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Differential immunogenicity of HLA mismatches in clinical transplantation

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Abstract

Although HLA matching is beneficial in clinical transplantation, it is not feasible to select a completely HLA matched donor for every potential recipient because of the enormous polymorphism of the HLA system. As a consequence, the majority of the recipients will be transplanted with a mismatched donor organ or hematopoietic stem cell transplant. For this large group of patients it is important to take advantage of the differential immunogenicity of HLA mismatches and to select for them a donor with HLA mismatches of low immunogenicity, the so-called acceptable mismatches. The differential immunogenicity of HLA mismatches can be determined by either retrospective analysis of graft survival data or by in vitro assays measuring T-cell and B-cell alloreactivity.

A recently developed computer algorithm (HLAMatchmaker) can be instrumental in selecting donors with HLA mismatches, which do not lead to alloantibody formation. The theoretical background and practical implications of this acceptable mismatch approach are discussed.

Keywords: Differential immunogenicity; HLA mismatches; Clinical transplantation

1. Introduction

Transplantation in the absence of immunosuppressive drugs is only possible if donor and recipient are monozygotic twins. In all other situations, confrontation with allogeneic cells or tissues will lead to both humoral and cellular immune responses, which are directly responsible for complications such as graft rejection [1] and, in case of haematopoietic stem cell transplantation, graft-versus-host disease [2].

These detrimental immune responses are mainly directed at the allogeneic HLA molecules but also minor histocompatibility antigens may serve as targets for these alloimmune reactions [3].

Considering the dominance of the major histocompatibility antigens, selection of an HLA identical sibling- or HLA identical unrelated donor may prevent a severe alloimmune response as suggested by the beneficial effect of HLA matching in clinical transplantation. As the genes coding for the HLA molecules are clustered on the short arm of chromosome 6 and are often inherited as a fixed haplotype, the chance to find a completely HLA identical family donor is about 25–30 %. However, finding a donor is far more difficult for patients, who do not have a suitable family donor.

The enormous polymorphism of the HLA system makes it impossible to find an HLA identical unrelated donor for every patient. The recent introduction of molecular typing techniques has led to a more reliable HLA typing but has increased the complexity of the HLA system as well.

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Several of the serologically defined HLA antigens can now be subdivided in many different alleles, for instance HLA-A2, for which more than 50 different alleles have been described [4].

The practical implications of the complexity of the HLA system for the selection of HLA matched unrelated donors are well illustrated by the international efforts to establish a large pool of potential hematopoietic stem cell donors. Even with more than 9 million HLA typed potential donors available, it is impossible for many patients in need for a bone marrow or hematopoietic stem cell transplantation, and especially for non-Caucasoid patients with rare HLA types, to find a well-matched donor [5].

In this perspective, it is not realistic to aim at a completely HLA matched unrelated organ donor for most of the patients on the waiting list.

2. Alternative strategies for donor selection

It is clear that most of the patients will be transplanted with an HLA mismatched donor, while early acute graft rejection will be prevented by the current potent immunosuppressive therapy. However, even in the presence of these immunosuppressive drugs, it is important to minimize the degree of HLA incompatibility as this will result in a better graft survival and provide the possibility to taper the immunosuppressive treatment.

Recent data suggest that the immunogenicity of HLA mismatches may differ and that some HLA mismatches, the so-called acceptable mismatches, are hardly recognized by the immune system of the recipient. This knowledge should lead to a different strategy for donor selection. In the past donor selection was based on structural matching (donor and recipient must have exactly the same HLA molecules), while future donor selection should be based on functional matching (the immune system of the recipient should not, or only weakly, react to the donor).

There are essentially two approaches to determine which donor/recipient combinations are likely to meet the criteria of a functional match.

One is based on population studies where the immunogenicity of specific HLA mismatches is determined by retrospective analyses of graft survival data. The other is a more individual approach, where the alloimmune repertoire of the patient is monitored in vitro with functional assays that are predictive for the in vivo immune response by alloreactive T and B lymphocytes.

3. The identification of acceptable and taboo mismatches in clinical kidney transplantation

Retrospective analyses of graft survival data showed that the HLA phenotype of the recipient, and especially the HLA-DR antigens, may be predictive for the strength of an alloimmune response.

HLA-DR6 positive recipients have been described to be high responders as they are more likely to reject HLA mismatched kidney grafts [6] whereas HLA-DR1 positive recipients have been suggested to be low responders [7].

Similarly, certain HLA-DR mismatches in the donor, such as HLA-DR6 have shown to be less immunogenic than other ones [8].

However, such association studies focussing on only a recipient or a donor antigen are too simplistic and do not take into consideration that a specific immune response is the product of the interaction between the immune system of the recipient and the specific target antigen involved. For this reason, the immunogenicity of an HLA mismatch should always be considered in the context of the patients own HLA antigens. On basis of this principle, Maruya et al. [9] were able to define acceptable or, as they call them, permissible HLA class I mismatches. Donor–recipient combinations with permissible mismatches had a similar graft survival as completely HLA identical grafts.

On the other hand Doxiadis et al. [10] could demonstrate that certain HLA class I mismatches are highly immunogenic in patients with some HLA phenotypes and not in patients with another phenotype.

For instance graft survival of kidneys with a single HLA-B7 mismatch was significantly lower in patients, who are HLA-A1 positive (45% after 5 years) compared to HLA-A1 negative recipients (75% after 5 years). These studies have led to the definition of several taboo mismatches, which are specific donor/recipient combinations that lead to a very poor graft survival and should be avoided.

It should be noticed that in these population studies, acceptable and taboo mismatches are defined by a better or poorer graft survival when groups of patients are compared (Fig. 1) These definitions do not have a direct implication for the individual patient. Patients with acceptable mis-

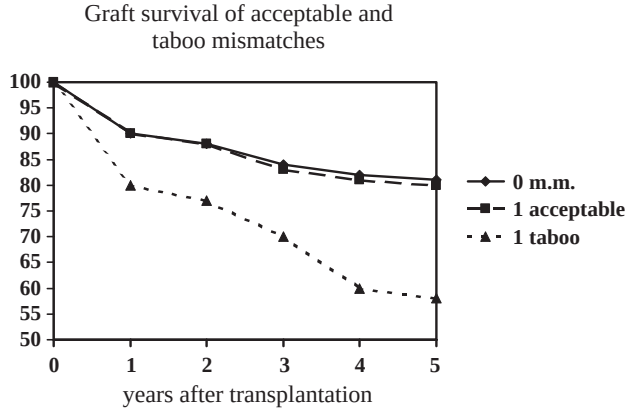

Fig. 1. Graft survival of kidneys from donors with a single HLA match qualified as taboo mismatch () is significantly poorer than that of HLA identical grafts. In contrast, survival of kidneys from donors with a single HLA mismatch not qualified as a taboo mismatch (i.e. acceptable mismatch (■)) has a similar graft survival as HLA identical donor kidneys.

matches may reject their grafts while patients with taboo mismatches may experience an excellent graft survival.

The immunological history of the individual patient, which plays a determinative role in shaping the immune repertoire, is not taken into account.

Prior exposure to a taboo mismatch in the form of a non-inherited maternal HLA antigen (NIMA) may have down regulated the immune repertoire of a child [11] while previous blood transfusions may have affected the alloimmune repertoire of the recipient in a similar way [12].

On the other hand, presensitization of the patient may lead to a more vigorous immune response to the acceptable mismatches. Here viral infections may play a role as well. Antiviral T-cell responses can have a direct impact on the alloreactive T-cell repertoire [13] confirming that T-cell alloreactivity against mismatched HLA antigens is based on crossreactivity of T cells recognizing foreign viral peptides in the context of self HLA molecules.

Therefore, retrospective population studies can give an indication of the chance that graft survival of a particular donor–recipient combination is good or bad, but other parameters are necessary to select the optimal HLA mismatched donor for an individual patient.

4. Inventory of the alloreactive T-cell repertoire

As T cells play a crucial role in early acute graft rejection, it is an alternative option to select an HLA mismatched donor on basis of a low T-cell alloreactivity of the patient. The classical assays to measure T-cell alloreactivity against HLA class I and class II mismatches. CML (Cell-Mediated-Lympholysis) and MLR (Mixed Lymphocyte Reactivity) are not really quantitative assays and have only a limited prognostic value for the in vivo situation [14,15].

With the introduction of limiting dilution assays, it became possible to quantify alloreactive T-cell precursor frequencies (CTLp; [16,17]. These assays revealed that different individuals have different T-cell precursor frequencies for the same HLA mismatches and every individual has different T-cell precursor frequencies against different HLA mismatches [18]. A clinical relevance for these assays is suggested by the fact that cytotoxic T-cell precursor frequencies against acceptable mismatches are significantly lower than CTLp against immunogenic HLA mismatches [19,20]. Several studies suggest that a very low CTLp frequency is predictive for a low incidence of graft-versus-host disease after HLA mismatched bone marrow transplantation [21,22] while a recent study in renal transplant recipients showed that successful tapering of immunosuppression is possible if the patient has very low donor specific CTLp frequencies. In case of high donor specific CTLp frequency rejection often occurred [23]. What is the reason that T-cell precursor frequencies against HLA alloantigens differ so much?

First of all, the number of epitopes mismatched between the target antigen and the recipients HLA antigens is a determinative factor. In case of only a limited number of amino acid mismatches and a similar peptide binding repertoire in the pocket, hardly any CTLps can be detected in, e.g. the combination of Cw*0303 versus Cw*0304 [24]. On the other hand, the degree of mimicry between the mismatched HLA antigen and the combination of autologous HLA molecules and viral peptides may play a determinative role. A classical example is the high frequency of CTLs directed against HLA-B14 and HLA-B35 in EBV infected HLA-B8 positive individuals [25].

Earlier exposure to the mismatched HLA antigen may influence the T-cell repertoire in two ways. It may either lead to activation of the T-cell repertoire and the formation of memory cells or to the induction of regulatory T cells, a topic which is discussed elsewhere in this volume.

With regard to presensitization, it may be useful to distinguish the contribution of primed versus naïve CTLs to the cytotoxic activity in the LDA assay.

Primed CTLs have a higher avidity for the target antigens and are resistant to anti-CD8 antibodies, whereas the reactivity of naïve CTLs is blocked by this antibody treatment [26]. Furthermore primed CTLs can be distinguished from naïve CTLs on basis of their resistance to Cyclosporine A [27].

The situation is far more complex when the options for a predictive assay for alloreactivity by $CD4^+$ T cells are considered. This is not surprising since different types of $CD4^+$ T cells may become activated in an allogeneic situation. Some of these T cells react to donor class II antigens on the basis of direct recognition, others recognize donor peptides in the context of self HLA class II, a phenomenon called indirect recognition. Furthermore, $CD4^+$ T cells may both function as helper cells (Th1/Th2) but also as regulatory cells.

Therefore it is obvious that the detection of acceptable mismatches for $CD4^+$ T cells is far more complicated than the detection of acceptable HLA class I antigens.

5. Inventory of the alloreactive B-cell repertoire

Besides to alloreactive T cells, alloantibodies may play a crucial role in graft rejection. While hyperacute rejection is due to preformed donor specific HLA antibodies [28], chronic graft rejection is often associated with HLA antibody formation as well [29]. It would be convenient to predict before transplantation that certain HLA mismatches can be considered as acceptable mismatches in order to limit the risk that the patient will form antibodies against the mismatched donor antigens.

HLA antigens that belong to a cross-reactive group (CREG) with the patient's own HLA antigens have a lower chance to induce alloantibodies [30] but transplantation with a donor carrying CREG mismatches is not a guarantee that

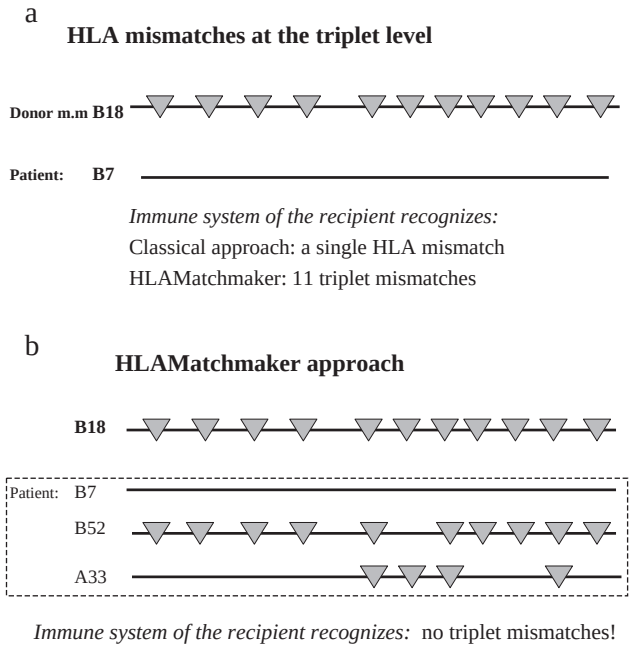

Fig. 2. (a) Classical view of the alloimmune response to a single mismatch (patient HLA-B7, donor HLA-B18): the patient will recognize HLA-B18 as a foreign antigen. HLAMatchmaker view: 11 triplet epitopes on HLA-B18 are potential targets for alloantibodies. (b) Principle of HLAMatchmaker is inter and intra-comparison of triplets. In this particular patients all triplets mismatched between HLA-B18 and HLA-B7 are shared by the other HLA alleles of the patient. Final interpretation: no foreign triplets on B18 and therefore no alloantibody induction.

no antibodies will be formed and certainly not a guarantee that no alloreactive T cells will become activated [31].

Similar to the in vitro tests for alloreactive T cells, limiting dilution assay has been established to quantify B lymphocytes that are able to produce specific antibodies against a particular HLA mismatches [32]. However, these assays are only successful in presensitised recipients, as precursor frequencies are only found for those HLA antigens toward which the patients had preformed HLA alloantibodies. They have no predictive value in non-immunized recipients, who receive a mismatched transplant.

A recently developed computer algorithm, taking advantage of the fact that the amino acid sequences of all HLA molecules are known, is more useful in this respect. The algorithm of this HLA matchmaker program [33] is based on the concept that immunogenic epitopes for B cells are represented by amino acid triplets on those parts of the HLA molecules that are accessible for antibodies. The principle of the program is that a patient does not make antibodies to self-triplets.

By both intra- and interlocus comparisons of triplets on the patients own HLA molecules and the mismatched HLA antigen, the program can predict the immunogenicity of a particular mismatch [34]. If no triplet mismatches are present, the patient is not supposed to make antibodies (Fig. 2). We have validated this theoretical concept by analysing the results of extensive antibody screening and crossmatch results, which are routinely performed in our laboratory to define acceptable mismatches for highly sensitized patients [35]. In case of donors with an HLA antigen mismatch but no triplet mismatches, antibodies were never detected. This program gives also an explanation why HLA-A2 recipients do not make antibodies against an HLA-A28 mismatch (no unique triplets on HLA-A28) whereas HLA-A28 positive recipients do make antibodies against HLA-A2 (unique triplets, not share by other HLA molecules) [36].

Serum analysis of highly sensitized patients has permitted the identification of highly immunogenic triplets that frequently induce specific antibodies whereas many other triplets do not appear immunogenic [37]. Mismatching for triplets with low immunogenicity has shown to increase the number of compatible platelet donors for refractory thrombocytopenic patients [38].

This HLAMatchmaker program explains and is useful to predict the presence of acceptable HLA mismatches. However, epitope differences between the mismatched HLA antigen and the HLA antigens of the patients are not the only prerequisite for the effective induction of alloantibodies. Although B lymphocytes are able to produce IgM antibodies on their own, $CD4^+$ T helper cells are needed for the induction of IgG alloantibodies.

Binding of allopeptides derived from the mismatched HLA molecule into the groove of the class II molecules from the patient is essential for the activation of these $CD4^+$ T helper cells, which by their interaction with B lymphocytes will induce a class switch from IgM to IgG alloantibodies.

As different HLA class II molecules can bind different peptides, antibodies against specific HLA mismatches are preferentially formed in patients with specific HLA-DR antigens, e.g. antibodies against the Bw4 epitopes are mainly found in HLA-DR1 and HLA-DR3 positive individuals [39].

6. Conclusion

Acceptable mismatches can be determined on the basis of retrospective data on graft survival and by analysis of in vitro T-cell reactivity while the HLA matchmaker algorithm is especially instrumental for determination of acceptable HLA mismatches with regard to the B alloimmune response.

Transplantation with HLA mismatches of low immunogenicity will lead to enhanced graft survival and a lower incidence of (highly) sensitized retransplant candidates.

References

[1] Opelz G, Wujciak T, Dohler B, Scherer S, Mytilineos J. HLA compatibility and organ transplant survival. Collaborative Transplant study. Rev Immunogenetics 1999;1(3):334–42.

[2] Mickelson EM, Petersdorf E, Anasetti C, Martin P, Woolfrey A, Hansen JA. HLA matching in hematopoietic cell transplantation. Hum Immunol 2000;61(2):92–100.
[3] Simpson E, Roopenian D, Goulmy E. Much ado about minor histocompatibility antigens. Immunol Today 1998;19(3):108–12.
[4] Robinson J, Waller MJ, Parham P, Bodmer JG, Marsh SG. IMGT/HLAdatabase—a sequence database for the human major histocompatibility complex. Tissue Antigens 2000;55(3):280–7.
[5] Oudshoorn M, Cornelissen JJ, Fibbe WE, de Graeff-Meeder ER, Lie JL, Schreuder GM, et al. Problems and possible solutions in finding an unrelated bone marrow donor. Results of consecutive searches for 240 Dutch patients. Bone Marrow Transplant 1997;20(12):1011–7.
[6] Hendriks GFJ, Schreuder GMTh, Claas FHJ, D'Amaro J, Persijn GG, Cohen B, et al. HLA-DRw6 and renal allograft rejection. Br Med J 1983;286(6359):85–7.
[7] Cook DJ, Cecka JM, Terasaki PI. HLA-DR1 recipients have the highest kidney graft survival. Transplant Proc 1987;18(1):675–7.
[8] Hendriks GFJ, D'Amaro J, Persijn GG, Schreuder GM, Lansbergen Q, Cohen B, et al. Excellent outcome after transplantation of renal allografts from HLA-DRw6 positive donors even in HLA-DR mismatches. Lancet 1983;2(8343):187–9.
[9] Maruya E, Takemoto S, Terasaki PI. HLA matching: identification of permissible HLA mismatches. Clin Transpl 1993511–20.
[10] Doxiadis IIN, Smits JMA, Schreuder GMTh, Persijn GG, van Houwelingen HC, van Rood JJ, et al. Association between specific HLA combinations and probability of kidney allograft loss: the taboo concept. Lancet 1996;348(9031):850–3.
[11] Van Rood JJ, Claas FHJ. Both self and non-inherited maternal HLA antigens influence the immune response. Immunol Today 2000;21(6):269–73.
[12] Roelen DL, Van Rood JJ, Brand A, Claas FHJ. Immunomodulation by blood transfusion. Vox Sang 2000;78(Suppl. 12):273–5.
[13] Burrows SR, Khanna R, Silims SL, Moss DJ. The influence of anti-viral T cell responses on the alloreactive repertoire. Immunol Today 1999;20(5):203–7.
[14] Goulmy E, Stijnen T, Groenewoud AF, Persijn GG, Blokland E, Pool J, et al. Renal transplant patients monitored by the cell-mediated lympholysis assay. Evaluation of its clinical value. Transplantation 1989;48(4):559–63.
[15] Reinsmoen NL, Kaufman D, Matas AJ, Sutherland D, Najarian JJ, Bach FH. A new in vitro approach to determine acquired tolerance in long term kidney allograft recipients. Transplantation 1990;50(5):783–90.
[16] Lindahl KF, Wilson DD. Histocompatibility antigen activated cytotoxic T lymphocytes: II. Estimates of the frequency and specificity of precursors. J Exp Med 1977;145(3):508–22.
[17] Theobald M, Nierle T, Bunjes D, Arnold R, Heimpel H. Host-specific interleukin-2 secreting donor T cell precursors as predictors of acute graft-versus host disease in bone marrow transplantation between HLA identical siblings. N Engl J Med 1992;327(23):1613–7.
[18] Zhang L, Li SG, Vandekerckhove B, Termijtelen A, Van Rood JJ, Claas FHJ. Analysis of cytotoxic T cell precursor frequencies directed against individual HLA-A and -B alloantigens. J Immunol Methods 1989;121(1):39–45.
[19] Roelen DL, Stobbe I, Young NT, van Bree SP, Doxiadis II, Oudshoorn M, et al. Permissible and immunogenic HLA-A mismatches: cytotoxic T-cell precursor frequencies reflect graft survival data. Hum Immunol 2001;62(7):661–7.
[20] Van der Meer A, Joosten I, Schattenberg AV, de Witte TJ, Allebes WA. Cytotoxic T-lymphocyte precursor frequency (CTLp-f) as a tool for distinguishing permissible from non-permissible class I mismatches in T-cell-depleted allogeneic bone marrow transplantation. Br J Haematol 2000;11(2):685–94.
[21] Kaminski E, Hows J, Man S, Brookes P, MacKinnon S, Hughes T, et al. Prediction of graft versus host disease by frequency analysis of cytotoxic T cells after unrelated donor bone marrow transplantation. Transplantation 1989;48(4):608–13.
[22] Roosnek E, Hogendijk S, Zawadynski S, Speiser D, Tiercy JM, Helg C, et al. The frequency of pretransplant donor cytotoxic T cell precursors with anti-host specificity predicts survival of patients transplanted with bone marrow from donors other than HLA-identical siblings. Transplantation 1993;56(3):691–6.
[23] Van Besouw NM, Van der Mast BJ, De Kuiper P, Smak Gregoor PJ, Vaessen LM, Ijzerman JN, et al. Donor-specific T-cell reactivity identifies kidney transplant patients in whom immunosuppressive therapy can be safely reduced. Transplantation 2000;70(1):136–43.
[24] Oudshoorn M, Doxiadis IIN, Van den Berg-Loonen PM, Voorter CEM, Verduyn W, Claas FHJ. Functional versus structural matching: can the CTLp test be replaced by HLA allele typing? Hum Immunol 2002;63:176–84.
[25] Burrows SR, Silins SL, Cross SM, Peh CA, Rischmueller M, Burrows JM, et al. Human leukocyte antigen phenotype imposes complex constraints on the antigen-specific cytotoxic T lymphocyte repertoire. Eur J Immunol 1997;27(1):178–82.
[26] Roelen D, Datema G, Van Bree S, Zhang L, Van Rood J, Claas F. Evidence that antibody formation against a certain HLA alloantigen is associated not with a quantitative but with a qualitative change in the cytotoxic T cells recognizing the same antigen. Transplantation 1992;53(4):899–903.
[27] Roelen DL, Van Bree FP, Schanz U, Van Rood JJ, Claas FH. Differential inhibition of primed alloreactive CTLs in vitro by clinically used concentrations of cylosporine and FK506. Transplantation 1993;56(1):190–5.
[28] Patel R, Terasaki PI. Significance of the positive crossmatch test in kidney transplantation. N Engl J Med 1969;280:735–9.
[29] McKenna RM, Takemoto SK, Terasaki PI. Anti-HLA antibodies after solid organ transplantation. Transplantation 2000;69(3):319–26.
[30] Papassavas AC, Iniotaki-Theodoraki A, Boletis J, Kostakis A, Stavropoulous-Giokas C. Epitope analysis of HLA class I donor specific antibodies in sensitized renal transplant recipients. Transplantation 2000;70(2):323–7.
[31] Stobbe I, Van der Meer-Prins EMW, De Lange P, Oudshoorn M, Doxiadis IIN, Claas FHJ. In vitro CTL precursor frequencies do not reflect a beneficial effect of Cross Reactive Group (CREG) matching. Hum Immunol 2000;61:879–83.
[32] Mulder A, Kardol MJ, Kamp J, Uit het Broek C, Schreider GM, Doxiadis II, et al. Determination of the frequency of HLA antibody secreting B-lymphocytes in alloantigen sensitized individuals. Clin Exp Immunol 2001;124(1):9–15.
[33] Duquesnoy RJ. HLAMatchmaker: a molecularly based algorithm for histocompatibility determination: I. Description of the algorithm. Hum Immunol 2002;63(5):339–52.
[34] Duquesnoy RJ, Howe J, Takemoto S. HLA matchmaker: a molecularly based algorithm for histocompatibility determination: IV. An alternative strategy to increase the number of compatible donors for highly sensitized patients. Transplantation 2003;75(6):889–97.
[35] Claas FH, De Meester J, Witvliet MD, Smits JM, Persijn GG, Doxiadis II. Acceptable HLA mismatches for highly immunized patients. Rev Immunogenet 1999;1(3):351–8.
[36] Dankers MK, Roelen DL, Van Der Meer-Prins EM, de Lange P, Witvliet M, Sandkuijl L, et al. Differential immunogenicity of HLA mismatches: HLA-A2 versus HLA-A28. Transplantation 2003;75(3):418–20.
[37] Duquesnoy RJ, Marrari M. HLAMatchmaker: a molecularly based algorithm for histocompatibility determination II: verification of the algorithm and determination of the relative immunogenicity of amino acid triplet-defined epitopes. Hum Immunol 2002;63:353–63.
[38] Nambiar A, Duquesnoy RJ, Adams S, Oblitas J, Leitman S, Stroncek, D. et al. HLAMatchmaker-driven analysis of response to HLA typed platelet transfusions in alloimmunized patients. Blood in press.
[39] Fuller TC, Fuller A. The humoral immune response against an HLA class I allodeterminant correlates with the HLA-DR phenotype of the responder. Transplantation 1999;68(2):173–82.