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Rat pancreatic islet pretreatment with anti-MHC class II monoclonal antibodies and culture: in vitro MLIC test response does not predict islet allograft survival

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Abstract. Antigen presenting cells (APC) expressing MHC class II antigens have been attributed with stimulatory capacity for initiating islet allograft rejection (direct pathway). Therefore, we evaluated the effect of pretreating isolated islets with different monoclonal antibodies against MHC class II antigens and complement, with and without culture at 22 °C or 37 °C, on MHC class II antigen expression, on the allogeneic proliferative response in the mixed lymphocyte islet culture (MLIC) and on islet allograft survival in adult rats. Experiments were performed in two different strain combinations incompatible for MHC class II antigens and either incompatible or compatible for MHC class I antigens, in order to elucidate further the impact of class I antigens on islet allograft rejection. In terms of class II antigen suppression, pretreatment with anti-MHC class II antibodies together with complement and a 5-day (37 °C) culture period proved most effective. After this procedure 92.7% of the islets of LEW rats and 91.1% of the islets of LEW.1WR2 rats were negative for MHC class II antigens, as demonstrated by indirect immunofluorescence. Transfer of successfully pretreated islets to a MLIC in vitro test system provoked a significantly reduced allogeneic T-cell proliferative response in the case of additional MHC class I disparity (ratio 1.3 vs 4.7) and a response as low as that of a syngeneic setting when stimulator islets and allogeneic responder lymphocytes shared MHC class I antigens (ratio 1.0 vs 1.6). However, these encouraging in vitro results could not be confirmed in vivo after intraportal allotransplantation into streptozotocin-induced diabetic rats, neither in a strain combination incompatible for MHC class I antigens nor in a compatible combination. In conclusion, these findings provide evidence that an in vitro MLIC test response has a limited value for predicting in vivo islet allograft survival. In addition, the results are consistent with the notion that even near-total suppression of MHC class II antigens seems insufficient to prolong islet allograft survival; an indirect pathway coexisting in vivo may be involved in the antigen molecule processing and presentation by recipient APCs.

Key words: Antibody pretreatment – Culture pretreatment – Experimental diabetes – Islet allotransplantation – Mixed lymphocyte islet culture (MLIC)

Indroduction

The use of exogenous insulin therapy has not prevented the long-term secondary complications of type 1 (insulin-dependent) diabetes mellitus [1]. Total endocrine replacement therapy has long been considered an option for the better treatment of type 1 diabetes. This is in principle possible by transplantation either of vascularized pancreatic organ allografts or of islets of Langerhans as free grafts, and has recently been successfully performed also in man [2, 3]. Perfect metabolic control following islet transplantation may also prevent the long-term morbidity and mortality associated with type 1 diabetes [4]. Although the point is controversial, previous studies in animal models of the disease have shown that islet transplantation may prevent the development of organ lesions or halt its progression, and may even reverse secondary complications [5].

However, in experimental and clinical islet allotransplantation there is the problem of rapid islet allograft rejection requiring long-term conventional immunosuppressive treatment, which is potentially hazardous to the diabetic recipients [6]. Tissue immunogenicity may be derived from antigen-presenting cells (APC) or passenger leucocytes within the graft rather than from alloantigens per se, which proved to be rather weak immunogens [7, 8]. These MHC class II (Ia)-antigen positive APC, passenger leukocytes or macrophages and dendritic cells, are supposed to present antigens to the CD4+ T-cells of the host thus initiating islet allograft rejection [9]. By contrast, class I-antigens are recognized by CD8+ T-cells but their

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cytotoxic effects are usually dependent on interleukin-2 released by activated CD4 cells. Briefly, two signals are required for T-cell activation [10]: one provided by binding of the T-cell receptor and the other by an inductive molecule, or co-stimulator, produced by metabolically active stimulator cells (passenger leucocytes, dendritic cells) [11].

Therefore, attempts have been made to modulate islet graft immunogenicity in vitro prior to transplantation by eliminating or inactivating APC or by blocking their MHC class II antigen expression, with the eventual aim of transplanting the pretreated islet tissue without the recipient requiring immunosuppressive treatment (or at most only a brief course). Successful pretreatment regimens in terms of decreasing in vitro immunogenicity and, in several cases, prolonging islet allograft survival, have included 37 °C, low temperature culture, low pH media and high oxygen (95%) culture, incubation with gangliosides or with deoxyguanosine, ultraviolet irradiation, gamma irradiation, cryopreservation and a combined cryopreservation-culture method [12].

Other successful measures consisted of mouse pancreatic islet pretreatment with monoclonal antibodies directed against donor-strain class II antigens [13, 14] or antigens specific for dendritic cells [15], which resulted in a significantly prolonged survival of islet allografts across a major histocompatibility barrier in mice. Studies with rat pancreatic islets have also observed a significant reduction of islet immunogenicity by antibody pretreatment in vitro, but failed to establish prolonged islet allograft survival [16–19]. The failure to prolong allograft survival with anti-class II antigen treatment may be simply quantitative, since recent studies have demonstrated that more than 95% of donor class II positive cells must be eliminated to achieve allograft prolongation and that 2–3% of remaining class II positive cells per islet may be sufficient to stimulate a T-cell response in the MLIC [20, 21]. Furthermore, it has recently been shown that in the absence of class II antigens differences in the MHC class I allotype alone are capable of provoking an allogeneic response – which has, on the other hand, been successfully blocked by antibody pretreatment against MHC class I antigens [22, 23]. It is difficult to predict by in vitro tests the outcome of pancreatic islet allotransplantation, and mixed lymphocyte culture (MLC) may sometimes overestimate the risk of allograft rejection [24].

Therefore, we tested the effect of a combined treatment using a panel of relevant monoclonal antibodies directed against MHC class II antigens along with complement and 5 days of tissue culture at 37 °C to remove islet immunogenicity in the hope of avoiding immunosuppressive treatment of the recipients. In order to discriminate between the effects of class I and class II antigens we performed islet pretreatment and allograft studies in two different strain combinations incompatible for MHC class II with concomitant incompatibility or compatibility for MHC class I. The efficacy of islet pretreatment was assessed in vitro by analysis of class II antigen expression on islets and the T-cell response in the MLIC. Furthermore, we assessed the reliability of the T-cell response in the MLIC for predicting islet allograft survival in diabetic rats.

Table 1. Major histocompatibility complex (MHC) haplotype of rats used as donors and recipients

Strain	Haplotype	A region (class I)	B/D region (class II)
LEW	$RT1^{l}$	l	l
LEW.1WR2	$RT1^{r6}$	u	a
BDII	$RT1^{u}$	u	a

Materials and methods

Animals

Donors were adult male rats of the inbred strain Lewis (LEW) and Lewis.1WR2 (LEW.1WR2) with a body weight of about 250 g (Zentralinstitut für Versuchstierzucht, Hannover, Germany). Recipients were adult male rats of the inbred strain BDII (BDII) (Savo-Ivanovas, Kisslegg, Germany) and adult male LEW rats or LEW.WR2 rats for control studies, weighing about 250 g. The MHC haplotype is given in Table 1. Rats were made diabetic by intravenous injection of 55 mg/kg body weight streptozotocin (STZ, Upjohn, Kalamazoo, Mich.) under ether anaesthesia. Rats of the same inbred strains were used as lymphocyte donors for MLIC assessment.

Pancreatic islet isolation and transplantation

Pancreatic islets were isolated as described in detail elsewhere [19]. Diabetic BDII or LEW rats (blood glucose >16.8 mM for 2 weeks) were anaesthetized with ether. About 1000 antibody-pretreated cultured islets or untreated islets were injected into the liver via portal vein cannulation as described in detail previously [6]. Fasting blood glucose was measured every morning and rejection was suggested by fasting blood glucose levels >8.4 mM for two consecutive bleedings after a period of graft-induced normoglycaemia.

Monoclonal antibodies and sera

Isolated islets were treated with the anti-Ia mouse monoclonal antibody (mAb) OX-6 (IgG1; Camon-Serotec, Wiesbaden, Germany), the I-e antibody 29A1 (IgG1), kindly donated by Karin Ulrichs, Kiel, Germany and the monoclonal antibody 2MC3 (IgG2a), kindly provided by Juergen Neppert, Kiel, Germany. The mAb 2MC3 is directed against a monomorphic epitope on human HLA class II molecules (especially on the A chain). We found cross-reactivity of this antibody with rat, pig and dog antigens. Working dilution of OX-6 was 1:500 in RPMI 1640 with 10% fetal calf serum (FCS; Gibco, Eggenstein, Germany) in both the indirect immunofluorescence assay and islet pretreatment with anti-class II antibody in vitro. Antibodies 29A1 and 2MC3 were used as culture supernatant for islet pretreatment. Since mouse IgG1 does not fix complement (C), we used rabbit anti-mouse immunoglobulins (ram Ig; Dakopatts, Hamburg, Germany) as secondary antibody in a dilution of 1:20. Cytotoxicity was mediated by low-tox guinea pig complement (Camon-Cedarlane, Wiesbaden, Germany).

Islet pretreatment

About 1000 LEW or LEW.1WR2 islets were cultured for 5 days in RPMI 1640 with 10% FCS, 1% penicillin (5000 IU/ml), streptomycin (5000 µg/ml) and 1% L-glutamine (200 mM) (Flow, Neckenheim, Germany) at 22 °C or 37 °C in a 95% humidified atmosphere of 5% CO_2. Alternatively, the islets were incubated in 200 µl of one of the mAbs at 4 °C for 45 min, washed twice in RPMI 1640 with

10% FCS and incubated in ram Ig (1:20) at 4 °C for 45 min if the class II moab belonged to the IgG1 subclass. After washing twice, low-tox guinea pig complement was added at 37 °C for 45 min. After final washing the islets were either used for the different in vitro and in vivo tests or a 5-day culture period described above was added.

Immunofluorescence studies

Indirect immunofluorescence assessment was performed on whole islets. In brief, pretreated or untreated control islets were incubated with the mAb OX-6 followed by an incubation with fluorescein isothiocyanate (FITC)-labelled ram Ig. In each assay 1000 islets from random fields were examined with a Leitz fluorescence microscope for the number of cells per islet positive MHC class II antigen.

Mixed lymphocyte islet culture (MLIC)

The MLIC was performed as described previously [19]. In detail, 100 stimulator islets pretreated with mitomycin C (5 μg/ml; Sigma, Deisenhofen, Germany) were co-cultured together with 10^5 responder spleen lymphocytes for 3 days at 37 °C in a 95% humidified atmosphere of 5% CO_2. After 3 days of culture [^{3}H]thymidine (0.5 μCi per sample; Amersham and Buchler, Braunschweig, Germany) was added and cells were harvested after a further 24 h. Samples were counted in a Kontron scintillation counter. All counts were corrected for background radioactivity of islets and lymphocytes and results were expressed as mean counts per minute (cpm) of triplicate cultures. For the determination of the islet and lymphocyte background radioactivity, 100 islets and 10^5 lymphocytes of each rat strain were cultured. Pretreated and untreated control islets were co-cultured with allogeneic and syngeneic lymphocytes. The ratio of the allogeneic proliferative response to the syngeneic proliferative response was taken as an index for islet (allo-)immunogenicity.

Statistics

Results were expressed as mean ± SEM. For statistical analysis the parameter-free Mann-Whitney U-test was used. Level of significance was defined as $P<0.01$.

Results

MHC class II antigen expression of islets pretreated with monoclonal antibody, complement and culture

MHC class II antigen expression was visualized by indirect immunofluorescence using the monoclonal Ia antibody OX-6. Untreated isolated adult LEW and adult LEW.1WR2 islets exhibited different degrees of antigen expression. Approximately 6.5% of the LEW islets were completely negative for MHC class II antigen-positive cells, compared with 25.8% of the LEW.1WR2 islets (Table 2). In contrast, only 6.8% of the LEW.1WR2 islets contained more than 10 positive cells, compared with 38.0% of the LEW islets. In both strains MHC class II positive cells belonged to two morphologically different cell types: rather small and round leukocyte-like cells and dendritic cells.

Pretreatment of the islets with different monoclonal antibodies directed against MHC class II antigens provoked slightly different results within the two rat strains. In LEW rats islet pretreatment with the monoclonal antibody 29A1 along with ram Ig and complement was most effective, leading to a 86.2% of islets being negative for class II antigens (Table 2). After the pretreatment no islet contained

Table 2. MHC class II antigen expression of pretreated and untreated LEW and LEW.1WR2 islets

Islet pretreatment	MHC class II negative islets (%)		Islets with ≥ 10 class II positive cells (%)		Number of tests (n)	
	LEW	LEW.1WR2	LEW	LEW.1WR2	LEW	LEW.1WR2
None	6.5±2.2*	25.8±3.1	38.0±5.5	6.8±2.9	5	6
mAb OX-6+ ram Ig+C	65.4±2.5	75.1±4.0	0.3±0.2	0	10	8
mAb 29A1 +ram Ig+C	86.2±2.5[a]	59.1±4.5	0	1.7±0.6	6	6
mAb 2MC3+C	73.3±4.0	80.1±4.5[a]	4.4±1.1	0	7	6
5-day culture at 22 °C	74.0±2.7		0		5	
5-day culture at 37 °C	89.6±3.0[a]		0		7	
5-day culture at 22/37 °C	81.1±1.2		0		7	
mAb 29A1+ram Ig +C+22 °C	91.4±3.0[a]		0		6	
mAb 29A1+ram Ig +C+37 °C	92.7±1.8[a]		0		8	
mAb2MC3+C +37 °C		91.1±0.5[a]		0		6

Values are means ± SEM and represent average cumulative results from the number of tests indicated; 100 islets were examined per test.

[a] No significant differences between these different pretreatment protocols

* $P<0.001$ as compared with pretreated islets

mAb, Monoclonal antibody; ram Ig, rabbit anti-mouse immunoglobulin; C, complement

Table 3. Proliferative response of allogeneic T-cells to pretreated and untreated isolated islets of LEW rats (for comparison response in a syngeneic system is given)

Stimulator islets	Responder lymphocytes	Islet pretreatment		
		None	mAb 29A1 +ram Ig+C	mAb29A1+ramIg +C+5-day culture at 37 °C
LEW	BDII	3751.1	1558.9	1805.8
LEW	LEW	800.1	538.6	1423.4
Ratio		4.7	2.7*	1.3*

Proliferative response of BDII and LEW spleen lymphocytes following 3 days of co-culture with allogeneic and syngeneic, pretreated (antibody or antibody with culture) and untreated islets, respectively. Results are expressed as cpm of [^{3}H]thymidine incorporated in a 24-h proliferation assay corrected for background incorporation of islets and lymphocytes. The ratio of the allogeneic proliferative response to the syngeneic proliferative response is taken as an index for islet (allo-)immunogenicity

* $P<0.01$ as compared with untreated islets

Table 4. Proliferative response of allogeneic T-cells to pretreated and untreated isolated islets of LEW.1WR2 rats (for comparison response in a syngeneic system is given)

Stimulator islets	Responder lymphocytes	Islet pretreatment	
		None	mAb 2MC3+C+5-day culture at 37 °C
LEW.1WR2	BDII	3113.7	784.6
LEW.1WR2	LEW.1WR2	1925.0	780.6
Ratio		1.6	1.0*

Proliferative response of BDII and LEW.1WR2 spleen lymphocytes following 3 days of co-culture with allogeneic and syngeneic, pretreated (antibody with culture) and untreated islets, respectively. Results are expressed as cpm of [^{3}H]thymidine incorporated in a 24-h proliferation assay corrected for background incorporation of islets and lymphocytes. The ratio of the allogeneic proliferative response to the syngeneic proliferation response is taken as an index for islet (allo-)immunogenicity

* $P<0.01$ as compared with untreated islets

Table 5. Survival of pretreated and untreated LEW and LEW.1WR2 islet allografts in BDII rats (for comparison survival of syngeneic islet grafts given)

Donor	Recipient	Islet pretreatment	Days of normoglycemia	Mean graft survival time (days)	No. of rats transplanted (*n*)
LEW	BDII	mAb 29A1+ ram Ig+C	4, 6, 6, 6, 7	5.8[a]	5
LEW	BDII	5 days culture at 37 °C	5, 7, 7, 8, 10	7.4[a]	5
LEW	BDII	mAb 29A1+ ram Ig+C+5 days culture at 37 °C	7, 8, 9, 9, 10	8.6*	5
LEW	BDII	None	3, 4, 4, 5, 5	4.2	5
LEW	LEW	mAb 29A1+ ram Ig+C	5×100	>100	5
LEW.1WR2	BDII	mAb 2MC3+C +5 days culture at 37 °C	6, 7, 7, 7, 8	7.0[a]	5
LEW.1WR2	BDII	None	3, 5, 5, 6, 7	5.2	5
LEW.1WR2	LEW.1WR2	mAb 2MC3+C	5×100	>100	5

Islet graft survival following transplantation into the liver via portal vein cannulation of rats made diabetic with streptozotocin (55 mg/kg body weight intravenously)

[a] No significant difference as compared with untreated islets

* $P<0.01$ as compared with untreated islets

more than 10 positive cells (Table 2). By comparison, the maximum effect on LEW.1WR2 islets was obtained by treatment with the monoclonal antibody 2MC3 together with complement. This resulted in 80.1% of the islets being negative for class II antigen expression (Table 2). Islet culture methods were similarly effective in decreasing the percentage of class II antigen-positive islets. After 5 days of culture at 37 °C 89.6% of LEW islets were negative for class II antigen (Table 2). A combined antibody-culture pretreatment was marginally more effective: in LEW rats the percentage of islets completely depleted of class II antigens rose to a mean of 91.4% (22 °C culture) and 92.7% (37 °C culture) (Table 2). No significant differences were observed between islets treated with either moab 29A1, 5 days of culture at 37 °C or a combination of the two procedures. In comparison, the optimum protocol for LEW.1WR2 rat islets was combined pretreatment with mAb 2MC3 plus complement and a 5 days of culture at 37 °C. This protocol resulted in 91.1% of islets being negative for class II antigens (Table 2). Again, there was no significant difference between islet treatment with mAb 2MC3 and complement alone or in combination with a 5-day culture.

Proliferative response of allogeneic T-cells to pretreated and untreated islets in the MLIC

Lymphocytes derived from BDII, LEW or LEW.1WR2 rats were used as responder lymphocytes. Isolated adult islets from LEW or LEW.1WR2 rats served as stimulator islets. These islets were either untreated or pretreated by the most effective protocols as assessed by the studies on class II antigen expression. The proliferative response was studied in a test set of either combined incompatibility for class I and MHC class II antigens (LEW rats and BDII rats; Table 3) or incompatibility for MHC class II antigens but compatibility for MHC class I antigens (LEW.1WR2 rats and BDII rats; Table 4). The allogeneic T-cell proliferative response was significantly stronger when the two strains were incompatible for class I and class II than when they were incompatible for class II only. Untreated islets provoked a ratio of the allogeneic proliferative response to the syngeneic proliferative response of 4.7 in the LEW-BDII strain combination (Table 3) which is almost three times the ratio of 1.6 found in the LEW.1WR2-BDII strain combination (Table 4).

Pretreatment of the islets significantly reduced the allogeneic T-cell response in all cases. Using MHC class I and MHC class II incompatible LEW islets pretreated with mAb 29A1 + ram Ig and complement a 43% reduction in the MLIC ratio to 2.7 was observed (Table 3). Additional pretreatment of the islets with 5 days of culture at 37 °C further reduced the MLIC ratio to 1.3 (Table 3). Using MHC class II incompatible but class I compatible LEW.1WR2 islets pretreated with mAb 2MC3, complement and 5 days of culture at 37 °C, no remaining allogeneic proliferative response was found (MLIC ratio 1.0); the test response was equal to the MLIC response observed in a syngeneic setting (Table 4).

Islet transplantation

In neither the LEW-BDII nor the LEW.1WR2-BDII strain combination did pretreatment of the islets produce long-term allogeneic graft survival in any of the animals following intraportal implantation into streptozotocindiabetic rats (Table 5). Only the combined pretreatment of LEW rat islets with mAb 29A1, ram Ig and C together with a 5-day culture period at 37 °C caused a prolongation of the islet allograft survival which reached the level of statistical significance ($P<0.01$) (Table 5). However, this result does not represent long-term or indefinite islet allograft acceptance. In order to exclude any deleterious effects of antibody pretreatment with mAb 29A1 and mAb 2MC3 on the islets, the same protocols were tested on syngeneic isolated adult LEW and LEW.1WR2 islets. In contrast, all syngeneic islet grafts demonstrated long-term graft survival of more than 100 days (Table 5).

Discussion

This study compared the influence of in vitro islet pretreatment with different monoclonal antibodies against MHC class II antigens and complement, with and without a period in culture, on the MHC class II antigen expression of adult rat islets and its impact on islet allogenicity in vitro and on islet allograft survival in vivo after intraportal transplantation into diabetic rats. Experiments were performed in two different strain combinations, each incompatible for MHC class II antigens but either compatible or incompatible for MHC class I antigens, because there is controversy surrounding the influence of MHC class I and class II antigens on the outcome of allotransplantation.

As dendritic cells are more potent stimulators of cell-mediated immunity than are macrophages, the presence of these MHC class II (Ia-)positive cells within a graft was thought to be responsible for allograft rejection [8, 25]. The release of MHC class II antigen determinants from active stimulator cells triggered by engagement of the MHC complex is believed to stimulate CD4-positive lymphocytes to initiate the rejection process, whereas MHC class I antigens are affected by CD8-positive cytotoxic T-lymphocytes causing the deterioration of the graft [11, 26]. This hypothesis has recently been supported by the observations that treatment with depleting levels of anti-CD4 monoclonal antibody allowed indefinite survival of islet allografts in mice, that anti-CD8 abrogates this effect, and also that treatment with antibodies directed towards the interleukin-2 receptor exclusively expressed on activated immunocompetent cells (T-cells, B-cells and macrophages) indefinitely prolongs islet allograft survival in rats [27–29]. Further insight into the mechanisms of T-cell recognition of cell surface antigens has been provided recently by the detection of the role of adhesion molecules in that process [30, 31]. Following this concept Faustman et al. [13] were the first selectively to eliminate Ia-positive cells from mouse islets by in vitro pretreatment of the islets with antibodies directed against MHC class Ia positive cells together with complement to achieve long-

term islet allograft acceptance. In this study we tried to adapt this successful protocol to the rat islet allograft model.

Detection of class II antigens with the monoclonal antibody OX-6 in an immunofluorescence assay demonstrated different degrees of islet immunogenicity within the two strains used. Only 6.5% of untreated LEW islets were negative for MHC class II antigens compared with 25.8% of LEW.1WR2 islets. With respect to islet immunogenicity represented by MHC class II antigen expression, we found combined treatment with the monoclonal antibody 29A1, complement and a subsequent 5-day culture period at 37 °C to be most effective in suppressing class II antigen expression when adult islets from LEW rats were tested. More than about 90% of the islets were found to be negative for class II antigens after this procedure. In case of the LEW.1WR2 a 90% yield was also obtained when the monoclonal antibody 2MC3 and a 5-day culture period at 37 °C were used.

Transferring the optimal pretreatment protocol to the MLIC studies, different results were observed. Successfully pretreated LEW islets (92.7% of the islets were negative for class II antigens) provoked a significantly reduced allogeneic T-cell proliferative response compared with untreated islets (ratio 1.3 vs 4.7). However, differences still remained between the allogeneic and the syngeneic test responses. This may be explained by observations that 2–3% of residual MHC class II antigen-positive cells are sufficient to simulate the allogeneic T-cell proliferative response in the MLIC [21]. On the other hand, successfully pretreated LEW.1WR2 islets (91.1% of the islets negative for class II) produced complete suppression of the allogeneic response (ratio 1.0 vs 1.0). This is in contrast to recent findings in mouse studies by Stock et al. [32]. They were unable to inhibit the allogeneic T-cell proliferative response in the MLIC after total immunodepletion of class II positive cells from pancreatic mouse islets. It is noteworthy that they used a strain combination incompatible for MHC class I in their MLIC test setting, whereas in our MLIC testing of LEW.1WR2 stimulator islets against BDII responder lymphocytes no disparity for MHC class I antigen existed. Furthermore, the same group demonstrated that in the absence of MHC class II antigen expression, differences in the MHC class I allotype alone are able to provoke an allogeneic T-cell response [22]. However, in our strain combination that was concordant for class I even LEW.1WR2 islets provoked only a mild allogeneic BDII T-cell response which was about one third that of the LEW-BDII combination. This leads to the suggestion that in the rat strain-dependent differences may exist; in particular LEW islets seem to be much more immunogenic than LEW.1WR2 islets when exposed to lymphocytes of allogeneic BDII rats. But the influence of MHC class I disparity remains unclear. Activation and proliferation of $CD4^+$ T-cells is induced by MHC class II antigens. However, it seems to be necessary for these MHC class II antigens to be expressed on an active antigen-presenting cell (APC) [11, 33, 34]. Thus, APC-induced activation of $CD4^+$ T-cells may also induce activation and proliferation of $CD8^+$ T-cells by secretion of cytokines such as interleukin-2 (IL-2), interferon gamma (IFN-γ) and tumour necrosis factor (TNF). This effect would presuppose MHC class I antigen disparity.

This interpretation, however, is not sufficient to explain the results of intraportal allotransplantation of pretreated islets into diabetic rats. In none of the cases was long-term allograft survival achieved following intrahepatic islet lodging via portal venous cannulation. As a control, viability and preserved endocrine function of pretreated islets were demonstrated by syngeneic transplantations, which had graft survival times of more than 100 days. Even shedding of islet MHC class II antigens along with donor and recipient concordance for MHC class I antigens was not followed by prevention of islet allograft rejection in vivo, despite the absence of any allogeneic T-cell response in vitro (LEW.1WR2-BDII MLIC system). There was no significant difference in allograft survival between the strain combinations concordant and discordant for MHC class I. In this study at least, the in vitro result of the MLIC did not correlate with the outcome of allotransplantation in vivo. In particular, the risk of an islet allograft being rejected was underestimated, whereas mixed lymphocyte culture (MLC) may sometimes overestimate the risk of allograft rejection [24], thus limiting the predictive value of such in vitro test systems. Similarly, Gores et al. [17] did not achieve prolonged rat islet allograft survival even after total depletion of class II antigen-positive cells from the islets. They explain induction of allograft rejection by a so-called indirect pathway in which shed alloantigens can be indirectly presented by responder APCs to $CD4^+$ T-cells, thus producing allograft rejection; the role of MHC class I antigens was interpreted on the basis of in vitro studies by Singer's group [35–38]. Our findings may support this hypothesis.

This again raises the questions of the role of MHC antigens in islet allograft rejection and of the main trigger mechanisms. The failure to see prolonged allograft survival after anti-class II antigen islet pretreatment may be simply quantitative, since it was stated that 95% of donor class II positive cells have to be eliminated [20]. In our hands a maximum of 92.7% of the islets were depleted of MHC class II antigen, which might not have been sufficient. On the other hand even total depletion of class II antigen expression does not unconditionally promise success [17]. Responder $CD4^+$ T-cells may infiltrate the pretreated class II negative graft and may induce novel class I and class II antigen expression by the secretion of cytokines such as IFN-γ even on non-endocrine cells [39]. IFN-γ and probably TNF induce T-cell activation and proliferation. IFN-γ was found to be necessary for the induction of MHC class I antigen expression in the graft and this step was said to be mandatory to render the target cells sensitive to the cytotoxic activity of specific $CD8^+$ T-cells. This is in contrast to findings that islet allograft rejection is not enhanced even after class I antigen induction prior to allografting [40].

Apart from previous controversial results there is now a body of evidence that islet immunomodulation by MHC class II antigen depletion prior to transplantation may facilitate islet allografting – but cytokine-mediated processes may trigger allograft destruction, counteracting the effects of immunomodulation. Thus, combined islet pre-

treatment together with treatment of the recipient with cyclosporin to block the production of cytokines and the activation and maturation of T-lymphocytes [41, 42] may solve the problem. However, treatment of the recipient with cyclosporin would counteract the aim of circumventing the hazards of general immunosuppression. Future protocols should consist of measures blocking the direct and indirect pathways of alloantigen presentation by effective islet in vitro pretreatment and recipient treatment with highly selective substances such as anti-L3T4 monoclonal antibody [21] or anti-IL-2 receptor antibody [29] that block cytokine production and T-cell recruitment but leave the immune system as a whole unaffected.

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