

THE NUMBER OF AMINO ACID TRIPLET DIFFERENCES BETWEEN PATIENT AND DONOR IS PREDICTIVE FOR THE ANTIBODY REACTIVITY AGAINST MISMATCHED HUMAN LEUKOCYTE ANTIGENS¹

MARLIES K. A. DANKERS,^{2,5} MARIAN D. WITVLIET,² DAVE L. ROELEN,² PETER DE LANGE,² NELLEKE KORFAGE,² GUIDO G. PERSIJN,³ RENÉ DUQUESNOY,⁴ ILIAS I. N. DOXIADIS,² AND FRANS H. J. CLAAS

Background. The correlation between antibody production against mismatched donor human leukocyte antigens (HLA) and the number of amino acid sequence mismatches was analyzed in patients who rejected a kidney transplant (n=146).

Methods. A similar analysis was performed for the antibody production of women against the paternal HLA antigens of their child (n=1,397). The amino acid sequence (triplet) differences were analyzed using the HLAMatchmaker algorithm.

Results. In both groups, a positive correlation was found between the number of triplet mismatches and the percentage of individuals producing antibodies ($P < 0.0001$). If zero triplet mismatches were present, no antibodies were formed in all cases. When 11 or 12 triplet mismatches were present, 94% of the transplant patients produced antibodies against the donor. In pregnancy, 11 or 12 triplet mismatches led to 27% of the women producing specific antibodies.

Conclusions. These results indicate that the immunogenicity of the fetus is lower than that of a rejected kidney and that analysis of the number of triplet mismatches can predict the antibody reactivity against the mismatched HLA antigens.

Donor-specific human leukocyte antigen (HLA) antibodies can be detected in individuals who have been immunized as a result of pregnancies, failed transplants, or blood transfusions. During pregnancy, women can produce antibodies directed against the paternal HLA antigens of the child. The role of these antibodies in pregnancy is unclear. It is suggested that recognition of paternal HLA antigens of the child might be involved in a maternal immunologic adaptation required to protect the fetal allograft (1). Antibodies directed against the paternal HLA antigens of the child are generally detected in only 15% to 30% of women. Potential factors contributing to the induction of antipaternal antibodies are the number of fetal cells passing through the placenta, the number of HLA mismatches between child and mother, the

development of anti-idiotypic antibodies directed against anti-HLA antibodies (2), or cytokine polymorphism in the mother (3).

In cadaveric transplantation, a serologic crossmatch test between kidney donor and transplant recipient is routinely performed to avoid hyperacute rejection caused by donor HLA-specific antibodies. However, acute rejection leading to early graft loss and chronic rejection remain major problems. A better understanding and prediction of the humoral response may be of benefit for renal transplant recipients.

In previous studies, we could demonstrate that the immunogenicity of a particular HLA mismatch, as measured by the induction of alloantibodies, depends on the HLA phenotype of the donor as well as that of the recipient (4–6). This differential immunogenicity might be explained by specific amino acid sequence differences between the HLA alleles of donor and recipient. These amino acid sequence differences can be analyzed by a computer algorithm developed by Duquesnoy (7) that defines the HLA-A and HLA-B mismatches by triplets of amino acid residues on alloantibody accessible sites of HLA molecules. The program converts each HLA class I allele into a string of linear amino acid triplets and then determines which donor amino acid triplets are shared or not shared between donor and recipient. The program is based on the concept that no antibodies are formed against triplets of amino acids that are shared between donor and recipient.

In the present study, we determined whether in patients who rejected a kidney transplant a correlation exists between the antibody production against the mismatched donor HLA antigens and the number of triplet mismatches between patient and donor. A similar approach was used for the incidence of antibody production by women against the mismatched paternal HLA antigens of the child.

PATIENTS AND METHODS

Sera were collected from renal transplant patients who underwent transplantation between 1973 and 2000 and who came back onto the Eurotransplant waiting list after transplant failure (n=144) and from women who, from 1968 on, had their delivery at the Leiden University Medical Center (n=1,397). The transplant patient cohort consisted of 61 women and 83 men. In 48 patient and donor combinations, a single HLA class I mismatch was present; in 93 combinations, two HLA class I mismatches were present; in 37 combinations, three HLA class I mismatches were present; and in 12 combinations, four HLA class I mismatches were present. Until 1985, the patients (n=35) were treated with prednisone and Imuran or prednisone and cyclosporine. After 1985, the patients (n=109) were treated with cyclosporine, prednisone, and antithymocyte globulin followed by different protocols including anti-CD25, OKT3, tacrolimus, and mycophenolate mofetil.

¹This work was supported by the J. A. Cohen Institute for Radiopathology and Radiation Protection and the National Reference Center for Histocompatibility.

²Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, The Netherlands.

³Eurotransplant Foundation, Leiden, The Netherlands.

⁴University of Pittsburgh Medical Center, Pittsburgh, PA.

⁵Address correspondence to: Marlies K. A. Dankers, M.S., Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Building 1, E3Q, P.O. Box 9600, 2300 RC Leiden, The Netherlands. Email: m.k.a.dankers@lumc.nl.

Received 2 September 2003.

Revision requested 3 October 2003. Accepted 3 November 2003.

The sera of the pregnant women were tested by a serologic cross-match against the lymphocytes of the child or the father. Furthermore, all sera were tested by a screening against a panel of 50 HLA-typed individuals, using the standard National Institutes of Health complement-dependent cytotoxicity assay, to identify whether specific antibodies were formed against the donor or against the mismatched paternal antigens. All patients, donors, women, fathers, and children were typed for HLA-A and HLA-B using the standard complement-dependent cytotoxicity assay technique.

The number of triplet mismatches was calculated using the HLA-Matchmaker program developed by Duquesnoy (7). It analyzed whether a correlation exists between the number of triplet mismatches and the percentage of patients producing specific antibodies. The same approach was used for all combinations of women and mismatched paternal antigens of the child. A linear regression analysis, using GraphPad InStat (Graphpad Software, Inc., San Diego, CA) was performed to analyze whether a significant correlation was present.

RESULTS

Transplant Patients

A strong correlation between the number of triplet mismatches between patient and donor antigen and the percentage of patients that produce donor-specific antibodies was found ($P < 0.0001$, $r^2 = 0.99$) (Fig. 1). When zero triplet mismatches were present, no specific antibodies against the donor antigen were formed; and when 11 or 12 triplet mismatches were present, almost all patients (94%) produced specific antibodies against the donor. Because HLA matching is an important allocation parameter within Eurotransplant, the number of cases with more than 12 triplet mismatches was too low ($n = 7$) to be included in the analysis. To analyze the influence of the different immunosuppression protocols, the cohort was split into two groups: one that underwent transplantation before and one after 1985. The triplet analysis was performed in both groups, and the same significant correlation could be found.

Pregnant Women

Similarly, as shown in Figure 2, the percentage of women that produce specific antibodies against their child increases when a higher number of triplet mismatches between mother and child are present ($P < 0.0001$, $r^2 = 0.95$). When zero triplet mismatches were present, no antibodies were formed in all cases; and when 11 or 12 triplet mismatches were present, approximately 27% of the women produced specific antibodies

against their child, showing that the immunogenicity of an HLA mismatch during pregnancy was lower than in cases of graft rejection. This is further substantiated by Figure 3, which shows the correlation coefficients between the number of triplet mismatches and the percentage of individuals producing HLA alloantibodies.

The triplet mismatches in all women with fewer than five triplet mismatches were analyzed in more detail ($n = 122$). We compared the positions and the amino acid sequence of the triplet mismatches in women that produced specific antibodies against their child with those of women that did not produce specific antibodies against their child and the same HLA mismatch was expressed by the child. No differences in the position or the amino acid sequence of the triplet mismatches was found between the two groups (data not shown).

DISCUSSION

To validate the HLA-Matchmaker algorithm, the induction of alloantibodies in case of graft rejection and pregnancy was analyzed in relation to the number of triplet mismatches defined by the HLA-Matchmaker computer algorithm. HLA-Matchmaker considers only triplets in antibody-accessible positions of the HLA molecule. HLA-specific antibodies play a major role in graft rejection; therefore, matching at the triplet level can be expected to decrease the antibody production and improve graft survival. A former analysis using HLA-Matchmaker already showed that HLA-A,B-mismatched kidneys that were compatible at the triplet level exhibited almost identical graft survival rates as HLA-A,B-matched kidneys (8).

Our results show that a strong correlation can be found between the number of triplet mismatches and antibody production. If zero triplet mismatches were present, no antibodies were formed in all cases, which implies that rejection of grafts from donors that do express HLA mismatches but no triplet mismatches will not lead to humoral sensitization of the recipient.

In contrast, combinations with 11 or 12 triplet mismatches will almost always lead to sensitization of the patient, as 94% of the patients with a failed transplant produced specific antibodies against the donor. A similar strong correlation between the number of triplet mismatches and antibody production was observed for pregnancy, although the immunogenicity of the fetus was significantly lower than that of a rejected kidney graft.

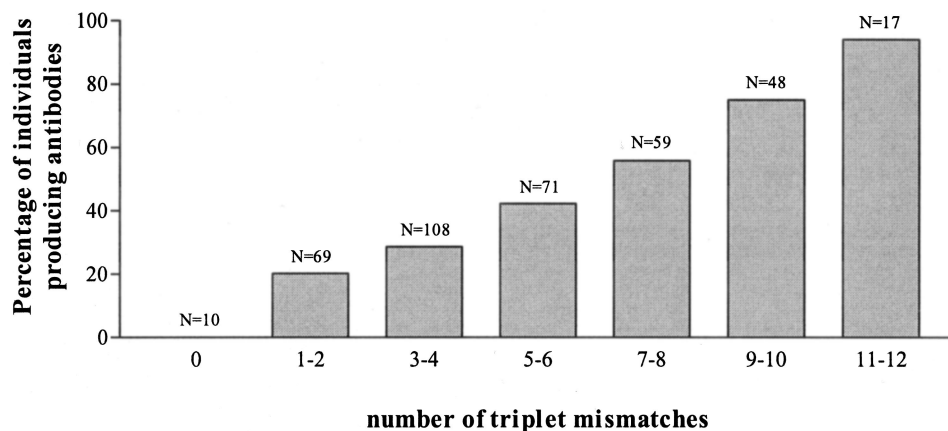


FIGURE 1. Correlation between the percentage of renal patients with failed transplants producing antibodies against the donor and the number of triplet mismatches between patient and donor.

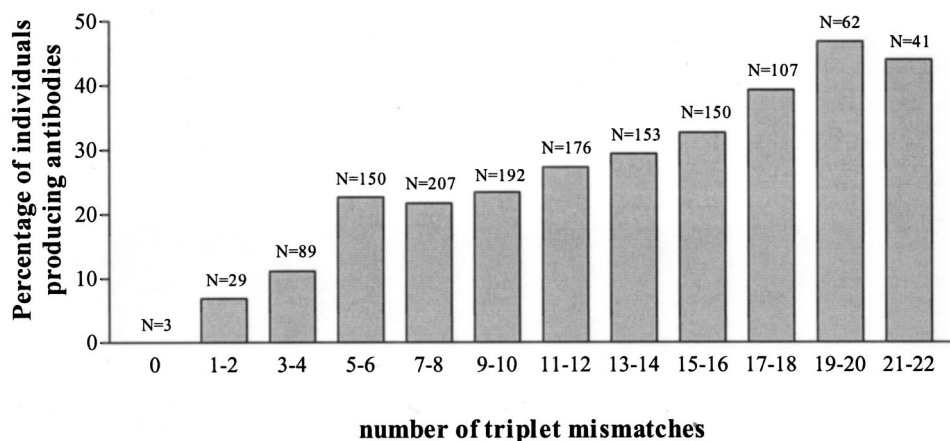


FIGURE 2. Correlation between the percentage of mothers producing antibodies against the mismatched paternal HLA antigens of the child and the number of triplet mismatches between mother and child.

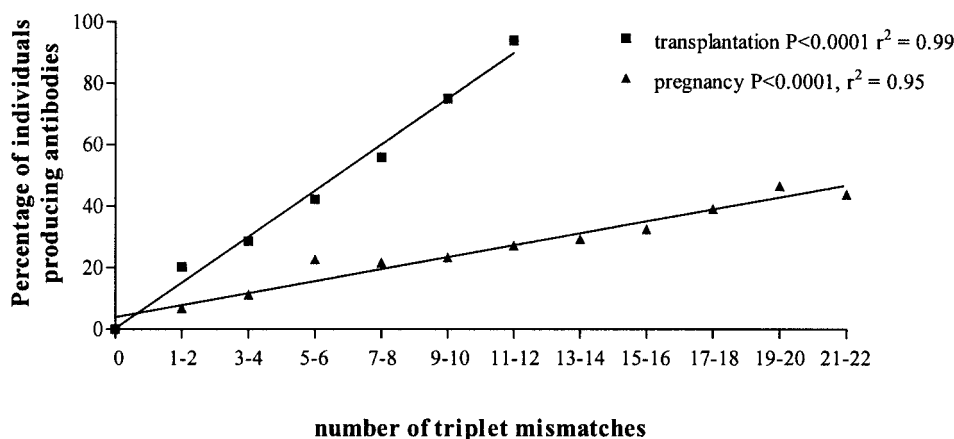


FIGURE 3. Correlation between the percentage of individuals producing antibodies in association with a failed transplantation or pregnancy and the number of triplet mismatches. Statistical analysis using linear regression showed a strong positive correlation between the number of triplet mismatches and the percentage of patients producing specific antibodies against the donor ($P < 0.0001$, $r^2 = 0.99$) and the percentage of mothers producing specific antibodies against their child ($P < 0.0001$, $r^2 = 0.95$).

Several factors can be responsible for this difference. During pregnancy, there is limited fetomaternal hemorrhage. The placenta acts as a barrier between mother and child and serves as a filter for the passage of antibodies to the fetus. The placental trophoblasts can be considered as an allograft and are the only cells that are in contact with maternal blood. However, these cells do not express MHC class I, thereby minimizing tissue incompatibility and reducing the presentation of paternal antigens to maternal T cells (9). In contrast, in transplantation, the endothelium of the graft expresses HLA class I and II molecules and comes into direct contact with blood of the patient, which implies a much higher antigenic stimulation compared with pregnancy.

Furthermore, multiple studies show that the maternal immune system is locally suppressed by various pregnancy-associated proteins, such as β -1 glycoprotein and α -fetoprotein, produced by the placental cells (10). Finally, the placenta can express Fas, which can induce apoptosis of lymphocytes by binding to Fas ligand (11).

The current results indicate that analysis of the number of triplet mismatches between patient and donor can predict the antibody reactivity against the mismatched HLA antigens of failed transplants. This study focused on the patients who rejected their graft. Therefore, in a future study, it would be interesting to analyze the effect of triplet mismatching in patients with functioning transplants to determine its effectiveness in reducing graft rejection.

CONCLUSION

The HLA Matchmaker program is already used by the Eurotransplant Reference Laboratory for the definition of acceptable mismatches for highly sensitized patients (12). The present study suggests that further implementation of triplet matching in a kidney allocation scheme might reduce the incidence of humoral graft rejection and minimize the sensitization grade of retransplant candidates.

Acknowledgments. The authors thank the technicians of the transplantation laboratory of the Leiden University Medical Center for typing and screening the sera from all patients studies, and Drs. A. Brand and G. M. Th. Schreuder for critically reading the manuscript.

REFERENCES

- Power DA, Mason RJ, Stewart GM, et al. The fetus as an allograft: Evidence for protective antibodies to HLA-linked paternal antigens. *Lancet* 1983; 2: 701.
- Suciu-Foca N, Reed E, Rohowsky C, et al. Anti-idiotypic antibodies to anti-HLA receptors induced by pregnancy. *Proc Natl Acad Sci USA* 1983; 80: 830.
- Babbage SJ, Arkwright PD, Vince GS, et al. Cytokine promoter gene polymorphisms and idiopathic recurrent pregnancy loss. *J Reprod Immunol* 2001; 51: 21.
- Dankers MKA, Roelen DL, Van der Meer-Prins EM, et al. Differential immunogenicity of HLA mismatches: HLA-A2 versus HLA-A28. *Transplantation* 2003; 75: 418.
- Dankers MKA, Roelen DL, Korfage N, et al. Differential immunogenicity

- of paternal HLA class I antigens in pregnant women. *Hum Immunol* 2003; 64: 600.
6. Dankers MKA, Roelen DL, Nagelkerke NJD, et al. The HLA-DR phenotype of the responder is predictive for the humoral response against HLA class I antigens. *Hum Immunol* 2004; 65: 13.
 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. Head JR, Drake BL, Zukerman FA. Major histocompatibility antigens on trophoblast and their regulation: Implications in the maternal-fetal relationship. *Am J Reprod Immunol Microbiol* 1987; 15: 12.
 10. Malan BI. Modulation of the humoral immune response by placental secretory factors. *Am J Reprod Immunol* 1996; 35: 529.
 11. Guller S. The role of placental Fas ligand in maintaining immune privilege at maternal-fetal interfaces. *Semin Reprod Endocrinol* 1999; 17: 39.
 12. Claas FHJ. Predictive parameters for in vivo alloreactivity. *Transplant Immunol* 2002; 10: 137.

0041-1337/04/7708-1239/0

TRANSPLANTATION

Copyright © 2004 by Lippincott Williams & Wilkins, Inc.

Vol. 77, 1239–1245, No. 8, April 27, 2004

Printed in U.S.A.

XANTHINE OXIDOREDUCTASE AND PRESERVATION INJURY IN HUMAN LIVER TRANSPLANTATION

RAMON MARTÍ,¹ ENRIC MÚRIO,² ENCARN VARELA,¹ ITXARONE BILBAO,² CARLES PASCUAL,¹ CARLOS MARGARIT,² AND ROSA M. SEGURA^{1,3}

Background. Preservation injury is a major cause of primary graft dysfunction in liver transplantation (LT). Oxidative damage is considered to be the first event leading to graft damage. Xanthine oxidoreductase (XOR) and neutrophil activation, two sources of reactive oxygen species, could play a role in the development of graft dysfunction.

Methods. We determined activities of XOR forms, polymorphonuclear elastase (PMN-E), aminotransferases, and hyaluronic acid in plasma of 20 patients undergoing LT. Samples were taken from the radial artery (RA) before the anhepatic phase; from the portal vein (PV) before reperfusion; from graft caval effluent (CE) at reperfusion; and from RA, PV, and the hepatic vein (HV) 10 and 90 min postreperfusion.

Results. The graft, but not recipient bowel, released XOR into blood (XOR in CE, median, 61.2 mU/g protein [range, 1.9–160.4 vs. undetectable in PV before reperfusion]). Circulating XOR was transformed from dehydrogenase to reversible oxidase (XO_{rev}) (XO_{rev}-to-XOR ratio, 48.1% in CE and 65.1% in HV 90 min postreperfusion). Neutrophil activation was detected in the recipients before reperfusion, and in liver at early postreperfusion (median PMN-E was 0.85 μg/g protein [range, 0.01–1.58] in RA before the anhepatic phase; 2.22 μg/g protein [range, 0.20–5.88] in PV prerreperfusion; and 3.60 μg/g protein [range, 0.48–6.78] in HV 10 min postreperfusion). XOR, but none of the other

markers, was higher in the CE of patients with moderate primary graft dysfunction than in those with slight primary graft dysfunction.

Conclusions. XOR release and neutrophil activation are produced during LT, and they are potentially injurious mechanisms associated with this therapy.

Liver transplantation (LT) is an effective treatment for end-stage liver disease. However, early graft dysfunction is still a serious complication in which preservation-reperfusion injury is considered to play a central role (1). There is some evidence to suggest that cold ischemia of the liver first affects the sinusoid lining cells and that the damage can later extend to parenchymal cells (2). After reperfusion, the production of reactive oxygen species (ROS) causes cell damage and triggers further processes, such as expression of adhesion molecules in leukocytes and endothelial cells, subsequent infiltration and adhesion of activated neutrophils to the endothelium, and microcirculatory disorders that can alter graft function (3). Xanthine oxidoreductase (XOR) and activated neutrophils are two accepted sources of ROS that cause oxidative injury in tissues after ischemia and reperfusion (4). Moreover, during activation, the infiltrated neutrophils release proteolytic enzymes that could enhance ischemia-reperfusion injury in transplanted organs.

XOR catalyzes the two final steps in the degradation of purines in humans, oxidizing hypoxanthine and xanthine to uric acid. The enzyme exists in two forms: xanthine dehydrogenase (XD), which has NAD⁺ as the electron acceptor, and xanthine oxidase (XO), which is oxygen dependent and generates ROS as products of its catalysis (5). In tissues, XD is the main form, and it can be transformed into XO through two mechanisms: reversible oxidation of thiol groups, generating reversible XO (XO_{rev}), or partial proteolysis, generating the irreversible form (XO_{irr}) (6). According to Roy and McCord (4), during the ischemic phase, the loss of ionic equilib-

¹ Biochemistry Department, Hospital Universitari Vall d'Hebron, Barcelona, Spain.

² Liver Transplantation Unit, Hospital Universitari Vall d'Hebron, Barcelona, Spain.

³ Address correspondence to: Rosa M. Segura, Ph.D., Servei de Bioquímica, Hospital Universitari Vall d'Hebron, Passeig Vall d'Hebron, 119-129, 08035 Barcelona, Spain. Email: rsegura@hg.vhebron.es.

Received 18 September 2003.

Revision requested 27 October 2003. Accepted 10 November 2003.

DOI: 10.1097/01.TP.0000120384.52033.BC