant Study. Rev Immunogenet. 1999;1:334-42.

[8] Mahdi BM. A glow of HLA typing in organ transplantation. Clin Transl Med. 2013;2:6.

[9] Elsner HA, Blasczyk R. Sequence similarity matching: proposal of a structure-based rating system for bone marrow transplantation. Eur J Immunogenet 2002; 29: 229-236.

[10] Elsner HA, DeLuca D, Strub J,Blasczyk R. HistoCheck: rating of HLA class I and II mismatches by an internet-based software tool. Bone Marrow Transplant. 2004; 33: 165-169.

[11] Zino E, Frumento G, Marktel S, Sormani MP, Ficara F, Di Terlizzi S, Parodi AM, Sergeant R, Martinetti M, Bontadini A, Bonifazi F, Lisini D, Mazzi B, Rossini S, Servida P, Ciceri F, Bonini C, Lanino E, Bandini G, Locatelli F, Apperley J, Bacigalupo A, Ferrara GB, Bordignon C, Fleischhauer K. A T-cell epitope encoded by a subset of HLA-DPB1 alleles determines nonpermissive mismatches for hematologic stem cell transplantation. Blood. 2004 Feb 15; 103(4):1417-24.

[12] Crocchiolo R, Zino E, Vago L, Oneto R, Bruno B, Pollichieni S, Sacchi N, Sormani MP, Marcon J, Lamparelli T, Fanin R, Garbarino L, Miotti V, Bandini G, Bosi A, Ciceri F, Bacigalupo A, Fleischhauer K; Gruppo Italiano Trapianto di Midollo Osseo, Cellule Staminale Ematopoietiche (CSE) e Terapia Cellulare; Italian Bone Marrow Donor Registry. Nonpermissive HLA-DPB1 disparity is a significant independent risk factor for mortality after unrelated hematopoietic stem cell transplantation. Blood. 2009 Aug 13; 114(7):1437-44.

[13] Fleischhauer K, Locatelli F, Zecca M, Orofino MG, Giardini C, De Stefano P, Pession A, Iannone AM, Carcassi C, Zino E, La Nasa G. Graft rejection after unrelated donor hematopoietic stem cell transplantation for thalassemia is associated with nonper-

missive HLA-DPB1 disparity in host-versus-graft direction.Blood. 2006 Apr 1; 107(7): 2984-92.

[14] Zino E, Vago L, Di Terlizzi S, Mazzi B, Zito L, Sironi E, Rossini S, Bonini C, Ciceri F, Roncarolo MG, Bordignon C, Fleischhauer K. Frequency and targeted detection of HLA-DPB1 T cell epitope disparities relevant in unrelated hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2007 Sep; 13(9):1031-40.

[15] Urlacher A, Dormoy A, Tongio MM. DP epitope mapping by using T-cell clones. Hum Immunol. 1992; 35:100-108.

[16] Cesbron A, Moreau P, Milpied N, Harousseau JL,Muller JY, Bignon JD. Crucial role of the third and fourth hypervariable regions of HLA-DPB1 allelic sequences in the mixed lymphocyte reaction. Hum Immunol. 1992; 33:202-207.

[17] Naruse TK, Nose Y, Kagiya M, et al. Cloned primed lymphocyte test cells recognize the fourth, fifth, and sixth hypervariable regions at amino acid positions 65-87 of the DPB1 molecule. Hum Immunol. 1995; 42:123-130.

[18] Brown JH, Jardetzky TS, Gorga JC, Stern LJ, Urban RG, Strominger JL, Wiley DC. Three-dimensional structure of the human class II histocompatibility antigen HLA-DR1. Nature 1993; 364: 33-9.

[19] Garboczi DN, Ghosh P, Utz U, Fan QR, Biddison WE, Wiley DC. Structure of the complex between human T-cell receptor, viral peptide and HLA-A2. Nature 1996; 384: 134-41.

[20] Hennecke J, Carfi A, Wiley DC. Structure of a covalently stabilized complex of a human alphabeta T-cell receptor, influenza HA peptide and MHC class II molecule, HLA-DR1. EMBO J 2000; 19: 5611-24.

[21] Risler JL, Delorme MO, Delacroix H, Henaut A. Amino acid substitutions in structurally related proteins. A pattern recognition approach. Determination of a new and efficient scoring matrix. J Mol Biol 1988; 204: 1019-29.

[22] Saper MA, Bjorkman, PJ, Wiley DC. Refined structure of the human histocompatibility antigen HLA-A2 at 2.6 Å resolution. J Mol Biol 1991; 219: 277-319.

[23] Sayle RA, Milner-White EJ. RASMOL: biomolecular graphics for all. Trends Biochem Sci. 1995; 20: 374.

[24] Stern LJ, Brown JH, Jardetzky TS, Gorga JC, Urban RG, Strominger JL, Wiley DC. Crystal structure of the human class II MHC protein HLA-DR1 complex with an influenza virus peptide. Nature 1994; 368: 215-21.

[25] Opelz G, Terasaki PI. International study of histocompatibility in renal transplantation. Transplantation. 1982, 33:87-95.

[26] Marsh SGE, Albert ED, Bodmer WF, et al. Nomenclature for factors of the HLA system, 2010. Tissue Antigens. 2010, 75:291-455.

[27] http://hla.alleles.org/

[28] Hansen JA, Petersdorf EW, Lin MT, Wang S, Chien JW, Storer B, Martin PJ. Genetics of allogeneic hematopoietic cell transplantation. Role of HLA matching, functional variation in immune response genes. Immunol Res. 2008, 41:56-78.

[29] Rubinstein P. HLA matching for bone marrow transplantation: How much is enough? N Engl J Med. 2001, 345: 1842-1844.

[30] Petersdorf EW, Hansen JA, Martin PJ, Woolfrey A, Malkki M, Gooley T, Storer B, Mickelson E, Smith A, Anasetti C. Major histocompatibility complex class I alleles and antigens in hematopoietic2cell transplantatio. N Engl J Med. 2001, 345:1794-1800.

[31] Petersdorf EW, Kollman C, Hurley CK, Dupont B, Nademanee A, Begovich AB, Weisdorf D, McGlave P. Effect of HLA class II gene disparity on clinical outcome in unrelated donor hematopoietic cell transplantation for chronic myeloid leukemia: the US national marrow donor program experience. Blood. 2001, 98:2922.

[32] McGlave PB, Shu XO, Wen W, Anasetti C, Nademanee A, Champlin R, Antin JH, Kernan NA, King R, Weisdorf DJ. Unrelated donor marrow transplantation for chronic myelogenous leukemia: 9 years' experience of the national marrow donor program. Blood. 2000, 95:2219-2225.

[33] Hadhoud A, Abdulaziz AM, Menawi LA, Shaheen FA, Abdulghaffar A, Abas FA, Al Mobrak MF. The relationship between HLA typing and HCV infection and outcome of renal transplantation in HCV positive patients. Exp Clin Transplant. 2003, 1:19-25.

[34] Erlich HA, Opelz G, Hansen J. HLA DNA typing and translation. Immunity, 2001, 14: 347- 356.

[35] Brown M, Wittwer C. Flow cytometry: principles and clinical application in hematology. Clin Chem. 2000, 46:1221-1229.

[36] Terasaki PI , Cho Y, Takemoto S et al. Twenty-year follow-up on the effect of HLA matching on kidney transplant survival and prediction of future twenty year survival. Transplant Proc. 1996, 28:1144-1145.

[37] Takemoto S, Terasaki PI. HLA compatibility can be predicted by matching only three residues with outward oriented sidechains.Transplant Proc. 1996, 28:1264-1266.

[38] Takemoto SK. HLA amino acid residue matching. Clin Transpl. 1996, 28:397-425.

[39] Takemoto SK, Terasaki PI, Gjertson DW,et al. Twelve years'experience with national sharing of HLA-matched cadaveric kidneys for transplantation. N Engl J Med. 2000,343: 1078-1084.

[40] Hata Y, Cecka JM, Takemoto S, et al. Effects of changes in the criteria for nationally shared kidney transplants for HLA-matched patients. Transplantation. 1998, 65: 208-212.

[41] Takemoto S, Cecka JM, Terasaki PI. Benefits of HLA-CREG matching for sensitized recipients as illustrated in kidney regraftes. Transplant Proc. 1997, 29:1417-1418.

[42] Orofino MG, Argiolu F, Sanna MA, Rosatelli MC, Tuveri T, Scalas MT, Badiali M, Cossu P, Puddu R, Lai ME, Cao A. Fetal HLA typing in beta thalassaemia: implications for haemopoietic stem-cell transplantation. Lancet. 2003, 362: 41-42.

[43] Karabon L, Polak M, Pacuszko T, Karabon L, Polak M, Pacuszko T. HLA typing for donor-recipient matching in unrelated donor hematopotetic stem cell transplantation.Transplant Proc. 2002, 34: 668-670.

[44] Torío A, Moya-Quiles MR, Muro M, Montes-Ares O, Ontañon J, Minguela A, Marin L, Alemany JM, Sánchez-Bueno F, Alvarez-López MR. Discrepancies in HLA-C typing in transplantation: comparison of PCR-SSP and serology results. Transplant Proc. 2002, 34: 419-420.

[45] Arrieta A, Maruri N, Rinon M, Riñón M, Fernández JR, Ortiz de Urbina J, García Masdevall MD. Confirmation of graft-versus-host disease by HLA typing after liver transplantation. Transplant Proc. 2002, 34: 278-279.

[46] Olerup O, Zetter H. HLA-DR typing by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours: an alternation to serological DR typing in clinical practice including donor-recipient matching in cadaveric transplantation. Tissue Antigens. 1992, 39: 225.

[47] Takemoto S, Terasaki PI. Refinement of permissible HLA mismatches. In: Terasaki PI, Cecka JM, eds. Clinical Transplants. Los Angeles: UCLA Tissue Typing Laboratory. 1994, 451.

[48] Stern LJ, Brown JH, Jardetzky TS, Gorga JC, Urban RG, Strominger JL, Wiley DC. Crystal structure of the human class II MHC protein HLA-DR1 complexed with an influenza virus peptide. Nature. 1994, 368:215-221.

[49] Smith PA, Brunmark A, Jackson MR, Potter TA. Peptide-independent recognition by alloreactive cytotoxic T lymphocytes (CTL). J Exp Med, 1997, 185: 1023-1033.

[50] Viret C, Wong FS, Janeway CA Jr. Designing and maintaining the mature TCR repertoire: the continuum of self-peptide: self-MHC complex recognition. Immunity. 1999, 10: 559.

[51] The MHC sequencing consortium: complete sequence and the gene map of a human major histocompatibility complex. Nature. 1999, 401:921.

[52] Jordan F, McWhinnie AJ, Turner S, Gavira N, Calvert AA, Cleaver SA, Holman RH, Goldman JM, Madrigal JA. Comparison of HLA-DRB1 typing by DNA-RFLP, PCR-SSO and PCR-SSP methods and their application in providing matched unrelated donors for bone marrow transplantation. Tissue Antigens. 1995, 45, 103-110.

[53] Zetterquist H, Bengtsson M, Backstrom G, Bäckström G, Egle-Jansson I, Ekdahl AM, Grunnet N, Gustafsson I, Knutsen I, Kuhle A, Rydberg L, Spurkland A, Steffensen R,

Storgärds M, Szojmer E, Söderholm G, Thuresson B, Turesson H, Olerup O. Report from the HLA class II typing by PCR-SSP Multicentre Study. Eur J Immunogenet. 1997, 24:191-199.

[54] Bryan CF, Harrell KM, Nelson PW, Pierce GE, Ross G, Shield CF 3rd, Warady BA, Aeder MI, Helling TS, Landreneau MD, Luger AM. HLA-DR and DQ typing by polymerase chain reaction using sequence-specific primer mixes reduces the incidence of phenotypic homozygosity (blanks) over serology. Transplantation. 1996, 62: 1819-1824.

[55] Suzuki Y, Orita M, Shiraishi M, Hayashi K, Sekiya T. Detection of ras gene mutations in human lung cancers by single-strand conformation polymorphism analysis of polymerase chain reaction products. Oncogene. 1990, 5:1037-1043.

[56] Hoshino S, Kimura A ,Fukuda Y, Dohi K, Sasazuki T. Polymerase chain reaction-single strand conformation polymorphism analysis of polymorphism in DPA1 and DPB1 genes :A simple ,economical and rapid method for histocompatibility testing. Hum Immunol. 1992, 33, 98-107.

[57] Pursall MC, Clay TM, Bidwell JL. Combined PCR-heteroduplex and PCR-SSCP analysis for matching of HLA-A, -B and -C allotypes in marrow transplantation. Eur J Immunogenet. 1996, 23: 41-53.

[58] Bradley BA, Bidwell JL. HLA-DQA1 and DQB1 genotyping by PCR-RFLP, heteroduplex and homoduplex analysis. Eur J Immunogenet, 1996. 23, 107-120.

[59] Saiki RK, Bugawan TL, Horn GT, ET AL. Analysis of enzymatically amplified beta-globin and HLA-DQ alpha DNA with allele-specific oligonucleotide probes. Nature. 1986, 324:163-166.

[60] Mickelson E, Smith A, McKinney S, Anderson G, Hansen JA. A comparative study of HLA-DRB1 typing by standard serology and hybridization of non-radioactive sequence-specific oligonucleotide probes to PCR-amplified DNA. Tissue Antigens. 1993, 41:86-93.

[61] Guo Z, Gatterman MS, Hood L, Hansen JA, Petersdorf EW. Oligonucleotide arrays for high-throughput SNPs detection in the MHC class I genes: HLA-B as a model system. Genome Res. 2002, 12:447-457.

[62] Consolandi C, Frosini A, Pera C,et al. Polymorphism analysis within the HLA-A locus by universal oligonucleotide array.Hum Mutat. 2004, 24:428-434.

[63] Li AX, Seul M, Cicciarelli J, et al. Multiplexed analysis of polymorphisms in the HLA gene complex using bead array chips.Tissue Antigens. 2004,63:518-528.

[64] Arguello JR, Little AM, Pay AL, et al. Mutation detection and typing of polymorphic loci through double-strand conformation analysis. Nature Genetics. 1998, 18:192-194.

[65] Arguello JR, Little AM, Bohan E, et al. A high resolution HLA class I and class II matching method for bone marrow donor selection. Bone Marrow Transplantation. 1998, 22:527-534.

[66] Lenz TL, Eizaguirre C, Becker S, Reusch TB. RSCA genotyping of MHC for high-throughput evolutionary studies in the model organism three-spined stickleback Gasterosteus aculeatus. BMC Evol Biol. 2009, 9:57.

[67] Sun Y, Zhao D, Jin L, Liu N, Liang F, Kong F, Xi Y. Human leukocyte antigens A and B Loci genotyping by reference strand-mediated conformation analysis in hematopoietic stem cell transplantation donor selection. Int J Hematol. 2007, 86:77-83.

[68] Shibata H, Yasunami M, Obuchi N, Takahashi M, Kobayashi Y, Numano F, Kimura A. Direct determination of single nucleotide polymorphism haplotype of NFKBIL1 promoter polymorphism by DNA conformation analysis and its application to association study of chronic inflammatory diseases. Hum Immunol. 2006, 67:363-73

[69] Kennedy LJ, Quarmby S, Fretwell N, Martin AJ, Jones PG, Jones CA, Ollier WE. High-resolution characterization of the canine DLA-DRB1 locus using reference strand-mediated conformational analysis. J Hered. 2005, 96:836-842.

[70] Ronaghi M, Elahi E. Pyrosequencing for microbial typing. J Chromatogr B. 2002, 782:67-72.

[71] Díaz S, Echeverría MG, It V, Posik DM, Rogberg-Muñoz A, Pena NL, Peral-García P, Vega-Pla JL, Giovambattista G. Development of an ELA-DRA gene typing method based on pyrosequencing technology. Tissue Antigens. 2008,72:464-468.

[72] Lank SM, Golbach BA, Creager HM, Wiseman RW, Keskin DB, Reinherz EL, Brusic V, O'Connor DH. Ultra-high resolution HLA genotyping and allele discovery by highly multiplexed cDNA amplicon pyrosequencing. BMC Genomics. 2012, 13(1): 378.

[73] Lank SM, Wiseman RW, Dudley DM, O'Connor DH. A novel single cDNA amplicon pyrosequencing method for high-throughput, cost-effective sequence-based HLA class I genotyping. Hum Immunol. 2010, 71:1011-1017.

[74] Gabriel C, Danzer M, Hackl C, Kopal G, Hufnagl P, Hofer K, Polin H, Stabentheiner S, Pröll J. Rapid high-throughput human leukocyte antigen typing by massively parallel pyrosequencing for high-resolution allele identification. Hum Immunol. 2009, 70: 960-964.

[75] Lu Y, Boehm J, Nichol L, Trucco M, Ringquist S. Multiplex HLA-typing by pyrosequencing. Methods Mol Biol. 2009, 496:89-114.

[76] Ringquist S, Styche A, Rudert WA, Trucco M. Pyrosequencing-based strategies for improved allele typing of human leukocyte antigen loci. Methods Mol Biol. 2007, 373:115-134.

[77] Loginova MA, Paramonov IV, Trofimova NP. Experience in using the LABType SSO reagent kits in the practice of a HLA typing laboratory. Klin Lab Diagn. 2011, 4:45-47.

[78] Testi M, Iannelli S, Testa G, Troiano M, Capelli S, Fruet F, Federici G, Bontadini A, Andreani M. Evaluation of DRB1 high resolution typing by a new SSO-based Luminex method. Mol Biol Rep. 2012, 39:13-16.

[79] Feng C, Putonti C, Zhang M, Eggers R, Mitra R, Hogan M, Jayaraman K, Fofanov Y. Ultraspecific probes for high throughput HLA typing. BMC Genomics. 2009, 10:85.

[80] Lee KR, Park E, Moon SH, Kim JM, Kwon OJ, Kim MH, Sohn YH, Ko SY, Oh HB. Development and clinical evaluation of a microarray for HLA-A and -DRB1 genotyping. Tissue Antigens. 2008, 72:568-577.

[81] Consolandi C. High-throughput multiplex HLA-typing by ligase detection reaction (LDR) and universal array (UA) approach. Methods Mol Biol. 2009, 496:115-127.

[82] Consolandi C, Frosini A, Pera C, Ferrara GB, Bordoni R, Castiglioni B, Rizzi E, Mezzelani A, Bernardi LR, De Bellis G, Battaglia C. Polymorphism analysis within the HLA-A locus by universal oligonucleotide array. Hum Mutat. 2004, 24:428-434.

[83] Pröll J, Danzer M, Stabentheiner S, Niklas N, Hackl C, Hofer K, Atzmüller S, Hufnagl P, Gülly C, Hauser H, Krieger O, Gabriel C. Sequence capture and next generation resequencing of the MHC region highlights potential transplantation determinants in HLA identical haematopoietic stem cell transplantation. DNA Res. 2011, 18:201-210.

[84] Noreen HJ, Trachtenberg E, Williams TM, Baxter-Lowe LA, Begovich AB, Petersdorf E, Selvakumar A, Stastny P, Hegland J, Hartzman RJ, Carston M, Gandham S, Kollman C, Nelson G, Spellman S, Setterholm M. A high degree of HLA disparity arises from limited allelie diversity: analysis of 1775 unrelated bone marrow transplant donor-recipient pairs. Hum mmunol. 2007, 68: 30-40

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