Mannose-binding lectin: The Dr. Jekyll and Mr. Hyde of the innate immune system.

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CHAPTER 5

Adaptive immunity in pancreatic islet transplantation

HLA Incompatibility and Immunogenicity of Human Pancreatic Islet Preparations Co-Cultured with Blood Cells of Healthy Donors

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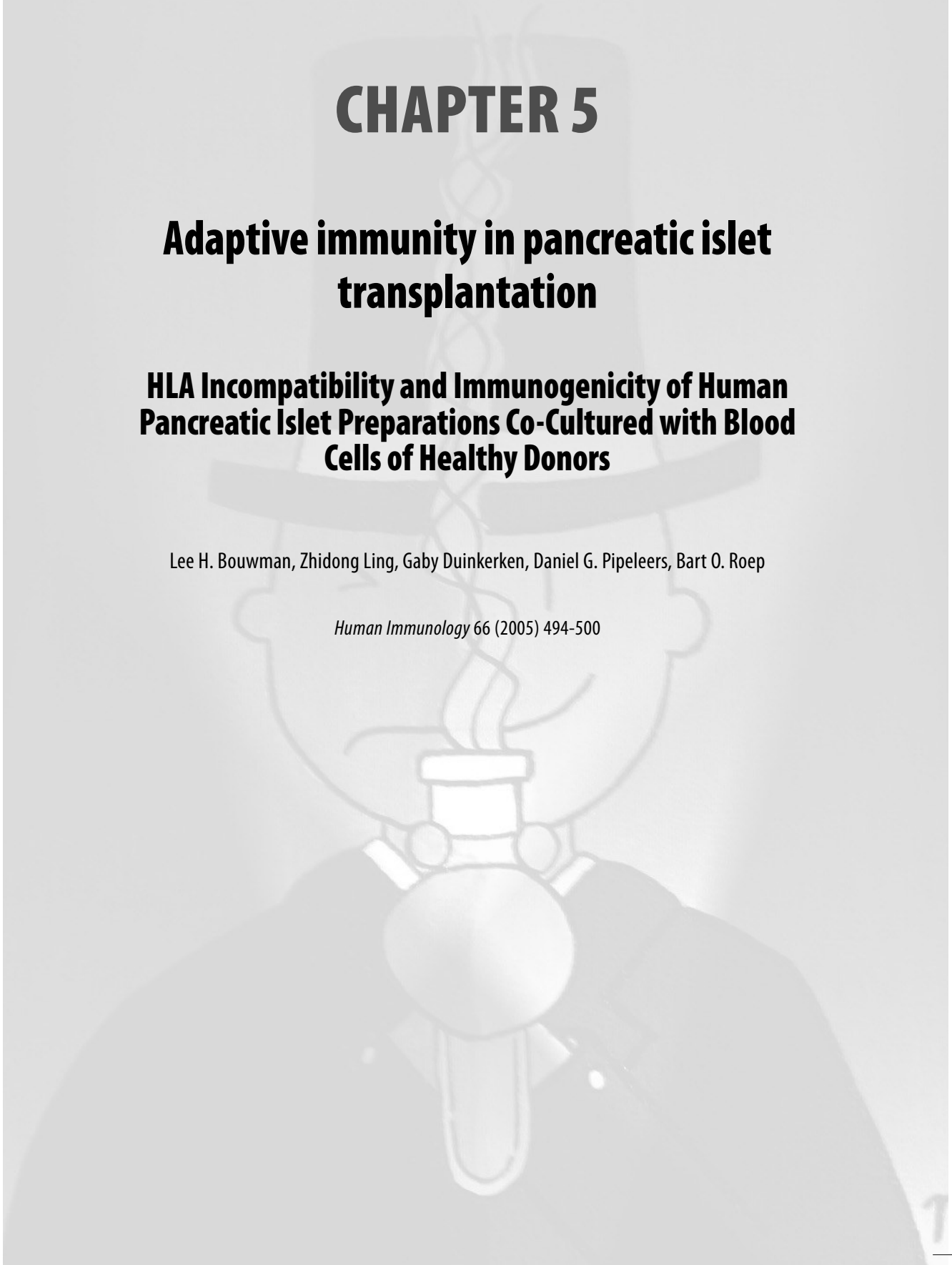

ABSTRACT

Type 1 Diabetes Mellitus (T1D) is a T-cell mediated autoimmune disease characterized by the destruction of beta cells in the pancreas. An attractive novel therapy for type 1 diabetes is pancreatic islet transplantation, provided that recurrent islet autoimmunity and allograft rejection can be prevented.

We analysed the response of peripheral blood mononuclear cells (PBMC) from healthy blood donors to human islet-cell preparations with a composition similar to that of islet grafts used in clinical transplantation trials. It was examined whether the degree of MHC incompatibility between PBMC and donor islet cells is related to the degree of proliferative T-cell responses during co-culture of HLA-matched and mismatched PBMC with human islet cell-preparations [i.e. mixed islet lymphocyte reaction (MILR)].

Prominent T-cell responses were observed in the vast majority of cases of double HLA class II-mismatches. Intermediate T-cell responsiveness was observed in single HLA class II mismatches, whereas HLA-matches did not induce a T-cell response.

Our results identify the potential immunogenicity of islet preparations transplanted between HLA-DR incompatible subjects regardless of an autoimmune background of the recipient.

INTRODUCTION

Since the major histocompatibility complex (MHC) molecule plays an essential role in the activation of T cell responses, the genes encoding these molecules have been implicated in susceptibility to serve T cell mediated autoimmune diseases. Several HLA alleles have been shown to be major genetic risk factors in development of type 1 diabetes. Vulnerability for type 1 diabetes is genetically dominated by the HLA gene region accounting for 42% of the familial inheritance of type 1 diabetes[1].

Type 1 (Insulin Dependent) Diabetes Mellitus (T1D) is an autoimmune disease characterized by the specific destruction of beta cells in the pancreas. The etiology of T1D is multifactorial, consisting of genetic predisposition and environmental factors including a variety of viruses and dietary components[2]. It has long been acknowledged that T-cells play a crucial role in the immunopathogenesis of type 1 diabetes, the hallmark of autoimmune diabetes being lymphocytic invasion of pancreatic islets[3-10].

A potential therapy for diabetes is transplantation of insulin-producing beta-cells of isolated pancreatic islets, provided that recurrence of T-cell autoreactivity against islet determinants, as well as induction of alloimmunity to donor antigens, is prevented[11-13]. This attractive and recently successful therapy for T1D is overshadowed by the need for permanent immune suppression. Without the administration of these non-specific and potentially harmful immunosuppressive drugs, graft failure seems inevitable. Islet transplantation is thus limited to diabetic patients already receiving immune suppression for a previous organ transplant, or to patients with severe hypoglycemia unawareness or uncontrollable hyperglycemia. The introduction of a new glucocorticoid free immunosuppressive regime, the so-called Edmonton protocol, has improved the outcome of islet transplantation considerably[14]. This protocol includes sirolimus, tacrolimus and dacluzimab. All these immunosuppressive drugs share the same basic quality that they all inhibit T-cell stimulation and proliferation, identifying once again T-cells as key-players in this rejection process.

Prediction and prevention of ongoing beta-cell destruction after islet transplantation, resulting in long-term graft survival is of utmost importance. In order to be able to optimise the current islet transplantation, it is essential to study the reaction of T-cells to islets.

In rat studies, it has been shown that T-cells obtained prior to islet transplantation can react against islet allografts in a mixed islet-leukocyte culture [15]. This reactivity was similar to that of T-cells isolated after grafting. We have previously demonstrated a marked increase in T-cell alloreactivity in immune suppressed type 1 diabetes patients rapidly upon implantation of human islet allografts from multiple donors[11]. This rise was exclusive to patients that acutely rejected the islet allograft, and absent

in cases of successful restoration of beta-cell function. The contribution of HLA class II to immunogenicity of islets was further underscored by our recent observation that islet autoantigens are processed and presented by vascular endothelial cells expressing MHC class II leading to activation of autoreactive T-cells [16], implying that MHC class II could be important in human islet graft-failure by autoreactivity[17]. Little is known about the extent that human islet preparations could be target of alloreactivity in relation with the degree of HLA mismatching with human healthy blood donors.

To evaluate the potential immunogenicity of human islet preparations under immunocompetent conditions, we investigated the ability of peripheral blood mononuclear cells (PBMC), isolated from immunologically uncompromised healthy blood donors, to react human islet preparations in relation with the degree of HLA-DR incompatibility. Mixed islets lymphocyte cultures [18,19] were performed with a panel of HLA-DR matched and mismatched healthy blood donors. For comparison, mixed lymphocyte reactions (MLR) using PBMC of blood donors with PBMC of the islet donor, were carried out and analysed in relationship to the pattern of reactivity found in the MILR.

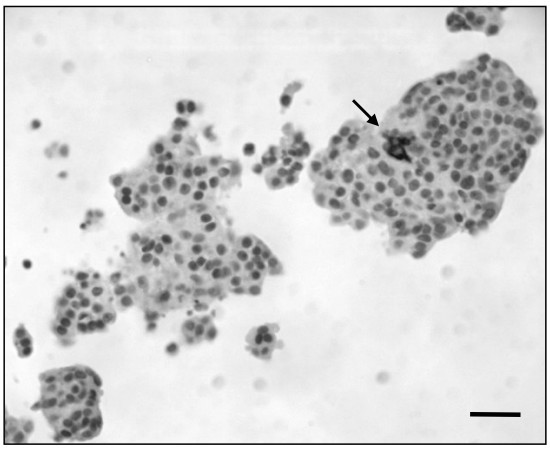

Figure 1: Representative image of human pancreatic cell preparations immunostained for HLA-DR. Positive cells (arrow) were present at a low percentage (<1%) and occurred usually as single units. The scale bar represents 50µm.

MATERIAL AND METHODS

Human islet isolation and culture

Human pancreata, were obtained from organ donors (eleven preparations from 10 donors, age 2-60 years) at European hospitals affiliated with ß-Cell Transplant, a European Concerted Action on islet cell transplantation in diabetes. Islets were

prepared in the Central Unit of this multicenter program (Medical Campus, Vrije Universiteit Brussel) [12]. Detailed description of methods of isolation, phenotypic and functional characterization of the islet preparations is available elsewhere [12]. This study was conducted with cultured Islet cell preparations that were comparable to those incorporated in grafts that are implanted in patients. They are composed of endocrine and non-endocrine duct cells with, in average, 48 percent insulin-positive cells (Table 1). Their content in MHC-II expressing cells is consistently lower than 1 percent (Figure 1). The available beta cell mass was too low for inclusion in grafts so that the preparations became available for approved research projects if they fulfilled the legal and ethical criteria that were set for such use. The isolated islet preparations were cultured in Ham's F10 medium[12] for 1-5 days before transfer to Leiden, where they were processed immediately for immunological studies.

Table 1: Characterization of human islet preparations.

Donors	Age	Sex	HLA-DR	Culture Time (days)	Cellular composition (% total)				
					Exocrine	Non-granulated	Endocrine	Dead	Insulin positive
1	17	M	DR4 DR6(13)	5	0	40	58	2	58
2	22	M	DR2(15) DR6(13)	1	15	31	50	4	42
3	40	F	DR2(16) DR3(17)	5	0	58	32	10	28
4	20	M	DR4	8	0	46	50	4	42
5	12	M	DR8 DR12	5	0	50	42	8	44
6	2	M	DR2 DR4	4	0	28	62	10	57
7	52	M	DR6 DR13	3	2	28	64	6	49
8	42	F	DR1	6	0	12	82	6	61
9	48	F	DR2 DR6	3	0	8	76	16	63
10a	60	M	DR4 DR6	4	0	31	66	3	54
10b	60	M	DR4 DR6	4	0	66	30	4	30

Peripheral Blood mononuclear cells (PBMC) isolation

PBMC were obtained from healthy blood donors visiting the blood bank in Leiden, the Netherlands by Ficoll separation after obtaining informed consent.

HLA-DR typing

The HLA-DR genotypes of both blood- and pancreas-donors were determined by conventional genotyping in the vast majority of cases as described in detail [20]. Incidentally, pancreata were HLA typed by serology only. Therefore, all HLA genotypes were translated to serotype to allow comparison.

Mixed islet lymphocyte reaction (MILR)

Islet preparations in a concentration range (10.000 to 150.000 endocrine cells per well) were co-cultured with 150.000 PBMC per well, in micro well tissue plates (Griener) for 5 days at 37°C, 5% carbon dioxide in air. Islet preparations and PMBC were HLA-DR matched or mismatched. RPMI culture medium was used containing 10% fetal calf serum and 100 units/ml penicillin and 100 µg/ml streptomycin. Samples were incubated in triplo. On day 5 [H^3]-Thymidine was added to each well for 16 hours in an end concentration of 1µCi/ml. The MILR response was measured by incorporation of [H^3]-Thymidine in proliferating lymphocytes using a liquid scintillation counter (LKB Wallac, Croydon UK). Dose-response curves differed between islet preparations, and optimal responses were determined individually. For standardization and comparison between islet preparations, the MILR was considered positive when the signal exceeded the average signal of stimulatory cells alone plus three times the standard deviation.

Furthermore, in two cases a double mismatched MILR was performed, in the presence and absence of monoclonal antibodies directed against HLA-DR (B8.11.2), HLA-DQ (SPV-L3) and HLA class 1 (W6.32). The human islet preparations were incubated in the presence of 10 µg/ml of monoclonal antibodies directed against HLA-DR (B8.11.2), HLA-DQ (SPV-L3) and HLA class 1 (W6.32).

Mixed lymphocyte reaction (MLR)

In the MLR, PBMC of both blood donors and islet donors were used. γ-irradiated PBMC (3000 R) of the islet donor were incubated with blood donor PMBC in a 1:1 ratio, 200.000 cells per well (Griener). Samples were incubated in triplicate for 5 days at 37°C, 5% carbon dioxide in air. On day 5 [H^3]-Thymidine was added to each well for 16 hours in an end concentration of 1 µCi/ml. The MLR response was measured by incorporation of [H^3]-Thymidine in proliferating lymphocytes. The MLR responses were considered positive when the signal exceeded the average signal of stimulatory cells alone plus 3 times the standard deviation.

Statistical analysis

Statistical analysis for group comparison was performed using Chi-square analyses with two-sided Fisher exact tests. Statistical significance was achieved if the *P* value was less than 0.05. Frequencies of both positive and negative MILR and MLR were compared with the chi-square test or the Fisher's exact probability test.

RESULTS

Comparison between MILR and HLA-DR haplotype

Mixed islet lymphocyte reactions (MILR) were performed with islet and PBMC preparations that were HLA-DR matched, single mismatched and doubled mismatched (Table 2). T cell responses were exclusively seen in HLA mismatch combinations (p=0.0004). In the group of double HLA-DR mismatches, 12/13 resulted in potent T cell proliferative responsiveness (double mismatched vs. matched: p=0.0003). The one combination without reactivity involved the islet preparation with the highest percent endocrine cells (82%). Intermediate T cell responses were observed in 6/9 tested combinations with a single HLA-DR mismatch (single mismatch vs. matched: p=0.017), whereas none of the 6 HLA-DR matched combinations induced a T cell response. In rare cases, sufficient numbers of islets were available to allow HLA blocking studies. The observed T cell proliferative responses were to a large extent suppressed when the mixed islet lymphocyte reactions were performed in presence of a monoclonal antibody directed against HLA-DR, whereas no inhibition was seen with monoclonal antibodies directed against HLA-DQ and HLA class 1 (data not shown).

Table 2: MILR in relation with HLA-DR matching. Indicated are positive or negative proliferative responses of HLA-matched, single or double mismatched PBMC of healthy donors against human islet-preparations, tested in the MILR ("nr." refers to the donor number indicated in Table 1).

MILR	HLA-DR mismatches		
	2	1	0
POS	DR3,3 x DR4,6 (nr.1)* DR3,3 x DR2,6 (nr.2) DR4,10 x DR 2,3(nr.3) DR1,8 x DR4,4(nr.4) DR6,13 x DR2,4(nr.6) DR3,3 x DR6,13(nr.7) DR2,6 x DR 1,1(nr.8) DR1,7 x DR2,6 (nr. 9) DR 15,15 x DR 4,6 (nr.10a) DR 1,1 x DR 4,6 (nr.10a) DR 16,11 x DR 4,6 (nr.10b) DR 3,7 x DR 4,6 (nr.10b)	DR6,6 x DR4,6(nr.1)* DR 2,6(14) x DR 2,6(13)(nr.2) DR2,2 x DR2,3(nr.3) DR1,8 x DR8,12(nr.5) DR2,6 x DR6,13(nr.7) DR1,8 x DR1,1(nr.8)	
NEG	DR 3,7 x 1,1(nr.8)	DR3,3 x DR2,3(nr.3) DR2,6 x DR2,4(nr.6) DR1,6 x DR 1,1(nr.8)	DR2,6 x DR2,6(nr.2) DR2,3 x DR2,3(nr.3) DR6,13 x DR6,13(nr.7) DR1,1 x DR1,1(nr.8) DR 4,6 x DR 4,6 (nr.10a) DR 4,6 x DR 4,6 (nr.10b)

Mismatch vs. Match:	p = 0,0004
Double mismatch vs. Match:	p = 0,0003
Single mismatch vs. Match:	p = 0,017

* No MLR done

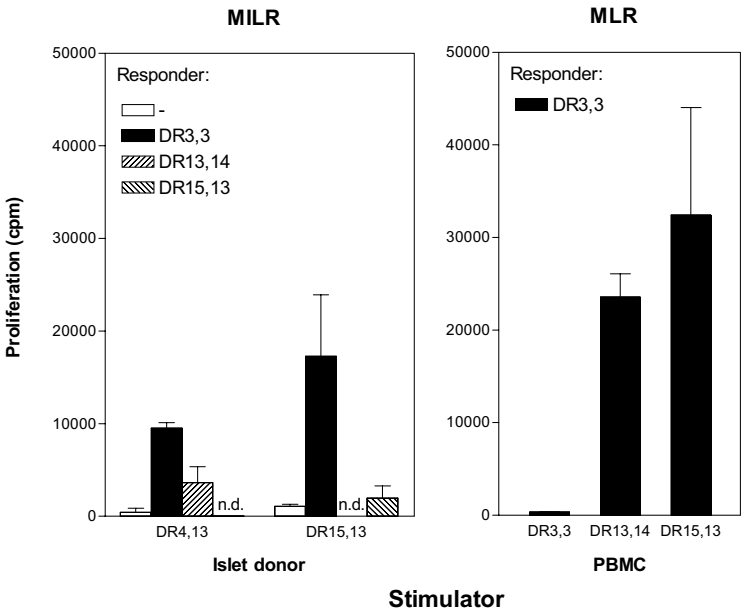

Figure 2: Representative comparison between MILR and MLR. Raw counts per minute (cpm) are indicated to illustrate background responses of islet preparations, stimulator or responder PBMC alone as well as the amplitude of the proliferative response to . (n.d.: not done)

Comparison between MILR and MLR

The lymphocyte reactivity to allogeneic islet cell preparations (MILR) was compared to that measured against lymphocytes (MLR) that were isolated from the islet cell donor (table 2). The MLR tests were positive for 15/16 cases where a positive MILR had been measured; the one MLR negative/ MILR positive case related to the only child (2y) that was tested as islet donor (Table 3). In the group of negative MILR tests, 3/10 exhibited a positive MLR. Overall, a significant relation was seen between recognition of islet donor PBMC and islet preparations by healthy donor derived PBMC (p=0.0013; Table 3). When the analysis was restricted to the 12 to 60 year old donor group the correlation between MILR and MLR was even stronger (p=0.0003).

Table 3: The correlation between the presence (POS) and absence (NEG) of proliferative T-cell responses in co-cultures of PBMC isolated from healthy blood donors with human islet preparation. Responsiveness was significantly concordant between MILR and MLR (p=0.0013).

MILR	MLR	
	POS	NEG
POS	DR 3,3 x DR 2,6	DR 6,13 x DR 2,4
	DR 4,10 x DR 2,3	
	DR 1,8 x DR 4,4	
	DR 3,3 x DR 6,13	
	DR 2,6 x DR 1,1	
	DR 1,7 x DR 2,6	
	DR 15,15 x DR 4,6	
	DR 1,1 x DR 4,6	
	DR 16,11 x DR 4,6	
	DR 3,7 x DR 4,6	
	DR 2,6(13) x DR 2,6(14)	
	DR 2,2 x DR 2,3	
	DR 1,8 x DR 8,12	
	DR 2,6 x DR 6,13	
	DR 1,8 x DR 1,1	
	DR 3,7 x DR 1,1	DR 2,6 x DR 2,4
NEG	DR 3,3 x DR 2,3	DR 2,6 x DR 2,6
	DR 1,6 x DR 1,1	DR 2,3 x DR 2,3
		DR 6,13 x DR 6,13
		DR 1,1 x DR 1,1
		DR 4,6 x DR 4,6
		DR 4,6 x DR 4,6

DISCUSSION

Our study demonstrates that cultured human islet cell preparations with a composition similar to that used in our clinical trial, have a strong immunogenic potential dependent of the degree of their HLA incompatibility with responder leukocytes. Importantly, matched islet preparation persistently led to absence of alloreactivity *in vitro*. This may bear implications for clinical islet cell transplantation. Human islet transplantation has been considered to be a promising therapy for T1D patients for the last 31 years[21,22]. A major obstacle that remains to be tackled in allograft islet transplantation, is the rejection process resulting in graft failure. The current knowledge of the immunogenesis of the rejection process is at best incomplete. This might be due to the fact that graft tissue is inaccessible after transplantation, making clinical and histological studies very difficult and demanding. Hence, most studies addressing the specific allogeneic rejection response are murine based and cannot be extrapolated directly to the human situation. Clinical trials studying the rejection process rely upon the presence of C-peptide, indicating beta cell function. The immunological status of the islet-transplantation however remains concealed[23].

Multiple donor islet grafts further cloud the different roles of involved actors in this immunological play. In the present study we selectively studied the degree of alloreactivity in PBMC of immunocompetent healthy blood donors against allogeneic islet preparation with different degrees of HLA incompatibility and composition.

HLA has long been recognized to be of clinical importance in solid organ transplantation as one of the primary causes of Th1/HLA class II antigen-rejection. However the importance of HLA in islet transplantation and rejection has been disputed. One study showed low or even absent T-cell response directed against pancreatic islets *in vitro* [23]. The authors hypothesized that low T-cell proliferative responses could be a direct result of the inhibitory effects of exocrine enzymes upon lymphocytes, utilizing the role of class II MHC. However, we have demonstrated that alloreactivity in type 1 diabetes patients transplanted with human islet allografts strongly correlated with acute rejection and loss of graft function *in vivo* [11]. Moreover, it has been suggested that HLA mismatching could provide better allograft survival of transplanted pancreatic islets in autoimmune diabetes, as was shown in mice. It has also been argued that avoidance of MHC class II sharing between donor and recipient, could avoid the recurrence of autoimmunity and subsequently the destruction of beta cells[24]. However, there is little evidence from clinical trials on islet transplantation that islet allografts benefit from mismatching with the recipient. Moreover, our present data clearly demonstrate that HLA matching significantly reduces the risk for alloreactivity, while induction of alloreactivity appears to be the most pronounced cause of graft failure[11]. We appreciate that in an era of donor shortage and multiple donors being required to obtain a sufficient islet load for transplantation, donor selection based upon HLA is inconceivable. Therefore, tailored immune suppresion is of utmost importance in order to optimize the outcome of islet transplantation.

In order to address the involvement of HLA in human islet transplantation, we studied the response of peripheral blood mononuclear cells (PBMC) from healthy blood donors to human islet-preparations. Healthy donors were chosen, rather than diabetic patients, to evaluate the primary T-cell response and exclude recurrent islet autoimmunity. Contrary to a previous report [23], a T-cell proliferative reaction could be invoked upon stimulation of healthy donor T-cells with double HLA-mismatched islet preparations in all but one mixed islet lymphocyte reactions. In contrast, none of MILR performed with HLA-DR matched T-cells and islet preparations displayed any T-cell responsiveness. Partial HLA-DR mismatching could invoke an intermediate T-cell response in the majority of cases, which accorded with the MLR.

As previously mentioned, there was only one exception where a positive MLR did not correlate with a positive MILR despite a complete HLA class II disparity. This occurred in the case of an islet preparation from a 2 year-old pancreas donor, suggesting that donor age may be a factor related to immunogenicity.

In conclusion, our data clearly demonstrate the immunogenic nature of islet preparations of clinical norm, even in healthy donors lacking auto-reactive islet T-cells. The immunogenicity is dependent largely on the degree of HLA matching with responder leukocytes. HLA-DR matching is accompanied by lack of immune reactivity against the islet preparation. Our findings underscore the difficulty of islet allotransplantation, but provide leads to reduce the extent of alloreactivity in clinical islet transplantation.

Acknowledgments

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