HLAMatchmaker Algorithm is not a Suitable Tool to Predict the Alloreactive Cytotoxic T-Lymphocyte Response in vitro

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Both donor-specific anti-human leukocyte antigen (HLA) antibodies and cytotoxic T lymphocytes are important mediators of graft rejection. HLAMatchmaker determines the amino acid triplets on antibody-accessible sites of the HLA molecule that 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 cytotoxic T-lymphocyte precursor (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 the case of zero triplet mismatches, a high CTLp frequency can be found. This lack of correlation is probably caused by the fact that HLA-Matchmaker considers only triplets on antibody-accessible positions, whereas CTLs also recognize other epitopes on the HLA molecule, including the bound peptides.

Keywords: CTL, Matchmaker, Triplet, HLA, Transplantation.

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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. This donor-specific humoral alloimmunity may exist in individuals who have been immunized as a result of blood transfusions, pregnancies, or failed transplants. Hyperacute graft rejection, which is the direct consequence of preexisting donor-reactive anti-HLA antibodies, has become rare after the introduction of the serologic crossmatch test (*1*). However, highly sensitized patients, with a panel reactive antibody value of 85% or more, remain on the waiting list with little prospect of a suitable (crossmatch-negative) donor.

To identify potential donors for highly sensitized patients, Duquesnoy developed a computer program called HLAMatchmaker. 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 (*2*). 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 hematopoietic 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 (*3*, *4*). In the present study, it was analyzed whether the number of triplet mismatches between donor and recipient is also predictive for the CTLp frequency.

MATERIALS AND METHODS

Patient and Donor Selection

Two groups of mismatched combinations were analyzed. The first group comprised 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 (SSP) and sequencing-based typing. 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 analyzed for alloreactive CTLp frequency in the graft-versus-host direction.

The second group comprised 21 healthy responder-stimulator combinations. All individuals were serologically typed for HLA-A, -B, and -DR using the standard NIH com-

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plement-dependent cytotoxicity assay. 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 analyzed 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. (*5*). The analysis of CTLp frequencies by limiting dilution assays in the healthy responder-stimulator combinations were performed as described by Bouma et al. (*6*).

In the chromium release assay, a negative CTLp test was defined as 1 or less CTLps per 10^6 peripheral blood lymphocytes (PBLs) and a high result as 10 or more CTLps per 10^6 PBLs. In the Europium release assay, a negative result was defined as 10 or less CTLps per 10^6 PBLs, an intermediate result as 11 to 100 CTLps per 10^6 PBLs, and a high result as more than 100 CTLps per 10^6 PBLs.

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 (*7*). It was analyzed whether 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 analyzing 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 or less and a CTLp frequency 10 or greater per 10^6 PBLs (P=0.04). However, a large overlap exists between both groups, and a CTLp frequency 10 or greater per 10^6 was also found in combinations with zero number of triplet mismatches (Fig. 1). When the single HLA-C mismatched combinations are included in the analysis, the difference between the two groups becomes even smaller and is no longer significant.

In healthy responder-stimulator combinations, only combinations with a CTLp frequency greater than 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 10 or less per 10^6 PBLs (P=0.04). Between combinations with a CTLp frequency 10 or less and a CTLp frequency between 11 and 100 per 10^6 PBLs, no significant difference in the number of triplet mismatches was found (Fig. 2). When the HLA-DR matched and HLA-DR mismatched combinations were analyzed 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 analyzed for all patient and donor combinations with zero or five or more triplet mismatches. The CTLp frequency against the zero–triplet-mismatched patients was not significantly lower compared with the combinations with five or more triplet mismatches (P=0.32) (Fig. 3).

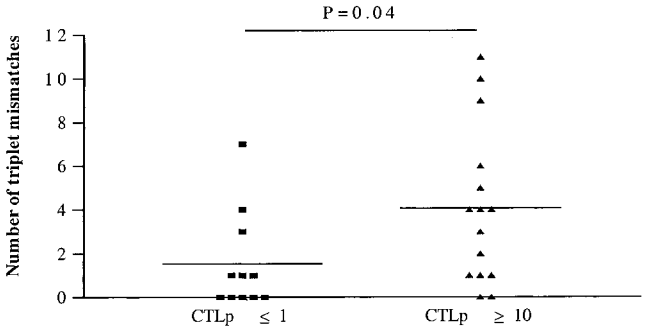

FIGURE 1. Number of triplet mismatches in patient-donor combinations from the donor registry of the Europdonor foundation with a single human leukocyte antigen (HLA)-A or -B mismatch and a cytotoxic T lymphocyte precursor (CTLp) frequency less than or equal to 1 (left) or a CTLp frequency greater than or equal to 10 (right) per 10^6 peripheral blood lymphocytes (PBLs). Mean of the group (*horizontal bars*). According to Mann-Whitney *U* test, a significant difference in the number of triplet mismatches was found between both groups (P=0.04).

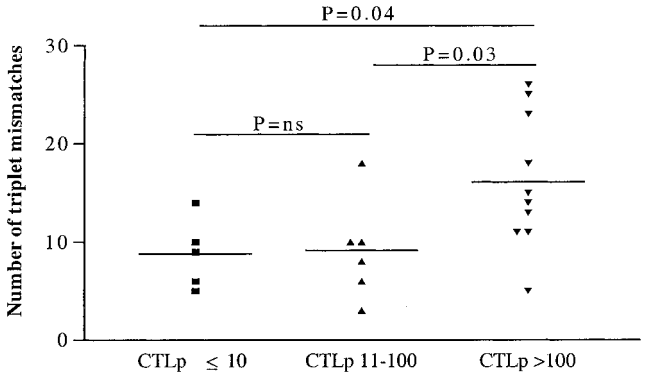

FIGURE 2. Number of triplet mismatches in healthy responder-stimulator combinations mismatched for at least one HLA class I antigen and a CTLp frequency of less than or equal to 10 (left), a CTLp frequency between 11 and 100 (middle), or a CTLp frequency greater than 100 (right) per 10^6 PBLs. Mean of the group (*horizontal bars*). According to Mann-Whitney *U* test, only the combinations with a CTLp frequency greater than 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 less than or equal to 10 (P=0.04) per 10^6 PBLs.

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 HLA-Matchmaker program was shown by its ability to identify acceptable mismatches for (highly) sensitized patients and to predict the outcome of crossmatch results (*1*). Furthermore, in case of HLA-DR compatibility between patient and kidney donor, HLA–A,B-mismatched grafts that were matched at

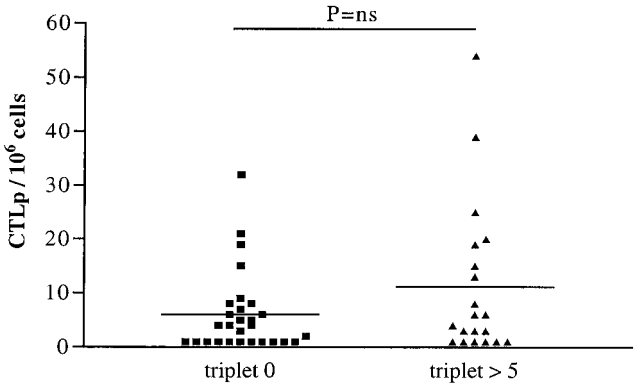

FIGURE 3. CTLp frequency in patient-donor combinations from the donor registry of the Europdonor foundation with a single HLA-A, -B, or -C mismatch and zero triplet mismatches (left) or greater than or equal to five triplet mismatches (right). Mean of the group (*horizontal bars*). According to Mann-Whitney *U* test, no significant difference was found between the groups ($P=0.32$).

the triplet level showed a similar graft survival as zero HLA–A,B-mismatched grafts (*7*).

HLAMatchmaker can also be used to optimize donor-recipient compatibility, thereby preventing or reducing antibody-mediated rejection of organ transplants (*2*). However, next to antibodies, CTLs play a major role in transplant rejection. As a tool to monitor alloreactivity in vitro, limiting dilution assays to quantify the number of CTL precursors frequencies have shown to be useful. 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 (*8*). However, high CTLp frequencies were also found in patients with a good functioning graft. In unrelated bone-marrow transplantation, CTLp frequencies has shown to be predictive for the occurrence of graft-versus-host disease (*3*).

In the present study, it was analyzed 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 (*9*). 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 or less and a CTLp frequency 10 or more per 10^6 PBLs. 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, whereas CTLs recognize different epitopes on the HLA molecule, often in context with the bound peptide. 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 recognized 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.

The present study indicates that it is not possible to select bone-marrow donors that will not form a CTLp response by analyzing 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 analyzed whether 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. (*4*) and Oudshoorn et al. (*10*). 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 of selecting bone-marrow donors by high-resolution DNA typing and amino acid analysis, without the need for the labor-intensive CTLp assay.

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REFERENCES

1. Lobashevsky AL, Senkbeil RW, Shoaf JL, et al. The number of amino acid residues mismatches correlates with flow cytometry crossmatching results in high PRA renal patients. Hum Immunol 2002; 63: 364.
2. Dankers MK, Witvliet MD, Roelen DL, et al. The number of amino acid sequence differences between patient and donor is predictive for the antibody reactivity against the mismatched HLA antigens. Transplantation 2004; 77: 1236.
3. Kaminski E, Hows J, Man S, et al. Prediction of graft versus host disease by frequency analysis of cytotoxic T cells after unrelated donor bone marrow transplantation. Transplantation 1989; 48: 608.
4. Ferrara GB, Bacigalupo A, Lamparelli T, et al. Bone marrow transplantation from unrelated donors: the impact of mismatches with substitutions at position 116 of the human leukocyte antigen class I heavy chain. Blood 2001; 98: 3150.
5. Zhang L, Li S, Vandekerckhove B, et al. Analysis of cytotoxic T cell precursor frequencies directed against individual HLA-A and -B alloantigens. J Immunol Meth 1989; 121: 39.
6. Bouma GJ, van der Meer-Prins EM, van Bree FM, et al. Determination of cytotoxic T-lymphocyte precursor frequencies using europium labeling as a non-radioactive alternative to labeling with chromium-51. Hum Imunol 1992; 35: 85.
7. Duquesnoy RJ. HLAMatchmaker: a molecularly based algorithm for histocompatibility determination. I. Description of the algorithm. Hum Immunol 2002; 63: 339.
8. Duquesnoy RJ, Takemoto S, de Lange P, et al. HLAMatchmaker: a molecularly based algorithm for histocompatibility determination. III. Effect of matching at the HLA-A,B amino acid triplet level on kidney transplant survival. Transplantation 2003; 75: 884.
9. Steinmann J, Leimenstoll G, Engemann R, et al. Clinical relevance of cytotoxic T cell precursor (p-CTL) frequencies in allograft recipients. Transpl Proc 1990; 22: 1873.
10. Oudshoorn M, Doxiadis II, van de Berg-Loonen PM, et al. Functional versus structural matching: can the CTLp test be replaced by HLA allele typing. Hum Immunol 2002; 63: 176.