Allogeneic haematopoietic stem cell donation and transplantation across the MHC class I barrier: "Faster is better than more. More is better than less".

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The HLAMatchmaker algorithm is not a suitable tool to predict the alloreactive cytotoxic T lymphocyte response in vitro

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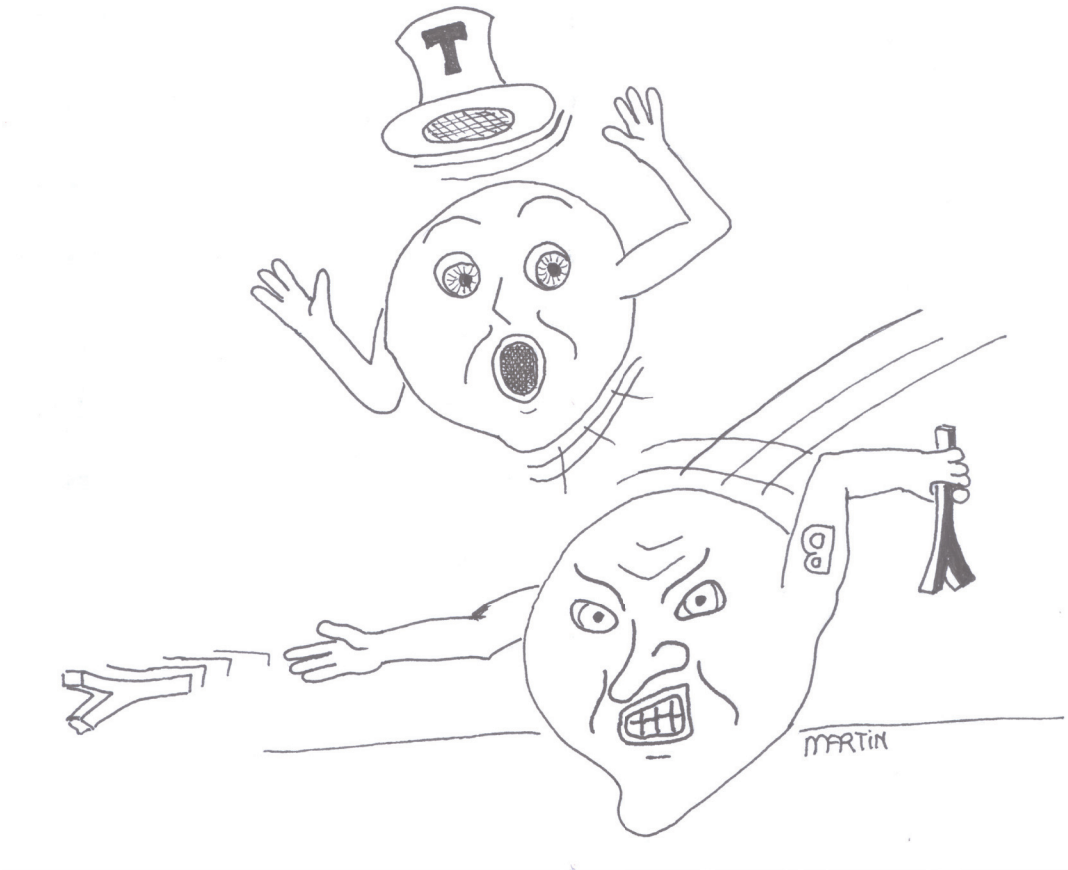
Transplantation 2004; 78:165-167



Difference of opinion

Abstract

Both donor specific anti HLA antibodies and cytotoxic T lymphocytes are important mediators of graft rejection. A recently developed computer algorithm, called HLAMatchmaker, determines the amino acid triplets on antibody accessible sites of the HLA molecule which are not shared between patient and donor. A previous study showed a strong positive correlation between the number of triplet mismatches and the percentage of individuals producing HLA antibodies. In the present study we tested whether the number of triplet mismatches is predictive for the CTLp frequency *in vitro*. The analysis was performed on 108 HLA-DRB1 and DQB1 identical patient/donor combinations registered by the Europdonor foundation, with a single HLA class I mismatch and in healthy responder/stimulator combinations mismatched for at least one HLA class I antigen.

The results show that there is no strong correlation between the number of triplet mismatches and the CTLp frequency. Even in case of zero triplet mismatches a high CTLp frequency can be found. This lack of correlation is probably due to the fact that HLAMatchmaker considers only triplets on antibody accessible positions while CTLs also recognise other epitopes on the HLA molecule including the bound peptides.

Introduction

The presence of donor specific anti human leukocyte antigen (HLA) antibodies in the circulation of a transplant recipient has a negative impact on transplantation outcome [1]. This donor specific humoral alloimmunity may exist in individuals who have been immunised as a result of blood transfusions, pregnancies or failed transplants [2-4]. Hyperacute graft rejection, which is the direct consequence of pre-existing donor reactive anti HLA antibodies, has become rare after the introduction of the serological crossmatch test [5]. However, highly sensitised patients, with a panel reactive antibody (PRA) value of 85% or more, remain on the waitinglist with little prospect of a suitable (crossmatch negative) donor.

To identify potential donors for highly sensitised patients, Duquesnoy developed a computer program, called HLAMatchmaker [6]. This computer based algorithm focuses on the structural basis of HLA class I polymorphisms so that HLA compatible donors can be identified for each patient without the need for extensive serum screening. HLAMatchmaker converts each HLA class I allele into a linear string of amino acid triplets, which are accessible to alloantibodies and then determines, by intralocus and interlocus comparison, which donor amino acid triplets are shared or not shared with the recipient. Its concept is that no antibodies are formed against triplets of amino acids that are shared between donor and recipient.

In a previous study we already showed a strong positive correlation between the number of triplet mismatches and the induction of alloantibodies [7]. Next to antibodies, T cells are important effector cells in graft rejection. In our department, the cytotoxic T lymphocyte

precursor (CTLp) test is routinely used for the selection of donors for patients who need a haematopoietic stem cell transplantation. The CTLp test provides insight into the frequency of donor CTLs capable of responding to HLA mismatches present on the patients cells [8,9]. In the present study it was analysed, whether the number of triplet mismatches between donor and recipient is also predictive for the CTLp frequency.

Material and Methods

Patient and donor selection

Two groups of mismatched combinations were analysed. The first group concerned 108 patient/donor combinations registered at the Europdonor foundation. All individuals were typed for HLA class I and HLA class II on a high resolution level by DNA-based typing using polymerase chain reaction sequence specific primers (PCR-SSP) and sequencing based typing (SBT). All couples had a single HLA class I mismatch at the allele level and were matched for HLA-DRB1 and DQB1. The group consisted of 34 single HLA-A mismatched combinations, 12 single HLA-B mismatched combinations and 62 single HLA-C mismatched combinations. All patient donor combinations were analysed for alloreactive CTLp frequency in the graft versus host direction.

The second group concerned 21 healthy responder/stimulator combinations. All individuals were serologically typed for HLA-A, -B and –DR using the standard NIH complement dependent cytotoxicity assay (CDC). All combinations were mismatched for at least one HLA class I antigen. The group consisted of 7 HLA-DR identical and 14 HLA-DR mismatched combinations. All combinations were analysed for alloreactive CTLp frequencies.

Cytotoxic T-Lymphocyte Precursor Test

The analysis of CTLp frequencies by limiting dilution assays in the graft versus host direction were performed as described by Zhang *et al.* [10]. Briefly, two fold diluted peripheral blood lymphocytes (PBL) from the donor (from 50,000 cells per well down to 781) were cultured in 24 replicates with 50,000 irradiated (30 Gy) stimulator cells (PBL from the patient) in a total volume of 0.2 ml. After 7 days of culture in RPMI 1640 (Gibco, Paisley, Scotland) supplemented with penicillin (100 U/ml, streptomycin 100 μg/ml), glutamin (2mM), 10% heat-inactivated pooled human serum and 15 U/ml of rIL-2 (Ortho Pharmaceutical Corp., Raritan, NJ, USA), the cytolytic activity againts ^{51}Cr-labeled target cells (PHA cells of the patient) was measured in each well. The supernatant was harvested using a Skatron harvesting system (Lies Norway) and the release of ^{51}Cr was assayed in a Packard gamma counter (Palo Alto, CA, USA).

The analysis of CTLp frequencies by limiting dilution assays in the healthy responder/stimulator combinations were performed as described by Bouma *et al.* [11]. Briefly, two fold

diluted responder PBL (from 40,000 cells per well down to 625) were cultured with 50,000 irradiated (3000 rad) stimulator cells. After 7 days of culture in RPMI 1640, supplemented with 10% heat inactivated pooled human serum and 20 U/ml of rIL-2 (Cetus, Amsterdam, The Netherlands), the cytolytic activity against Europium labelled target cells was measured in each well using a timeresolved fluorometer (Arcus 1234, Wallac).
In each test responder cells were cultured with irradiated responder cells as a negative control. Stimulator and responder cells were also cultured with HLA mismatched third party cells as positive controls. In the chromium release assay a negative CTLp test was defined as ≤ 1 CTLp per 10^6 PBL, a high result as 10 or more CTLp per 10^6 PBL. In the Europium release assay a negative result was defined as ≤ 10 CTLp per 10^6 PBL, an intermediate result as 11-100 CTLp per 10^6 PBL and a high result as > 100 CTLp per 10^6 PBL. Frequencies of CTL precursors were calculated using the Jackknife method according to Strijbosch *et al.* [12].

Triplet mismatch analysis
The number of triplet mismatches was calculated for every responder/stimulator combination with use of the HLAMatchmaker computer algorithm developed by Duquesnoy [6]. It was analysed if the number of triplet mismatches between responder and stimulator is predictive for the CTLp frequency against the stimulator. The Mann-Whitney *U* test was used for statistical comparison.

Results

When analysing all patient/donor combinations, registered at the Europdonor foundation, with a single HLA-A or -B mismatch, a statistically significant difference in the number of triplet mismatches was found between patient/donor combinations with a CTLp frequency ≤1 and a CTLp frequency ≥10 per 10^6 PBL (P=0.04). However, a large overlap exists between both groups and a CTLp frequency ≥10 per 10^6 was also found in combinations with zero number of triplet mismatches (figure 1). When the single HLA-C mismatched combinations are included in the analysis, the difference between the two groups becomes even smaller and is not significant anymore.
In healthy responder/stimulator combinations, only combinations with a CTLp frequency > 100 (N=10) showed a significantly higher number of triplet mismatches compared with combinations with a CTLp frequency between 11 and 100 (P=0.03) and a CTLp frequency

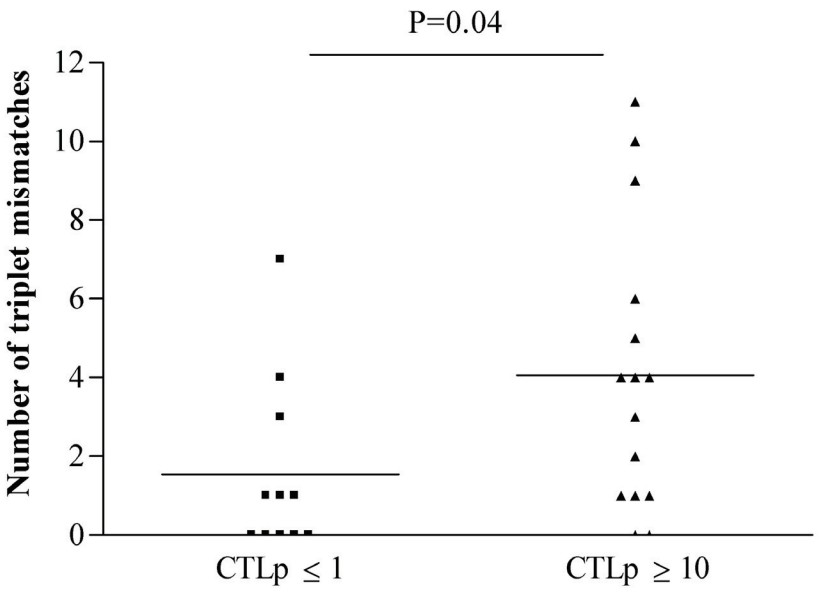


Figure 1. Number of triplet mismatches in patient/donor combinations from the donor registry of the Europdonor foundation with a single HLA-A or -B mismatch and a CTLp frequency ≤ 1 (left panel) or a CTLp frequency ≥ 10 (right panel) per 10^6 PBL. *Horizontal bars represent the mean of the group. According to Mann-Whitney U test, a significant difference in the number of triplet mismatches was found between both groups (P=0.04).*

≤ 10 per 10^6 PBL (P=0.04). Between combinations with a CTLp frequency ≤ 10 and a CTLp frequency between 11 and 100 per 10^6 PBL, no significant difference in the number of triplet mismatches was found (figure 2). When the HLA-DR matched and HLA-DR mismatched combinations were analysed separately, similar results were obtained (data not shown).
Another analysis was performed using the patient/donor combinations from the Europdonor population with a single HLA-A, -B, or -C mismatch. The CTLp frequency was analysed for all patient and donor combinations with zero or ≥ 5 triplet mismatches. The CTLp frequency against the zero triplet mismatched patients was not significantly lower compared with the combinations with 5 or more triplet mismatches (P=0.32) (figure 3).

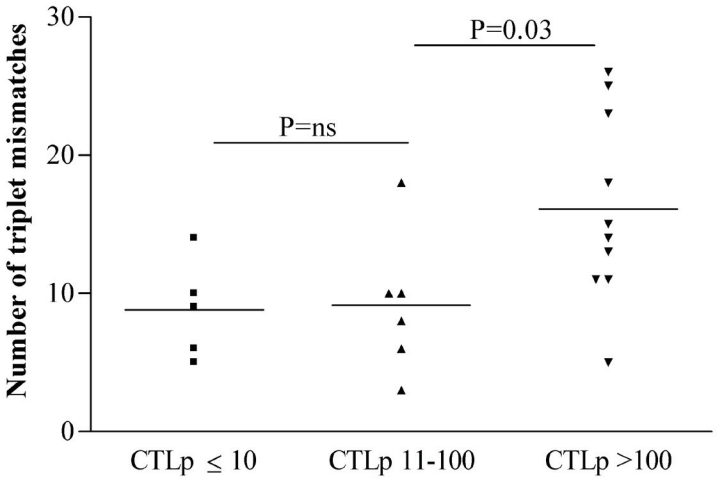


Figure 2. Number of triplet mismatches in healthy responder/ stimulator combinations mismatched for at least one HLA class I antigen and a CTLp frequency ≤ 10 (left), a CTLp frequency between 11 and 100 (middle) or a CTLp frequency > 100 (right) per 10^6 PBL. *Horizontal bars represent the mean of the group. According to Mann-Whitney U test only the combinations with a CTLp frequency >100 showed a significantly higher number of triplet mismatches compared with the combinations with a CTLp frequency between 11 and 100 (P=0.03) and a CTLp frequency ≤ 10 (P=0.04) per 10^6 PBL.*

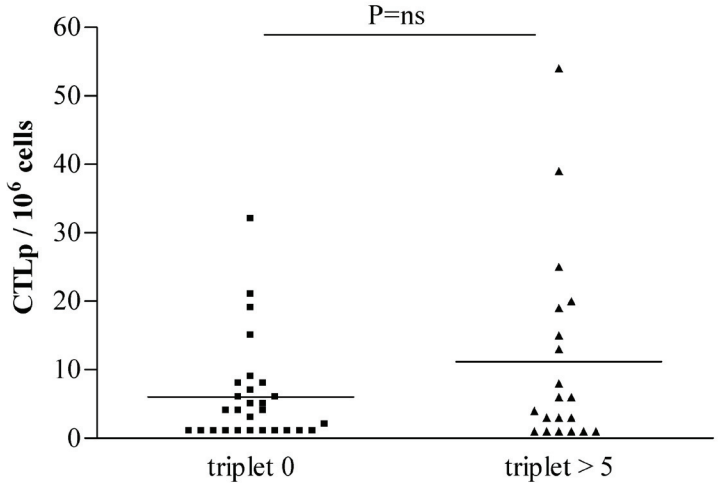


Figure 3. CTLp frequency in patient/donor combinations from the donor registry of the Europdonor foundation with a single HLA-A, -B or -C mismatch and zero triplet mismatches (left panel) or ≥ 5 triplet mismatches (right panel). *Horizontal bars represent the mean of the group. According to Mann-Whitney U test no significant difference was found between both groups (P=0.32).*

Discussion

On basis of the HLAMatchmaker algorithm certain HLA class I mismatched combinations may be fully compatible at the triplet level. The clinical relevance of the HLAMatchmaker program was shown by its ability to identify acceptable mismatches for (highly) sensitised patients and to predict the outcome of crossmatch results [13,14]. Furthermore, in case of HLA-DR compatibility between patient and kidney donor, HLA-A,B mismatched grafts that were matched at the triplet level showed a similar graft survival as zero HLA-A,B mismatched grafts [15].

HLAMatchmaker can also be used to optimise donor-recipient compatibility, thereby preventing or reducing antibody mediated rejection of organ transplants [7]. However, next to antibodies, cytotoxic T lymphocytes (CTL) play a major role in transplant rejection. As a tool to monitor alloreactivity *in vitro,* limiting dilution assays (LDA) to quantify the number of CTL precursors frequencies have shown to be useful [9]. The correlation between the CTLp frequency and the outcome of solid organ transplantation is still controversial. Several studies showed a correlation between an increased CTLp frequency and graft rejection [16,17]. However, high CTLp frequencies were also found in patients with a good functioning graft [18,19]. In unrelated bone marrow transplantation, CTLp frequencies has shown to be predictive for the occurrence of GVHD [20].

In the present study it was analysed, whether the CTLp frequency against an HLA mismatch correlates with the number of triplet mismatches between donor and recipient. In a previous study we already showed that a strong positive correlation exists between the number of triplet mismatches and the percentage of individuals producing antibodies [7]. If zero triplet mismatches were present no antibodies were formed in all cases.

The present results show that such a strong correlation does not exist for the number of triplet mismatches and the CTLp frequency. A large overlap of the number of triplet mismatches was found between combinations with a CTLp frequency ≤ 1 and a CTLp frequency ≥ 10 per 10^6 PBL. In the healthy responder/stimulator combinations only in the group with a very high CTLp frequency more triplet mismatches were present. Furthermore, in case of zero triplet mismatches a high CTLp frequency can be found.

HLAMatchmaker considers only triplets in antibody accessible positions of the HLA molecule which is probably the main reason that the correlation with the CTL response is significantly poorer than that between triplet mismatching and antibody formation. The humoral immune response is directed at epitopes in antibody accessible positions while CTL recognise different epitopes on the HLA molecule often in context with the bound peptide. In order to become more predictive for CTL reactivity the HLAMatchmaker program should be adapted by including epitopes present in the peptide binding groove. However, even in that case it is not known which specific peptides are presented by the HLA molecule and recognised by the CTL. Furthermore, in case of zero triplet mismatches, minor HLA antigens can still be presented by shared HLA molecules and these are also targets for a CTL response [21].

The present study indicates that it is not possible to select bone marrow donors that will not form a CTLp response by analysing the number of triplet mismatches using the HLAMatchmaker algorithm. A low number of triplet mismatches can lead to either a low or a high CTLp frequency. In a future study it will be analysed if specific amino acid sequence differences between donor and recipient, located in the groove of the HLA molecule, are associated with a high or low CTL response as is suggested by a recent study of Ferrara *et al.* [22] and Oudshoorn *et al.* [23]. These studies showed that amino acids at different positions of the peptide binding groove of the HLA class I molecule play a crucial role in the outcome of the CTLp frequency and unrelated bone marrow transplantation. Hopefully, these studies will lead to the possibility to select bone marrow donors by high resolution DNA typing and amino acid analysis, without the need for the labour intensive CTLp assay.

Acknowledgements

The authors thank Prof. Dr. A. Brand and Dr. G.M.Th. Schreuder for critically reading the manuscript. This work was supported by the J.A. Cohen Institute for Radio-pathology and Radiation Protection (IRS) and the National Reference Center for Histocompatibility.

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