

## Humoral Sensitization Against Rejected Grafts: Specific Antibodies to Graft Immunogenic Amino Acid Triplets

A. Iniotaki-Theodoraki, E. Kalogeropoulou, M. Apostolaki, I.N. Doxiadis, and C. Stavropoulos-Giokas

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### ABSTRACT

Humoral sensitization against immunogenic amino acid (aa) triplets expressed on a rejected graft was analyzed in 83 retransplant candidates. All patients had lost a graft with HLA-A,-B mismatches. The alloantibodies were detected by a complement-dependent cytotoxicity (CDC) technique and an ELISA method in parallel; they were classified as HLA graft-specific (GS) and non-GS antibodies. The aa triplet specificity of the antibodies was assessed using the HLAMatchmaker algorithm. HLA class I antibodies were detected in 74 of 78 (94%) cases, including GS reactivity in 55 (74.3%) and non-GS in 72 (97.2%), either alone ( $n = 19$ ) or in parallel with GS antibodies ( $n = 53$ ). For all HLA-GS-antibody-reactive patients, we defined the specificity against immunogenic aa triplets on the previous graft. Moreover, antibodies specific to graft aa triplets were observed within the non-GS antibodies among 19 of 19 and 28 of 53 cases, respectively. Therefore, aa triplet-specific antibodies against the rejected graft were present in all 74 cases with HLA class I antibodies. Antibodies against aa triplets expressed on all HLA class I-mismatched graft antigens were present in 73% of cases. The high extent of humoral alloreactivity against a rejected graft supports the decision to avoid repeated exposure to immunogenic aa triplet mismatches on a second graft. An accurate analysis for performed antibodies in these cases may be beneficial to select the most suitable second donor.

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**A**PPROXIMATELY 96% of patients who reject a kidney allograft display serum alloantibodies.<sup>1</sup> These antibodies have been characterized as HLA graft- or non-graft-specific antibodies, depending on whether they react with graft-mismatched human leukocyte antigens (HLA).

It is well known that the HLA class I molecules are extremely polymorphic, expressing private and public epitopes. In a previous study we showed that HLA class I graft-specific (GS) and non-GS antibodies displayed by candidates for a second renal graft may be directed against private or public epitopes on foreign transplants.<sup>2</sup>

A new concept about the amino acid sequences that are recognized by antibodies has recently appeared. Each HLA molecule bears multiple antigenic determinants (epitopes) that are expressed as amino acid (aa) triplets. Each immunogenic epitope is recognized by distinct alloantibodies.<sup>3</sup> It is now possible to define the potentially immunogenic aa triplets on graft alloantigens using HLAMatchmaker, an HLA matching algorithm. This algorithm uses intralocus and interlocus comparisons of polymorphic triplet sequences of HLA antigens in the recipient versus donor to

define the spectrum of nonshared aa triplets.<sup>3,4</sup> The goal in the present study was to clarify the extent of sensitization against a previous graft among patients awaiting retransplantation by using the aa triplet specificity of the alloantibodies present in the patient's serum. This methodology permits an accurate analysis of the preformed alloantibodies displayed in patients awaiting retransplantation, possibly facilitating the selection of an appropriate second donor.

### PATIENTS AND METHODS

During the last 2 years sequential blood samples were collected from 83 renal transplant candidates for a second graft (58 men/25 women). All patients had lost a previous graft with  $\geq 1$  HLA-A and

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From the National Tissue Typing Center, General Hospital of Athens, Athens, Greece (A.I.-T., E.K., M.A., C.S.-G.); and Immunohaematology and Blood Transfusion, Leiden University Medical Center, Leiden, The Netherlands (I.N.D.).

Address reprint requests to Dr Aliki Iniotaki-Theodoraki, National Tissue Typing Center, General Hospital of Athens, 34 Davaki Street, 174 55, Alimos, Athens, Greece. E-mail: gtheo@bigfoot.com

**Table 1. Example for the Definition of Graft Triplet-Specific (GTS) Antibodies**

	Position												
	9	12	17	41	45	56	62	66	70	74	76	80	150
Donor mismatch													
HLA-A3												150AHe	
HLA-B7				45Ee*			62Rn	66qly	70aQa				
Antibodies													
HLA-A32												80rAlr†	
HLA-B27				45Ee*				66qlc	70aKa	76Ed			
HLA-B51				45Te			62Rn	66qlf			80rAlr†		
HLA-B52				45Te							80rAlr†		

The HLA phenotype of the recipient was HLA-A2; -A11; -B44, -B60. The mismatched HLA donor molecules were HLA-A3, -B7. The specificities of the detected antibodies were HLA-A32, -B27, -B51, -B52. The nonshared immunogenic triplets with the donor and with nondonor alloantigens, against which antibodies were detected, are shown. HLA-B27-specific antibodies seem to recognize the immunogenic triplet 45Ee (\*), also expressed on the HLA-B7 graft molecule (GTS). Moreover, HLA-A32, -B51, -B52-specific antibodies seem to recognize the immunogenic triplet 80rAlr (†), which is not expressed on the previous graft (sensitization event other than transplant).

≥1 HLA-B mismatched (MM) antigen. In 57 of 83 cases (68.7%), HLA-DR,-DQ MMs were also present. Moreover, 62 patients had received at least one blood transfusion. All recipients and donors had been typed for HLA class I and II antigens by serologic and molecular biologic techniques (PCR-SSP). HLA class I- and II-specific antibodies were detected and analyzed in parallel, using an antihuman globulin CDC method (90 T- and B-cell lines; LCT, One Lambda) and an ELISA technique (LAT-1288; One Lambda). Antibody specificity was determined by chi-square tests ( $R > .50$ ) using a computer program. The antibodies were characterized as HLA graft-specific (GS) and non-GS antibodies.

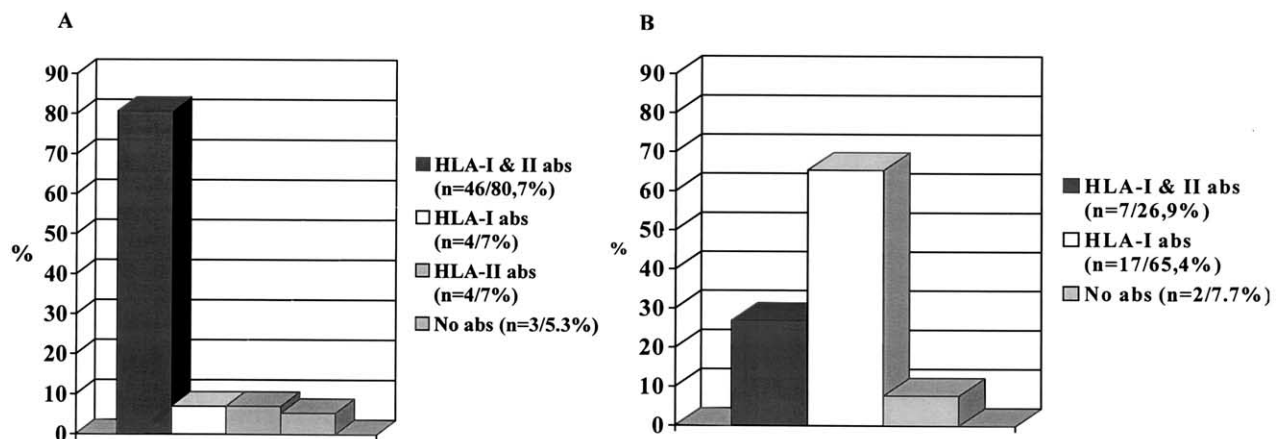
**Definition of Epitope Specificity**

The HLA Matchmaker computer algorithm was used to define the immunogenic aa triplets on the graft by comparing the polymorphic aa triplets on the HLA class I molecules of the recipient versus the previous donor. Because the program can also incorporate information about aa triplet sequences of HLA antigens against which the patient displayed antibodies, it was possible to define the presence of antibodies that may recognize polymorphic aa triplets on a potential donor. Following this approach we defined the aa triplet specificity of HLA GS antibodies and HLA non-GS antibodies. In the latter case, the alloantibodies that seemed to

recognize immunogenic aa triplets on a graft were considered as graft triplet-specific (GTS) antibodies. Thus, the GTS antibodies recognized shared aa triplets expressed on graft and nongraft HLA patients A molecules. (Table 1). Fisher's Exact *P* test was used for statistical analysis.

**RESULTS**

Alloantibodies were detected in 78 of 83 (93.9%) patients. The distribution of HLA class I-specific antibodies among patients who had lost a previous graft with or without an HLA class II incompatibility is shown in Fig. 1. HLA class I-specific antibodies were present in 74 of 78 (94%) cases. HLA class I GS antibodies were present in 55 of 74 (74.3%) cases; for all antibody reactivity patterns we defined the specificity against immunogenic aa triplets. Non-GS antibodies were present in 72 cases, either alone ( $n = 19$ ) or in parallel with GS antibodies ( $n = 53$ ). GTS antibodies were present in 19 of 19 and 28 of 53 cases of non-GS antibodies, respectively, suggesting that specific antibodies to the rejected graft were detected in all ( $n = 74$ ) cases with HLA class I antibodies. Moreover, antibodies specific to all HLA



**Fig 1.** Alloantibodies detected in patients ( $n = 83$ ) who lost a graft with mismatched alloantigens of HLA class I and II (A), or only HLA class I (B). Sixty-two patients had also received at least one blood transfusion.

**Table 2. All 74 Patients With HLA Class I-Specific Antibodies Were Found to Have Antibodies Against Polymorphic AA triplets on the Rejected Graft**

Antibodies against all or not all mismatches (MMs)	GS antibodies* only [n (%)]	GS and GTS antibodies [n (%)]	GTS antibodies only [n (%)]	Total [n (%)]
All MMs	20 (27%)	22 (30%)	12 (16%)	54 (73%)
Not all MMs	7 (9.5%)	6 (8%)	7 (9.5%)	20 (27%)
Total	27 (36.5%)	28 (37.8%)	19 (25.7%)	74 (100%)

\*GS, graft specific; GTS, graft triplet specific.

class I graft MM antigens were present in 73% (54 of 74) of cases when aa triplet specificity was assessed, but in only 27% (20 of 74) of cases when only GS (anti-HLA) antibodies were evaluated ( $P = .0000$ ) (Table 2).

Alloantibodies against aa triplets not expressed on donor alloantigens were present in 38 of 74 (51.3%) patients, in parallel with GS or GTS antibodies. These antibodies may have resulted from sensitization due to blood transfusions. In 9 of 83 (10.8%) cases, no HLA class I antibodies were detected despite an HLA-A or -B incompatibility with the rejected graft. In 3 of 9 cases without HLA class I antibodies, the aa triplet matching between donor and recipient revealed one or zero triplet mismatch between the specific HLA donor-recipient molecules.

## DISCUSSION

The common causes of sensitization in transplantation are blood transfusion, pregnancy, and allograft rejection. Sensitization by whatever route leads to activation of both the innate and the adaptive arms of the immune response. The inevitable consequences of this activation process are a more rapid immune response when the same antigen is encountered a second time.<sup>5</sup> Recently, humoral alloresponses toward immunogenic epitopes have been represented as aa triplets on the exposed protein sequences.<sup>3</sup> The goal of this study was to evaluate the extent of humoral sensitization against a rejected graft among patients awaiting retransplantation. This was performed by defining alloantibodies specific for aa triplets expressed on the previous graft.

A broad sensitization against the previous graft was revealed among patients awaiting retransplant. All patients with HLA class I antibodies were found to be sensitized toward specific immunogenic epitopes expressed on the rejected graft. In most cases (73%), these epitopes were present on all HLA class I-mismatched antigens. Apart from the HLA GS antibodies that recognize immunogenic triplets on graft alloantigens, non-GS anti-HLA antibodies were also discovered that may also recognize polymorphic triplets (GTS antibodies) on the previous graft. In the latter case, the GTS antibodies recognized share aa immunogenic triplets that are expressed on graft HLA molecules and nongraft HLA molecules.

It is unknown whether these results indicate an expansion of humoral alloreactivity (epitope spreading) against incompatible HLA graft molecules during the development

of chronic rejection, a phenomenon that has been described for the cellular alloimmune responses.<sup>6</sup>

It is well known that patients receiving a second graft show lower graft survival rates than nonsensitized recipients, even though sensitive crossmatch techniques facilitate second transplants with a lower risk of early graft loss.<sup>7</sup> Therefore, an accurate analysis of the performed antibodies among patients awaiting retransplant will help to select a suitable donor. In such cases, HLA aa triplet matching between the recipient and the second donor may reveal aa the second graft against which the host has preexistent antibodies, helping to exclude high-risk transplants.

In some cases we were not able to find HLA class I antibodies against specific alloantigens despite exposure of the recipient to foreign molecules. Whether an alloepitope will be presented by the professional antigen-presenting cells to allopeptide-specific T cells is determined by many factors, including the affinity of peptide binding to the HLA molecules, the peptide competition for antigen presentation or the precursor frequency of T-cell clones specific for a given determinant.<sup>8</sup> It is possible that some mismatch alloepitopes are not recognized across specific donor/recipients pairs. Moreover, three recipients in this study, with no antibodies against specific alloantigens, shared zero or one MM aa triplet with the donor, cases in which no production of antibodies was anticipated.<sup>4</sup> It is uncertain whether the absence of antibodies in other cases showing aa triplets mismatches was the result of recipient nonreactivity to specific donor alloantigens.

It is well known that almost all patients who reject a transplant become sensitized.<sup>9</sup> Patients who have already experienced graft loss are an immunologically unique group that has been exposed to a bolus of antigens from a graft and, in most cases, also from blood transfusions. In this study we defined the epitope specificity of the detected antibodies by distinguishing those specific to graft aa triplets from those produced from a sensitization event other than transplantation. Because the number of patients awaiting retransplantation is continuously increasing, an accurate analysis of preformed alloantibodies is becoming increasingly important. This study has shown that, among retransplant candidates, the definition of aa triplet specificity of alloantibodies indicates the extent of sensitization. This process may be beneficial to select a suitable second donor that allows one to achieve graft survival rates for the sensitized recipients equal to those of nonsensitized recipients.

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