

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

Marlies K. A. Dankers,<sup>1,4</sup> Martin B. A. Heemskerk,<sup>1,2</sup> Rene J. Duquesnoy,<sup>3</sup> Ilias I. N. Doxiadis,<sup>1</sup> Machteld Oudshoorn,<sup>1,2</sup> Dave L. Roelen,<sup>1</sup> and Frans H. J. Claas<sup>1</sup>

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 Eurodonor 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 HLAMatchmaker 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.

(*Transplantation* 2004;78: 165–167)

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 Eurodonor 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-

This work was supported by the J. A. Cohen Institute for Radio-pathology and Radiation Protection (IRS) and the National Reference Center for Histocompatibility.

<sup>1</sup> Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, The Netherlands.

<sup>2</sup> Eurodonor Foundation, Leiden, The Netherlands.

<sup>3</sup> University of Pittsburgh Medical Center, Pittsburgh, PA.

<sup>4</sup> Address correspondence to: Marlies K. A. Dankers, Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Building 1, E3Q, PO Box 9600, 2300 RC Leiden, The Netherlands. E-mail: dankers@ikr.nl

Received 18 November 2003. Accepted 16 January 2004.

Copyright © 2004 by Lippincott Williams & Wilkins

ISSN 0041-1337/04/7801-165

DOI: 10.1097/01.TP.0000133511.94487.D3

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

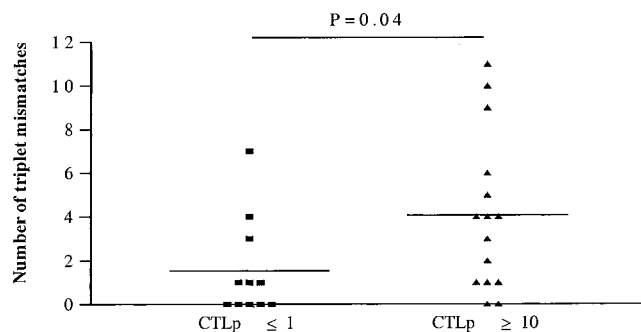
The number of triplet mismatches was calculated for every responder-stimulator combination with use of the HLA-Matchmaker computer algorithm developed by Duquesnoy (7). It was analyzed whether the number of triplet mismatches between responder and stimulator is predictive for the CTLp frequency against the stimulator. The Mann-Whitney *U* test was used for statistical comparison.

## RESULTS

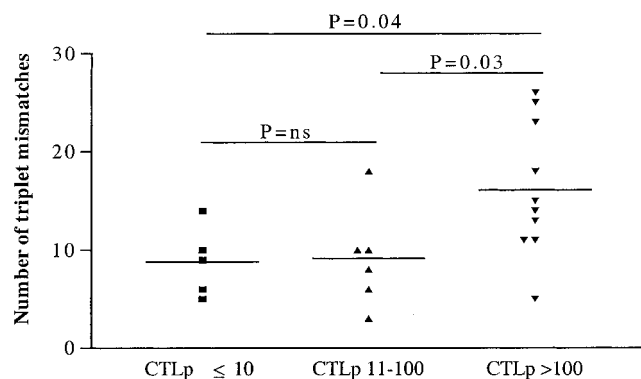
When analyzing all patient-donor combinations registered at the Europdonor foundation with a single HLA-A or -B mismatch, a statistically significant difference in the number of triplet mismatches was found between patient-donor combinations with a CTLp frequency 1 or less and a CTLp frequency 10 or 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



**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.

lower compared with the combinations with five or more triplet mismatches ( $P=0.32$ ) (Fig. 3).

## DISCUSSION

On basis of the HLA-Matchmaker 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

