Prevention of Xenograft Rejection by Masking Donor HLA Class I Antigens

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Destruction of target cells by cytotoxic T lymphocytes requires the presence of HLA (human lymphocyte antigen) class I antigens on the target cells for adhesion as well as for triggering of the antigen-specific T cell receptor. Rejection of xenogeneic human pancreatic islets and liver was circumvented by masking, before transplantation, donor antigens with $F(ab')_2$ antibody fragments to HLA class I or tissue-specific epitopes. This strategy eliminated the need for recipient immunosuppression and allowed islet xenograft survival beyond 200 days, as demonstrated functionally by C peptide secretion as well as by histology. These in vivo observations are consistent with the importance of donor HLA class I in eliciting graft rejection and have potential applicability to the successful transplantation of other HLA class I–bearing donor tissues.

CYTOTOXIC T CELLS (CTLs) INDUCE target cell lysis after at least three sequential activation steps (*1*). The lytic cascade is initiated when T cells bind to target cells by virtue of associations between CD2, CD8, and LFA-1 (lymphocyte function–associated antigen–1) molecules on T cells and LFA-3, HLA class I, and ICAM-1 (intercellular adhesion molecule–1) on the target (*2, 3*); antigen-specific activation follows, lytic steps are triggered, and target cell death ensues. This T cell activation process underlies the xenogeneic response that results in the rejection of transplants and presumably in the autoimmune rejection of tissues. Several treatment strategies that interfere with T cell–target cell adhesion have been attempted: T cell vaccines (*4, 5*), antibodies to the T cell receptor (*6, 7*), antibodies to HLA class II (*8, 9*), antibodies to CD4 (*10, 11*), enhancing antibodies (*12, 13*), and blocking peptides that occupy the relevant T cell receptor have all been tried (*14*). These diverse treatment strategies often result in recipient immune responses that interfere with therapy due to systemic treatment of the recipient.

In an alternative approach, the target cell is treated to avert T cell adhesion and activation, thereby eliminating the need for therapy of the host and its inevitable complications (*15–18*). We have used this approach to conceal the HLA class I antigens on pancreatic islets before transplantation.

Human islets primarily express HLA class I antigens and lack prominent expression of ICAM-1 (CD54) (*19*), CD29, and LFA-3 (CD58) (*20*) (Fig. 1). Islets, therefore, unlike many targets of CTLs, lack large quantities of two adhesion epitopes, LFA-3 and ICAM-1, thus decreasing the need to conceal these epitopes from CTLs and making the prominent HLA class I antigens the main candidate for masking.

BALB/c female mice (10 to 12 weeks old) (Jackson Laboratory, Bar Harbor, Maine) were transplanted with 2200 to 4500 fresh human islets ($\sim 1 \times 10^5$ β cells) that had been treated with an antibody to HLA class I (W6/32) (American Tissue Culture Collection), polyclonal antibodies to islet cells, or an antibody to CD29 (Coulter Corporation, Hialeah, Florida). $F(ab')_2$ fragments or whole antibodies were incubated with the islets [1 μg of antibody (20 to 30 μg/ml) per 1×10^6 islet cells] at room temperature. After incubation, the islets were washed once in Hanks' buffer and were then immediately transplanted under the kidney capsule by syringe injection. Human islets were transplanted within 3 days of isolation. At 30 or 200 days after transplantation, the mice were killed and the kidney containing the transplanted tissue was removed.

Treatment of donor xenogeneic islets with W6/32 $F(ab')_2$ resulted in complete survival of the islets in all five recipients evaluated at 30 days and at 200 days after transplantation (Table 1 and Fig. 2); in all ten mice, the large islet clusters were well granulated (Fig. 2, A and B). Untreated human islets were rejected within 7 days of transplantation, and no islet cells were visible on serial sections (Fig. 2C). The islet grafts treated with W6/32 $F(ab')_2$ were virtually free of adjacent lymphocyte deposits, even at 200 days after transplantation (Fig. 2B). The removal of the Fc domain from the W6/32 antibody to circumvent complement lysis appeared to be necessary, because mice that received islet grafts that had been treated with whole W6/32 had no surviving islet tissue at 30 or 200 days after transplantation (Table 1); these transplants showed only subcapsular kidney fibrosis at the transplantation site. In addition, the coating of donor islets with whole antibody or $F(ab')_2$ fragments to CD29 resulted in islet graft rejection by day 30 after transplantation (Table 1). The presence of the $F(ab')_2$ fragments to CD29 did not impair the beneficial response to treatment with W6/32 $F(ab')_2$ fragments.

We monitored function of transplanted human islets by evaluating human C peptide (a proinsulin processing product) concentrations in blood, 30 and 200 days after transplantation (Fig. 3) (in 20 recipients). At day 30 after transplantation, all 20 recipients that received human islets coated with W6/32 $F(ab')_2$ or with W6/32 $F(ab')_2$ and $F(ab')_2$ fragments to CD29 had human C peptide concentrations markedly above background values ($P < 0.002$ for all four groups compared to untransplanted mice). Human C peptide concentrations were also increased at 200 days in all ten recipients that received W6/32 $F(ab')_2$–coated human islets ($P = 0.003$ and $P = 0.002$ for groups 3 and 11, respectively, compared to untransplanted mice). In contrast, mice that re-

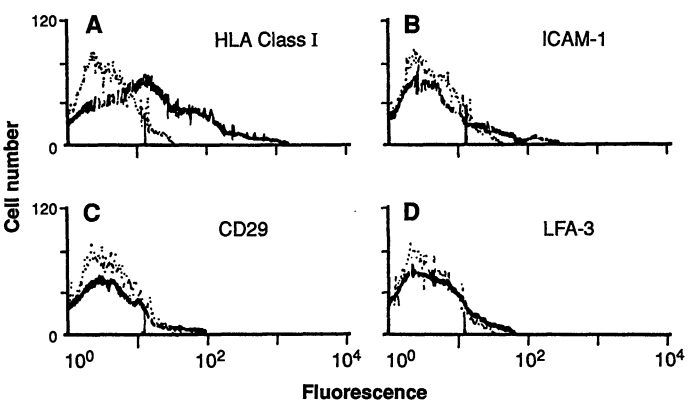

Fig. 1. Expression of (**A**) HLA class I (46% positive cells), (**B**) ICAM-1 (14%), (**C**) CD29 (9%), and (**D**) LFA-3 (10.2%) on freshly isolated whole human islets of Langerhans (90 to 97% pure) by indirect immunofluorescence and flow cytometry (heavy traces). Background fluorescence (9%) (broken traces) was established with FITC-conjugated goat antibodies to mouse immunoglobulin. An open gate with exclusion of dead cells and debris was used for flow cytometry. Islet preparations contaminated with large amounts of fibroblast overgrowth or endothelial cells (purity 60 to 75%) were positive for low levels of LFA-3 and ICAM-1.

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ceived untreated islets, or islets treated with whole W6/32 or whole antibody to CD29 or $F(ab')_2$ to CD29, had human C peptide concentrations similar to untransplanted mice ($P > 0.97$). Two mice with established transplants of xenogeneic human islets that had been treated with W6/32 $F(ab')_2$ were subjected to unilateral nephrectomy at day 31 to remove the subrenal transplanted islets; in both recipients, the concentrations of human C peptide returned to background values. The removed kidneys contained surviving human islet tissue that was free of lymphocytes. Therefore, the effectiveness of donor graft treatment with antibody fragments to HLA class I is supported by functional data as well as by histology.

To demonstrate the importance of HLA class I in transplant rejection, we produced polyclonal mouse antiserum to human islets in order to mask all donor islet antigens before transplantation. Masking of all donor human islet epitopes with $F(ab')_2$ fragments of this antiserum allowed human islet survival and function for up to 120 days after transplantation (Table 2). We then removed antibodies to HLA class I from the polyclonal mouse antiserum by adsorption with immunoprecipitated HLA class I antigens, and $F(ab')_2$ fragments were prepared from the remaining antibodies. Treatment of islets with this $F(ab')_2$ preparation did not prevent xenograft rejection by day 30 in all four recipients (Table 2). Adsorption of the polyclonal antiserum with CD29 had no effect on successful graft survival.

We confirmed by immunofluorescence that prolonged islet xenograft survival was due to antigen masking by W6/32 $F(ab')_2$ fragments (Table 3). Islets that had been treated with W6/32 $F(ab')_2$ or whole W6/32 lacked detectable HLA class I epitopes on their surface when evaluated with fluoroscein isothiocyanate (FITC)-conjugated W6/32. In addition, treatment of W6/32 $F(ab')_2$–coated islets with FITC–conjugated goat antibody to mouse immunoglobulin revealed $F(ab')_2$ antibody fragments on the cell surface of the islets (Table 3). After 2 days of culture, W6/32 $F(ab')_2$ fragments still masked islet HLA class I surface epitopes. In fact, $F(ab')_2$ fragments appeared to be more effective at continuously coating islets than whole antibody (Table 3).

To investigate whether graft-specific tolerance would be sufficient to allow the growth of a transplanted tissue, we transplanted untreated xenogeneic rat insulinoma RIN cells (*21*) (~5000 cells per recipient) under the kidney capsule of nonimmunosuppressed BALB/c mice. The transplanted cells were uniformly rejected when evaluated by histology at 30 days after transplantation ($n = 4$) (*22*). In contrast, treatment of RIN cells with $F(ab')_2$ fragments of mouse antibodies to RIN cells allowed tumor survival and visible tumor expansion for up to 4 months after transplantation. When fresh, untreated RIN cells were transplanted into the contralateral kidneys of mice bearing

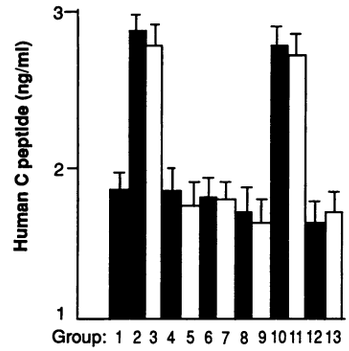

Fig. 3. Function of human xenografts evaluated by human C peptide concentrations. Group treatment: 1, untransplanted mice; 2, W6/32 $F(ab')_2$, day 30; 3, W6/32 $F(ab')_2$, day 200; 4, W6/32 antibody, day 30; 5, W6/32 antibody, day 200; 6, $F(ab')_2$ to CD29, day 30; 7, $F(ab')_2$ to CD29, day 200; 8, antibody to CD29, day 30; 9, CD29 antibody to CD29, day 200; 10, W6/32 $F(ab')_2$ and $F(ab')_2$ to CD29, day 30; 11, W6/32 $F(ab')_2$ and $F(ab')_2$ to CD29, day 200; 12, untreated islets, day 30; 13, untreated islets, day 200. Each bar represents the mean ± SD of values for five mice. Background C peptide concentrations in untransplanted BALB/c mice ranged from 1.6 to 1.8 ng/ml.

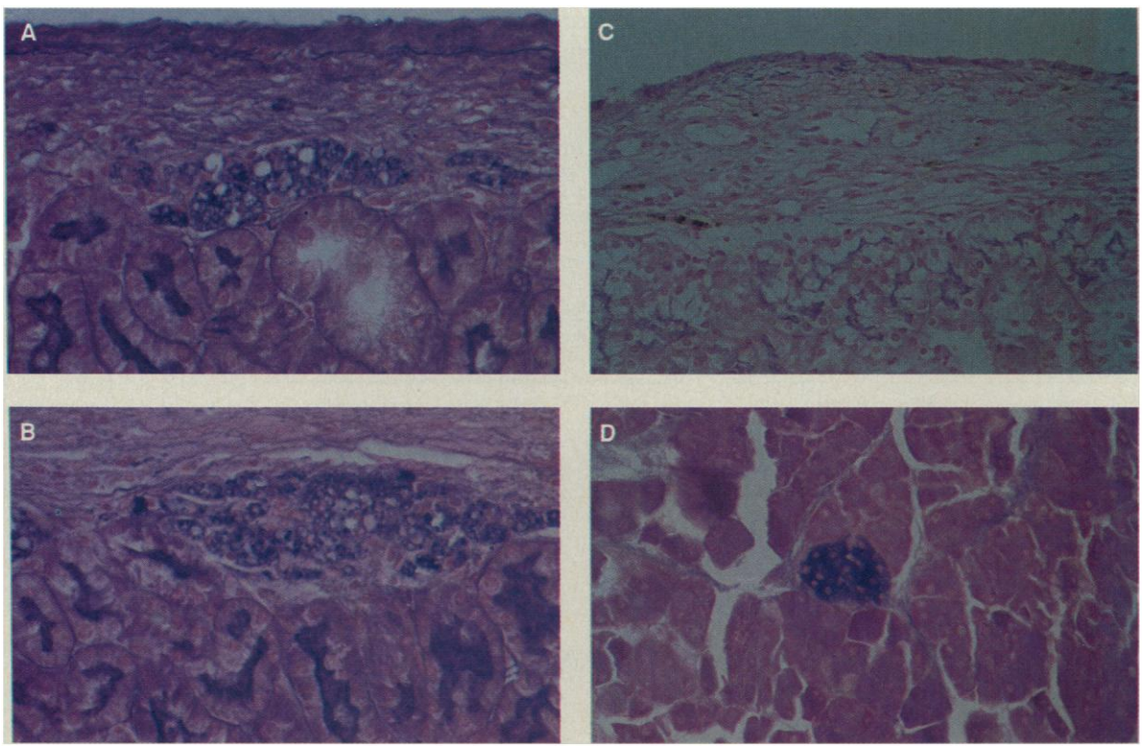

Fig. 2. Histological analysis of human islets transplanted under the kidney capsule of BALB/c recipients. (**A**) Human islet xenograft 30 days after implantation of islets that had been treated with W6/32 $F(ab')_2$ fragments. The aldehyde-fuchsin stain (×212) shows well-granulated islets under the kidney capsule. (**B**) Human islet xenograft 200 days after implantation of islets that had been treated with W6/32 $F(ab')_2$ fragments. Well-granulated islets are present under the kidney capsule. (**C**) A control BALB/c mouse was transplanted with untreated fresh human islets and then killed at day 30. No donor islets can be detected, but subcapsular fibrosis in the area where the islets were transplanted is apparent. (**D**) Aldehyde-fuchsin stain of a mouse islet in the mouse pancreas, demonstrating the characteristic purple granulation of healthy β cells.

Table 1. Effect of masking donor human xenografts with antibodies or $F(ab')_2$ fragments to HLA class I (W6/32) or to CD29 before transplantation. Islet xenograft survival was evaluated with hematoxylin-eosin and aldehyde-fuchsin staining. "Accepted" represents easily located, well-granulated islets under the kidney capsule, without lymphocyte foci. A transplant site with large lymphocyte accumulations or subcapsular fibrosis without islets constitutes rejection. Each group had a total of five transplants performed. $F(ab')_2$ fragments were generated with immobilized pepsin (Pierce Chemical). After digestion, the antibody fragments were dialyzed for 24 hours (50,000-dalton–threshold tubing). The progress of antibody digestion was monitored by silver staining of SDS–15% polyacrylamide gels. The final purification of the $F(ab')_2$ fragments was achieved by gel filtration.

Group	Islet treatment	Days after transplantation	Accepted grafts
1	W6/32 $F(ab')_2$	30	5
2	W6/32 $F(ab')_2$	200	5
3	W6/32 antibody	30	0
4	W6/32 antibody	200	0
5	$F(ab')_2$ to CD29	30	0
6	$F(ab')_2$ to CD29	200	0
7	Antibody to CD29	30	0
8	Antibody to CD29	200	0
9	W6/32 $F(ab')_2$ + $F(ab')_2$ to CD29	30	5
10	W6/32 $F(ab')_2$ + $F(ab')_2$ to CD29	200	5
11	None	30	0
12	None	200	0

30-day-established $F(ab')_2$-treated RIN transplants, the secondary transplants uniformly survived for at least 60 days, thus confirming the development of a systemic tolerant state that was sufficient for survival of unmodified tumor cells (*22*).

The effectiveness of treatment with $F(ab')_2$ fragments to HLA class I in preventing human xenogeneic liver cell rejection was also investigated (*22*). Approximately 5000 $F(ab')_2$-treated fresh cells from the parenchymal tissue of human liver were injected into the subcapsular space of the kidney of nonimmunosuppressed mouse recipients. Although liver cell function could not be evaluated, liver cell survival was determined by periodic acid Schiff's staining of the subcapsular sites. All five liver transplant recipients contained easily located, viable liver cells at the subcapsular site, 30 days after transplantation. Untreated human liver cells were uniformly rejected in all five mice by day 30 after transplantation.

Table 2. Treatment of human donor islets with polyclonal mouse antibodies to human islets before transplantation. Polyclonal mouse antibodies to human islets were produced by seven intraperitoneal immunizations of mice at weekly intervals with human islets. The antibody preparation was depleted of antibodies to HLA class I or CD29 as described (*26*). There were four mouse recipients in each group; human C peptide concentration was measured at day 30.

Islet treatment	C (ng/ml)	Histology
Polyclonal mouse antibodies to human islets	<2	No islets visible at 30, 60, 90, and 120 days after transplantation
Polyclonal mouse $F(ab')_2$ to human islets	>2.8	Well-granulated islets visible at 30, 60, 90, and 120 days after transplantation
Polyclonal mouse $F(ab')_2$ to human islets depleted of antibodies to HLA class I	<2	No islets visible at 30 days after transplantation
Untransplanted mice	<2	

Table 3. Immunofluorescence of islets after treatment with antibody W6/32 to HLA class I. Background immunofluorescence was set with FITC-conjugated mouse immunoglobulin (mIg-FITC) or FITC-conjugated goat antibodies to mouse immunoglobulin (anti-mIg-FITC). Parts of this experiment were repeated with ten different human islet preparations. W6/32-FITC, W6/32 antibody conjugated to FITC.

Treatment	HLA class I–positive cells (%)		
	W6/32-FITC	mIg-FITC	Anti-mIg-FITC
Fresh islets			
W6/32 $F(ab')_2$	7		62
W6/32 antibody	6.5		
None		7.5	12
Islets in culture for 2 days			
W6/32 $F(ab')_2$	10		70
W6/32 antibody	16		55
None	67	10	12

Our observations show that the simple interruption of recipient T cell recognition by concealment of foreign HLA class I determinants allows xenograft survival for at least 200 days. This strategy avoids the need for treatment of the recipient and preserves an intact immune system. Our results are consistent with the survival of tumor cells that express low levels of HLA class I and HLA class I–negative histoincompatible trophoblast cells of the placenta (*23, 24*). In addition, our results are consistent with the ability of an antibody to HLA class I (W6/32) alone to inhibit both in vitro human CTL adhesion and T cell receptor triggering of CTLs (*1*). Thus, it appears that masking of donor antigens or the possible elimination of HLA class I donor transplantation antigens (*25*) may be useful for islet transplantation as well as other xenogeneic and allogeneic transplant models.

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