has been observed. In this series with tacrolimus, we did not observe any episode of rejection during pregnancy, although there was a reduction in prednisone dosage and a decrease in the tacrolimus concentration by nearly 32%.

There was no increase in serum creatinine during the pregnancy. However, five (38%) mothers lost renal-allograft function 20 to 98 months postdelivery and 56 to 132 months posttransplantation. The impact of pregnancy on graft loss is difficult to evaluate, but the high rate of graft loss is clearly of concern. This observation is also different than what is observed in pregnancies after liver transplantation under tacrolimus (*10*, *11*).

Pregnancy after SPKTx has been previously reported. In this series, there were three deliveries in two mothers; one mother delivered twice and experienced pre-eclampsia on both occasions. She was managed medically and delivered successfully. Both mothers maintained pancreatic and renal function 42 to 62 months after the first delivery.

CONCLUSIONS

Pregnancy after KTx and SPKTx under tacrolimus-based immunosuppression has the same problems of prematurity, preterm delivery with low birth weight, and intrauterine growth retardation that have been observed with pregnancy after transplantation with other immunosuppressive agents. Although impaired renal function at the time of pregnancy should be considered an important risk factor, toxemia of pregnancy, deterioration of allograft function, or rejection were relatively rare during pregnancy.

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CRITICAL EVALUATION OF THE AMINO ACID TRIPLET-EPITOPE MATCHING CONCEPT IN CADAVER KIDNEY TRANSPLANTATION

GUNTER LAUX, JOANNIS MYTILINEOS, AND GERHARD OPELZ

Background. **A computer-based approach for determining human leukocyte antigen (HLA) compatibility between kidney donors and recipients on the basis of differences of amino acid sequences as motifs for immunogenic epitopes was proposed by Duquesnoy et al. The HLAMatchmaker algorithm focuses on HLA class I polymorphisms of serologically defined antigens encoded by the HLA-A and -B loci. HLA phenotypic mismatches that represent only a few mismatches at the amino acid triplet level are held to be not or only mildly immunogenic. This approach was proposed as being especially suitable for the allocation of donor kidneys to highly sensitized patients.**

Methods. **We reexamined this attractive concept using the data of the Collaborative Transplant Study. Intra- and interlocus comparisons for HLA-A and -B were performed according to the original HLAMatchmaker algorithm. To exclude the influence of HLA-DR, only transplants with no HLA-DR mismatch were considered. Patients who had one HLA-A and one HLA-B antigen mismatch were separated into subgroups, depending on the number of triplet mismatches as calculated by the HLAMatchmaker software. Separate**

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analyses were performed for first transplants, retransplants, and patients with a panel-reactive antibody activity of 50% or more. A total of 16,997 white patients matched for HLA-DR who received a cadaver kidney transplant between 1991 and 2001 formed the basis of this analysis.

Results. **Application of the HLAMatchmaker method could not be shown to result in any statistically significant effect on graft survival.**

Conclusions. **The HLAMatchmaker concept is theoretically attractive; however, it could not be shown to yield useful results in this analysis. Serologic HLA typing appears to provide an insufficient basis for applying epitope matching in clinical kidney transplantation.**

The identification of "acceptable" human leukocyte antigen (HLA) antigen mismatches for kidney-transplant candidates with a high panel-reactive antibody (PRA) activity is cumbersome because it requires extensive serum screening using large cell panels (*1*). Duquesnoy (*2*) presented an elegant alternative approach for the identification of kidney donors for highly immunized patients and found this approach advantageous for nonimmunized recipients as well. A computer program (HLAMatchmaker) based on molecular comparisons of amino acid triplets within exposed regions of HLA-A and -B chains as motifs for potentially immunogenic epitopes was developed. Because the genetic products at these loci are very similar in structure and amino acid sequence, intra- and interlocus comparisons are taken into account by the HLAMatchmaker software.

The primary premise of HLAMatchmaker is that each HLA antigen represents a composition of polymorphic triplets that can act as immunogenic epitopes. The second premise is that patients cannot mount an immune response against triplets expressed by their own HLA molecules. The underlying computer algorithm converts each HLA-A and -B antigen defined at the serologic-split resolution into a string of amino acid triplets and calculates the number of shared and nonshared triplets between donor and recipient. Published amino acid sequences of serologically defined HLA antigens are used for this conversion (*3*). Phenotypical HLA antigen mismatches that are fully compatible at the triplet level or have only few triplet mismatches (TMM) are considered to represent "permissible" mismatches. A detailed description of the HLAMatchmaker algorithm can be found elsewhere (*1*, *2*).

Duquesnoy et al. (*4*) reported that HLA-DR compatible cadaver kidney transplants exhibiting serologically defined HLA-A and -B antigen mismatches that were associated with no or only few amino acid TMM showed the same graft survival rates as HLA-A, -B, and -DR matched allografts. In an effort to verify this claim, we applied the HLAMatchmaker approach to the data of the international Collaborative Transplant Study (CTS).

METHODS

The impact of HLA-A and -B locus amino acid triplet matching on the outcome of cadaver-kidney transplants was analyzed using the CTS database (*5*). For each donor-recipient pair, the amino acid TMM for the loci HLA-A and -B were calculated according to the original HLAMatchmaker algorithm, and graft survival was analyzed in relation to the number of TMM.

Duquesnoy and Marrari (*6*) performed analyses to estimate the relative immunogenicity of different mismatched triplets. According to these estimations, certain triplets were regarded to be immunogenic, whereas others were considered to be innocuous for the graft. The latest version of HLAMatchmaker contains information concerning these triplets (*7*). We therefore implemented a separate analysis step to evaluate, in addition to the original concept of TMM, the impact of so-called immunogenic triplet mismatches (TMMI) on graft survival. The HLAMatchmaker software is freely accessible and downloadable (*7*).

To reduce any confounding influence of HLA-DR, only HLA-DR matched transplants were considered in this study. The typing results were reported by the participating centers to the CTS study, and most typings performed during the study period can be assumed to have been performed by molecular methods. We selected transplants matched for the specificities HLA-DR1 to HLA-DR10. The number of TMM and TMMI was calculated for the total of 16,997 white patients matched for HLA-DR and typed for HLA-A and -B antigens at the split resolution who received a cadaver-kidney transplant between 1991 and 2001. A subset of 4,038 patients fell into the category with one HLA-A and one HLA-B antigen mismatch, which formed the key subgroup for this analysis. Graft-survival rates were calculated according to the method of Kaplan and Meier (*8*). Statistical significance was estimated using the log-rank test. No exclusions were made for any reason.

RESULTS

Table 1 shows the results of the TMM analysis in first transplants, retransplants, and grafts into patients with a PRA value of 50% or more. Table 2 shows the corresponding analysis for TMMI. By definition, the number of immunogenic triplets (TMMI) per allele is lower than the number of polymorphic triplets (TMM). For presentation in the tables, we chose six TMM and five TMMI for the differentiation of "good" and "poor" matches.

We were unable to confirm a statistically significant correlation between triplet epitope matching and kidney-graft survival. The claim that a small number of mismatches at the HLA-A and -B amino acid triplet level would result in better graft outcome (*4*) is not supported by our results (Tables 1 and 2). In addition, we did not obtain statistically significant correlations when other threshold values (e.g., the threshold values used by Duquesnoy et al.) (*4*) for the differentiation between good and poor matches were chosen. Neither did the correlation improve when transplants performed in Western Europe or North America were analyzed separately, or when the time periods 1991 to 1995 and 1996 to 2001 were compared. Remarkably, in spite of carrying out multiple attempts, a significant difference in graft survival between patients with high or low numbers of TMM did not emerge in any of our analyses. For illustration, patients with 0 to 6, 7 to 9, 10 to 12, or greater than or equal to 13 TMM showed no significant differences in graft survival (at 5 years 73.31±2.90%, 71.54±2.13%, 71.00±1.87%, and 71.08±1.22%, respectively; P=NS) (Fig. 1). An analysis of TMMI also failed to show significant results (Fig. 2). In an attempt to further reduce the influence of confounding factors, we carried out an analysis of transplants with only a single phenotypic mismatch, either at the HLA-A or HLA-B locus (Fig. 3). Even in this analysis step, a significant effect of the number of TMM on graft survival could not be shown.

The claim that, among cadaver-kidney transplants with zero HLA-DR mismatches, those with phenotypical HLA-A and -B antigen mismatches but zero amino acid TMMs had

TABLE 1. Effect of amino acid triplet mismatches on kidney-graft survival

	Number of triplet mismatches (TMM)	Number of transplants	Graft-survival rate at 5 years (%±SE)
First transplants	0–6 TMM	357	73.31±2.90
	7–9 TMM	678	71.54±2.13
	10–12 TMM	898	71.00±1.87
	≥13 TMM	2,105	71.08±1.22
Retransplants	0–6 TMM	73	60.68±6.62
	7–9 TMM	137	57.10±6.16
	10–12 TMM	147	67.51±4.53
	≥13 TMM	316	67.99±3.12
Transplants PRA≥50%	0–9 TMM	40	50.08±8.97
	>9 TMM	92	63.05±5.54
First transplants Western Europe	0–6 TMM	279	76.30±3.11
	7–9 TMM	490	72.65±2.41
	10–12 TMM	649	71.32±2.14
	≥13 TMM	1,498	70.33±1.46
First transplants North America	0–6 TMM	44	65.61±10.32
	7–9 TMM	96	72.90±5.88
	10–12 TMM	36	72.44±10.23
	≥13 TMM	293	70.85±3.49
First transplants performed between 1991 and 1995	0–6 TMM	144	68.31±4.04
	7–9 TMM	286	73.13±2.75
	10–12 TMM	382	67.99±2.49
	≥13 TMM	864	68.40±1.67
First transplants performed between 1996 and 2001	0–6 TMM	213	76.96±4.75
	7–9 TMM	392	69.53±3.83
	10–12 TMM	516	73.69±3.11
	≥13 TMM	1,241	73.78±1.95

P=NS for all comparisons.
PRA, panel-reactive antibody.

TABLE 2. Effect of so-called immunogenic triplets (TMMI) on kidney-graft survival

	Number of triplet mismatches (TMMI)	Number of transplants	Graft-survival rate at 5 years (%±SE)
First transplants	0–5 TMMI	1,191	71.94±1.60
	6–8 TMMI	987	71.97±1.77
	9–11 TMMI	831	71.47±1.91
	≥12 TMMI	1,029	69.83±1.79
Retransplants	0–5 TMMI	231	62.47±4.06
	6–8 TMMI	169	65.66±4.40
	9–11 TMMI	127	68.06±4.99
	≥12 TMMI	146	66.65±4.52
Transplants PRA≥50%	0–5 TMMI	49	53.96±7.82
	>5 TMMI	83	62.96±5.93
First transplants Western Europe	0–5 TMMI	872	72.79±1.82
	6–8 TMMI	713	73.30±2.00
	9–11 TMMI	612	70.32±2.23
	≥12 TMMI	719	69.04±2.16
First transplants North America	0–5 TMMI	166	70.96±4.83
	6–8 TMMI	153	67.81±5.05
	9–11 TMMI	96	71.27±6.29
	≥12 TMMI	152	70.27±4.53
First transplants performed between 1991 and 1995	0–5 TMMI	494	70.77±2.14
	6–8 TMMI	420	71.79±2.29
	9–11 TMMI	359	66.71±2.61
	≥12 TMMI	403	66.33±2.51
First transplants performed between 1996 and 2001	0–5 TMMI	697	72.07±2.85
	6–8 TMMI	567	71.07±3.26
	9–11 TMMI	472	77.20±2.83
	≥12 TMMI	626	73.55±2.63

P=NS for all comparisons.
PRA, panel-reactive antibody.

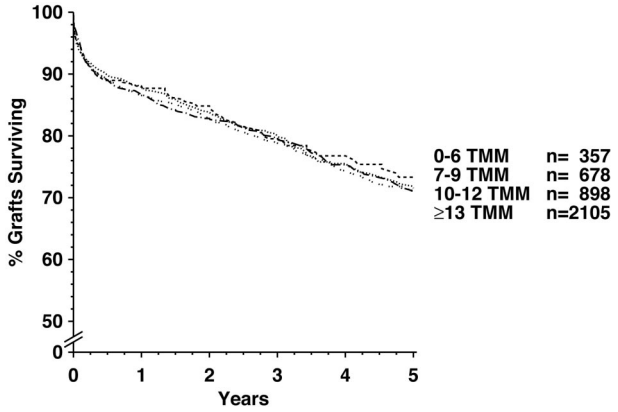

FIGURE 1. HLAMatchmaker evaluation. Effect of amino acid triplet mismatches (TMM) on graft survival of first cadaver kidney transplants. Numbers of transplants studied are indicated for each curve. *P*=NS.

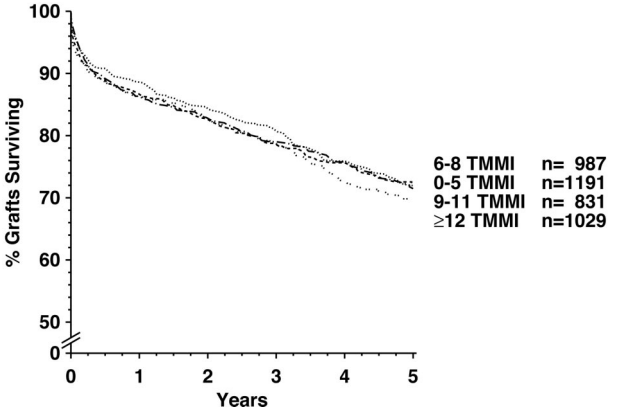

FIGURE 2. Effect of amino acid triplet mismatches on graft survival of first cadaver kidney transplants considering so-called immunogenic triplets (TMMI). No significant influence on graft survival was found. *P*=NS.

the same graft survival rate as HLA-A, -B, and -DR phenotypically compatible grafts (*2*) could not be verified. To the contrary, cadaver-kidney transplants with zero HLA-A and -B mismatches showed a significantly better graft outcome than transplants with zero TMMI (at 5 years 74.85±0.78% vs. 70.44±2.04%, $P=0.0058$) (Fig. 4).

DISCUSSION

Although the concept of identifying certain HLA mismatches as nondeleterious on the basis of the number of incompatible amino acid triplets is attractive, its practical implementation using the HLAMatchmaker program did not yield the desired result in this independent analysis. In particular, the following issues appear problematic:

1. The HLAMatchmaker amino acid triplet assignment is based on the most frequently occurring allele (as defined by DNA technology), which is part of a given serologically defined phenotypic HLA specificity. These assumed assignments are incorrect in all those instances where a patient carries an allele that is different from the most common one. We therefore believe that amino acid triplet comparisons must be performed on the basis of high-resolution DNA-typed alleles. In a recent study, we obtained good results with epitope-based HLA-DPB matching in cadaver-kidney retransplants using high-resolution DNA-typed DPB1 alleles as a basis for our calculations (*9*).

2. Under certain conditions, the HLAMatchmaker algorithm ignores mismatching sequences with more than three amino acids. Table 3 shows an example of a donor-recipient pair having no mismatches when only sequences with a length of three amino acids are considered because every triplet present in the donor is also present in the recipient. Actually, there exists an "8-let" (sequence with 8 amino acids) mismatch, because the amino acid sequence "LRAYLEGL" of the donor between positions 156 and 163, corresponding to allele B*5601, is absent in any of the recipient's alleles.

3. The definition of triplets with different degrees of immunogenicity (*6*) must be performed on the basis of large numbers of cases. In the article of Duquesnoy et al., immunogenicity estimations of polymorphic triplets sometimes were derived from fewer than 25 individuals.

4. We found that the approach chosen by Duquesnoy et al. contains an important pitfall. In contrast with Duquesnoy et al., we systematically considered HLA-DR matched patients with one HLA-A and one HLA-B antigen mismatch. This selection was done because there is a correlation between the

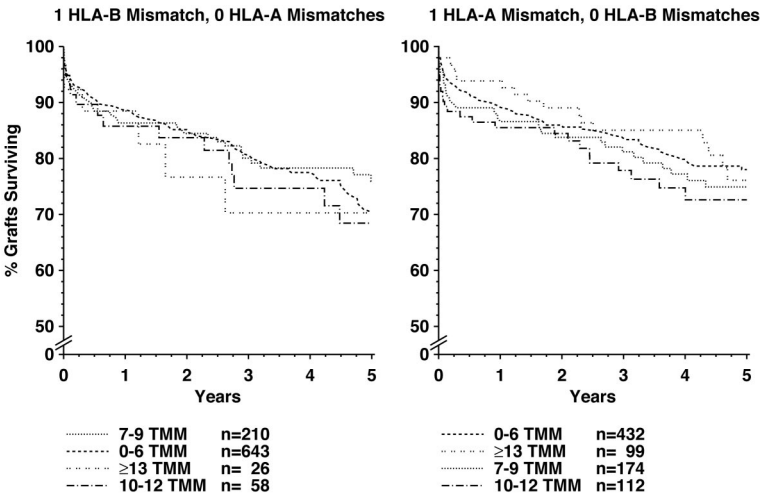

FIGURE 3. Effect of amino acid triplet mismatches on graft survival for patients with a single serologic human leukocyte antigen (HLA) mismatch. First cadaver transplants were studied. Numbers of triplet mismatches (TMM) and numbers of transplants analyzed are indicated. *P*=NS.

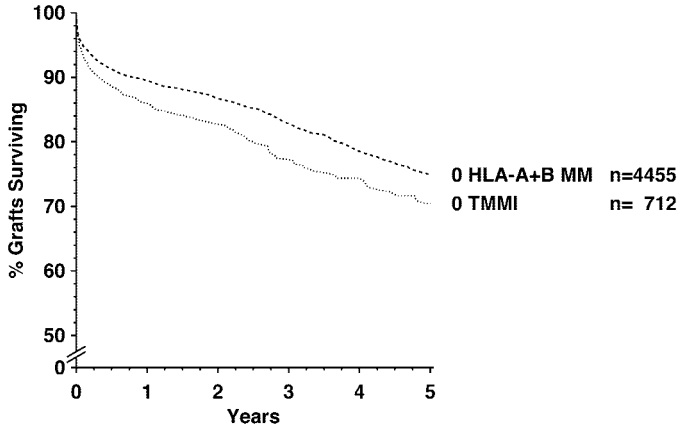

FIGURE 4. Impact of zero HLA-A and -B serologic mismatches versus zero immunogenic triplet mismatches (TMMI) on survival rate of first cadaver kidney transplants. All transplants were matched for HLA-DR. Transplants with zero HLA-A+B mismatches had a significantly better outcome. Log rank, $P=0.0058$.

number of TMM and the number of serologic antigen mismatches. Figure 5a shows the effect of amino acid TMM in HLA-DR matched patients without any restriction on the number of HLA-A and HLA-B antigen mismatches. A significantly better graft outcome can be observed for the group with 0 to 6 TMM as compared with 7 to 12 TMM (at 5 years 74.48±0.70% vs. 71.57±0.89%, respectively, $P=0.0002$). However, Figure 5b shows that this approach yields a misleading result. The group with 0 to 6 TMM (left chart) is much better matched at the serologic antigen level than the 7 to 12 TMM group (right chart). In the 0 to 6 TMM group, 91.8% of the patients had at the most one antigen mismatch, whereas the corresponding fraction in the 7 to 12 TMM group was 30.4% ($P<0.0001$). Figure 5c shows that the amino acid triplet matching effect disappears when only patients with the same antigen match grade are compared (at 5 years 73.31±2.90% vs. 71.23±1.40%, respectively; P=NS). Thus, the effect described by Duquesnoy et al. is mainly caused by the "conventional" HLA-class I antigen mismatch effect rather than by a specific influence of mismatches at the amino acid triplet level.

TABLE 3. Example of a zero TMM mismatch with mismatched sequence of eight amino acids

	HLA antigen	Assigned allele	Amino acid sequence (position 155–164)
Donor	A29	A*2902	QLRAYLEGTC
	A30	A*3001	QLRAYLEGTC
	B07	B*0702	QRRAYLEGEC
	B56	B*5601	QLRAYLEGLC
Recipient	A23	A*2301	QLRAYLEGTC
	A29	A*2902	QLRAYLEGTC
	B07	B*0702	QRRAYLEGEC
	B62	B*1501	QWRAYLEGLC
Mismatched antigens		A30, B56	
Mismatched triplets		None	
Mismatched sequence		LRAYLEGL	

TMM, triplet mismatch; HLA, human leukocyte antigen.

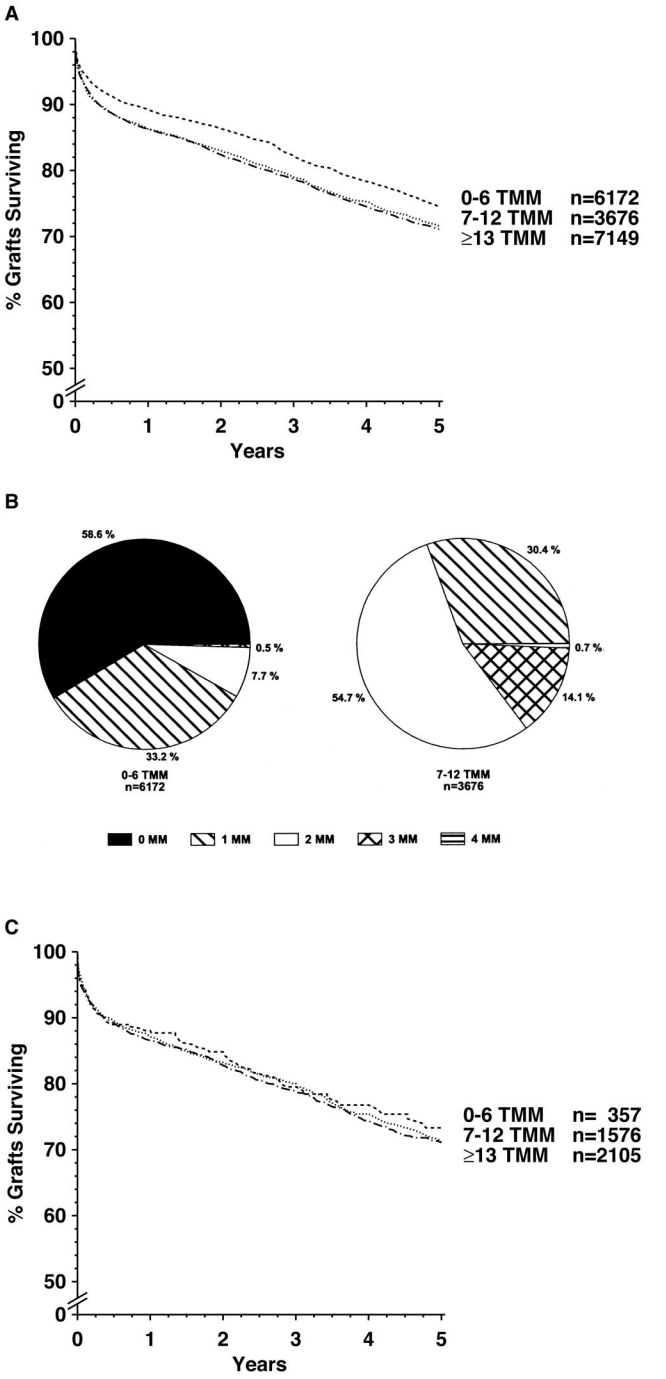

FIGURE 5. (a) Effect of amino acid triplet mismatches in HLA-DR matched patients without any consideration of HLA-A and HLA-B antigen mismatches. (b) Distribution of HLA-A and HLA-B antigen mismatches. Transplants with 0 to 6 TMM show a much more favorable distribution of conventional HLA antigen mismatches (MM) than transplants with 7 to 12 TMM. (c) Effect of amino acid triplet mismatches in HLA-DR matched patients with one HLA-A and one HLA-B antigen mismatch.

We conclude that the HLAMatchmaker concept as proposed by Duquesnoy et al. in its present form is not superior to conventional HLA matching for the allocation of cadaver kidneys. It is possible that a similar approach using high-

resolution DNA typing of HLA alleles, rather than serologic HLA typing, may allow a reliable definition of nonimmunogenic epitopes and the application of amino–acid-based matching in clinical transplantation.

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SURVIVAL OF MITOMYCIN C-TREATED PANCREATIC ISLET XENOGRAFTS IS MEDIATED BY INCREASED EXPRESSION OF TRANSFORMING GROWTH FACTOR-β[1]

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Background. **Mitomycin C (MMC) can trigger various intracellular signals. The authors previously showed that pretreatment of highly immunogenic crude pancreatic islets with MMC improved their survival in a rat-to-mouse transplantation model. The aim of this study was to investigate the role of transforming growth factor (TGF)-β in mediating MMC-induced survival of islet xenografts.**

Methods. **Collagenase-digested islets obtained from WS rats (RT1k) were incubated for 30 min with 10 μg/mL MMC and then transplanted into streptozotocin-induced diabetic C57BL/6 (H-2b) mice after 20 hr of culture at 37°C.**

Results. **Survival of xenografts was enhanced by pretreatment of islets with MMC. MMC-treated xenografts showed a mild inflammatory cell response and significantly minimal infiltration of macrophages, $CD4^+$ T cells, and $CD8^+$ T cells compared with untreated grafts. TGF-β mRNA was increased at 20 hr after MMC treatment, and TGF-β protein expression was also increased compared with untreated islet xenografts. TGF-β concentration in blebs formed around the xenografts (but not in the serum) was higher in animals that underwent transplantation with MMC-treated islets than with untreated islets. Simultaneous transplantation of MMC-treated and untreated islets separately in each kidney of recipient mice showed that protection was only found in MMC-treated islets. Treatment of islets before transplantation by neutralizing anti–TGF-β antibody suppressed the MMC-protective effects on graft survival, whereas no such effect was noted with isotype-matched immunoglobulin.**

Conclusions. **The authors' results indicate that MMC treatment effectively reduces local inflammatory response and that such effects are mediated by increase of TGF-β during the early period of islet transplantation.**

We have previously shown that inoculation of mitomycin C (MMC)-treated donor spleen cells induced specific unresponsiveness in rat cardiac allotransplantation (*1*). Other studies showed that MMC pretreatment of highly immunogenic crude islets leads to significant prolongation of graft survival in a rat-to-mouse model (*2*). This modality is also effective and can produce marked prolongation of islet graft survival when combined with host immunomodulation using antiad-

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