MHC Class II Antigen Expression on the Various Cells of Normal and Activated Isolated Pancreatic Islets

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It is the aim of this study to characterize and quantify the cells within isolated rat islets that express MHC class II antigens. A set of five monoclonal antibodies and two polyclonal antisera of defined specificity were used in combination with a newly devised procedure for three-dimensional immunofluorescence evaluation of intact islets. It is shown that in addition to passenger cells, such as lymphocytes, macrophages, and dendriticlike cells, vascular endothelial and endocrine cells are also capable of expressing class II antigens. This expression is strongly influenced by in vitro culture, pregnancy, streptozotocin-induced diabetes, transplantation trauma, and alloantigenic stimuli. The possible role of the above cells in antigen presentation related to islet transplantation is discussed.

Key words: pancreas islets, beta cells, islet transplantation, Ia antigens

INTRODUCTION

Major histocompatibility complex class II antigens, which have primarily been identified as transplantation antigens, are known to play an essential role in the initiation of immune responses [1]. These antigens, originally called Ia antigens, are regular structures on antigen-presenting cells (APC), such as dendritic cells and macrophages [2,3]. Furthermore, there is increasing evidence from the recent literature that class II antigens can also be expressed on the surface of other nonlymphoid but possibly immunogenic cells [4–11]. Though little is known about the meaning of such observations, it appears that the expression of these structures may be correlated to certain states of functional activity of the cells in question [4].

With regard to the pancreas, immunologic analyses of either frozen tissue sections [12,13] or isolated mouse [14] and canine [15] islets have indicated that class II antigens normally appear to be restricted to passenger leukocytes. Under the particular conditions of pancreas graft rejection, these antigens reportedly were also expressed on duct epithelia, acinar, and endothelial cells of the big vessels, but not on cells of the endocrine tissue within the pancreas [16]. Because the transplantation of pancreatic islets represents the increasingly attractive alternative to full-organ transplantation for a better therapy of diabetes mellitus, it is becoming more and more important to concentrate on a thorough diagnosis of the possible class II antigen expression of the various cell-types of such islets.

We reasoned that (a) isolated islets may react differently from those embedded within exocrine tissue of the whole organ, (b) different diagnostic tools (antibodies) and methods in different laboratories may account for different findings, and (c) a variety of functional conditions should be investigated. It is hoped that a better understanding of the occurrence of the highly immunogenic class II antigens and their manipulation will open new doors to more successful therapy via transplantation.

Therefore, in the present study a set of five monoclonal antibodies and two polyclonal antisera of defined specificity were applied to characterize lymphocytes, macrophages, and dendritic cells, vascular endothelial and endocrine cells of intact but isolated rat islets by indirect immunofluorescence in a newly devised procedure for three-dimensional evaluation. It will be shown that endothelial and beta cells are also able to express class II

antigens and that this expression may vary strongly within in vitro culture time and, more interestingly, with glucose metabolism-determined stimuli (such as pregnancy or diabetes) as well as other nonimmunologic or specific (allotransplantation-related) stimuli.

MATERIALS AND METHODS

Animals

CAP (RT1^c), DA (RT1^a), and LEW (RT1^l) rats of both sexes were bred and housed at the department of immunology and used at the age of 2–3 months.

Media

CMRL-1066 (cat. no. 41-1530, Gibco, Karlsruhe, FRG) was supplemented with 1 ml L-glutamin (200 mM), 10% heat-inactivated fetal calf serum, 1 ml penicillin/streptomycin (10,000 E/ml) and 150 mg% glucose for in vitro culture of isolated islets. Hanks' balanced salt solution (HBSS) (cat. no. 41-4020, Gibco, Karlsruhe, FRG) was used for washing isolated islets. Phosphate-buffered saline (PBS), was used for antibody/antiserum dilutions.

Diabetic Animals

Islet graft recipients were male LEW rats, made diabetic by a single i.v. injection of 55 mg/kg streptozotocin (Upjohn, Kalamazoo, MI).

Pancreatic Tissue

Rats were killed under ether anesthesia and total pancreata were removed, cut into pieces with a razor blade, snap-frozen, and stored in liquid nitrogen. Indirect immunofluorescence was performed according to standard techniques [12].

Islet Preparation

Islet isolation and purification were performed by collagenase digestion, Ficoll (Sigma Chemicals, Munich, FRG) density gradient centrifugation and by handpicking with the aid of a dissecting microscope using the green filter light technique [17]. A good isolation yielded 200 islets from a single donor.

Monoclonal Antibodies (MonAb)

The following mouse MonAb were used as primary antibodies in indirect immunofluorescence assays: MRC-0X2, directed against a monomorphic determinant expressed on rat thymocytes and endothelial cells; MRC-0X6, directed against a monomorphic determinant of the rat Ia antigen; MRC-0X8, which recognizes a determinant on the majority of thymocytes, likely to be the homologue of mouse Lyt-2 and human OKT8 (0X8-reactive cells are not labeled by the W3/25 MonAb); MRC-0X19, which binds to virtually all rat thymocytes, T-lympho-

cytes, and a maximum of 2% of B-lymphocytes [18]; W3/25, which recognizes a membrane glycoprotein present on rat T-helper cells, macrophages and, according to Steiniger et al [13], also on interdigitating dendritic cells. These MonAb were purchased as ascites from Camon, Wiesbaden, FRG, and used in dilutions ranging from 1:20 to 1:75 in PBS according to the activity of the individual batch.

Antisera

An antiporcine insulin antiserum produced in guinea pigs (cat. no. M8309, 1:300 dil., Novo Research Institute, Bagsvaerd, DK) crossreacted with rat insulin and was used without further dilution. A rabbit antirat lymphocyte serum was prepared, absorbed, and tested as described in detail elsewhere [19]. To prepare specified antilymphocyte serum (SAL), sequential absorptions with erythrocytes, peritoneal exudate cells, and fetal liver cells were performed [20]. It may be pointed out that each absorption was proved to be serologically exhaustive; the serum was used at a dilution of 1:4 in PBS and reacted only with the majority of the lymphocytes in the isolated islet. A rabbit antimouse dendritic cell serum, crossreacting with rat dendritic cells of the spleen, was obtained by immunizing rabbits with the mouse hybridoma cell line DCH-7 [21], a kind gift of Dr. Peters, Göttingen, FRG. The crude serum was absorbed with erythrocytes, thymocytes, lymphocytes, and adult liver cells. Thus, the specified anti-dendritic-cell serum SAD, diluted 1:2 in PBS, reacted positively with dendriticlike cells, macrophages, and vascular endothelial cells in the isolated rat islet, whereas the SAL reacted specifically with morphologically typical lymphocytes.

Second Antibodies

The following second antibodies were used: a fluorescein-linked goat antimouse IgG, F (ab')2 (cat. no. 4340, Medac, Hamburg, FRG), diluted 1:20 with PBS for the studies with 0X2, 0X6, 0X8, 0X19, and W3/25; a fluorescein-linked donkey antirabbit Ig (cat. no. N1034, Amersham-Buchler, Braunschweig, FRG), diluted 1:20 with PBS for the studies with SAL and SAD; a rhodamine-linked goat anti-guinea-pig IgG (cat. no. RA-2303, Medac, Hamburg, FRG), diluted 1:20 with PBS for double-labeling studies with pancreatic cryostat sections.

Preparation of Intact Islets for Immunofluorescence

About 150 thoroughly handpicked, clean islets of all sizes were kept in siliconized glass tubes and suspended in 100 µl of the primary antibody for 30 min at 24°C. After gentle washing (3×) with HBSS at 1,500 RPM for 10 sec, 100 µl of the second antibody was added and incubation performed at +4°C for 30 min in the dark. After

a final gentle washing (see above) islets were resuspended in veronal buffer, consisting of 50% glycerine plus 50% veronal buffer (5.5-diethylbarbituric acid sodium salt; buffer substance; MW 206.18; cat. no. 6318, Merck, Darmstadt, FRG). About 20 islets in 10 μl veronal buffer were transferred with a Pasteur pipette onto thoroughly cleaned standard microscopic glass slides. A cover slide was placed on top. With as little buffer as this, the cover slide guaranteed a gentle pressure on the biologic material. Thus, islets remained undamaged and their genuine three-dimensional infrastructure appeared nearly two-dimensional, which facilitated vertical screening. The four edges of the cover slide were completely sealed with Eukitt adhesive (Kindler, Freiburg, FRG) so that no water could evaporate from the buffer. The slides were then kept at +4°C in the dark for 1–2 hr to allow the adhesive to polymerize. Thereafter, each of the ten glass slides of one experiment was thoroughly screened for fluorescent islet cells with an inverted light fluorescence photo microscope (ICM 405, Zeiss, Oberkochen, FGR) at a magnification of × 250. The veronal buffer provided a stabilizing effect on fluorescence so that islets could still be screened 3–5 days after finishing preparation procedures.

Fluorescence Reading/Grading

Fluorescence was graded by its intensity: + + + +, very strong; + + +, strong; + +, medium; +, weak; (+), very weak; −, no detectable fluorescence. The gradings given in Results did not differ significantly among cells derived from different rat strains.

RESULTS

Macrophages and Dendriticlike Cells

Indirect immunofluorescence microscopy with isolated fresh islets of varying sizes from the three different inbred rat strains using the mouse monoclonal antirat Ia antibody MRC-0X6 revealed a population of Ia$^+$ cells which was composed of two subtypes: a very unregularly shaped cell which often had fingerlike protrusions or long plasmatic extensions, resembling the dendriticlike cells (Fig. 1a,c), which were recently described in mouse islets [14], and a smaller, more or less rounded cell that resembled control macrophages from peritoneal exudate cell suspensions (Fig. 1b). The Ia antigen expression of this population is strong, as judged from fluorescence intensity (grading: + + + to + + + +). Fluorescence was always distributed irregularly over the cell surfaces. Cell shape, fluorescence intensity, and distribution of Ia antigens were three characteristics of the MΦ-DC which made it easy to distinguish them from Ia$^+$ LY and other Ia$^+$ cells (see below). They were embedded rather regularly within the parenchyma of the islets and could be

counted easily as single cells when the islets were screened microscopically. The average number of MΦ-DC per freshly isolated islet varied remarkably with the rat strain (Table I).

Control studies performed with a variety of cell-group specific antisera and antibodies revealed the following (Table II): MΦ-DC were completely negative when stained with SAL. As expected, however, the great majority reacted strongly positively when stained with the specified antimouse dendritic cell serum which cross-reacts with rat macrophages and dendritic reticulum cells of the spleen. A positive reaction was also observed with W3/25, which is known to stain not only rat T-helper cells but also macrophages and interstitial dendritic cells [13].

Lymphocytes

A second population of Ia$^+$ cells detected with the 0X6 antibody consisted of small, round cells with very evenly distributed ring fluorescence (grading: + to + +), which was very similar to that of lymph node control cells (Fig. 1a). Interestingly, their average number per islet varied from rat strain to rat strain (Table I). However, in all strains the total number of LY per islet appeared to be two to three times the number of cells belonging to the Ia$^+$ subpopulation, as could be detected with a variety of cell-group specific antisera and antibodies in control studies (Table II). Usually, they were scattered regularly throughout the islets, but sometimes they appeared in groups of two to seven cells.

Vascular Endothelial Cells

Morphologic characteristics were judged from perfusion control experiments with SAD antiserum via the pancreatic artery in situ: The perfused pancreata were removed, islets isolated and incubated in antirabbit Ig·FITC. Fluorescence microscopy revealed a tight, three-dimensional capillary network in each individual islet (data not shown). About 10–30% of the total VE of isolated fresh rat islets expressed Ia antigens when exposed to 0X6 (Fig. 1b). 0X6- and SAD-positive structures appeared to be morphologically identical with the 0X2-reactive structures. Ia antigens were very evenly distributed all over the endothelial tissue of the three rat strains (grading: + +). The detailed serologic pattern of the islet VE is given in Table II.

Beta Cells

Fresh islets. An unexpected finding was that about 20% of all adult islets in the three rat strains expressed Ia on 20–40% of their parenchymal cells when studied in our newly devised procedure. Ia antigens were expressed comparatively weakly (grading: +) but were distributed evenly on the surfaces of the cells. From double-staining control studies with frozen pancreas tissue sections using
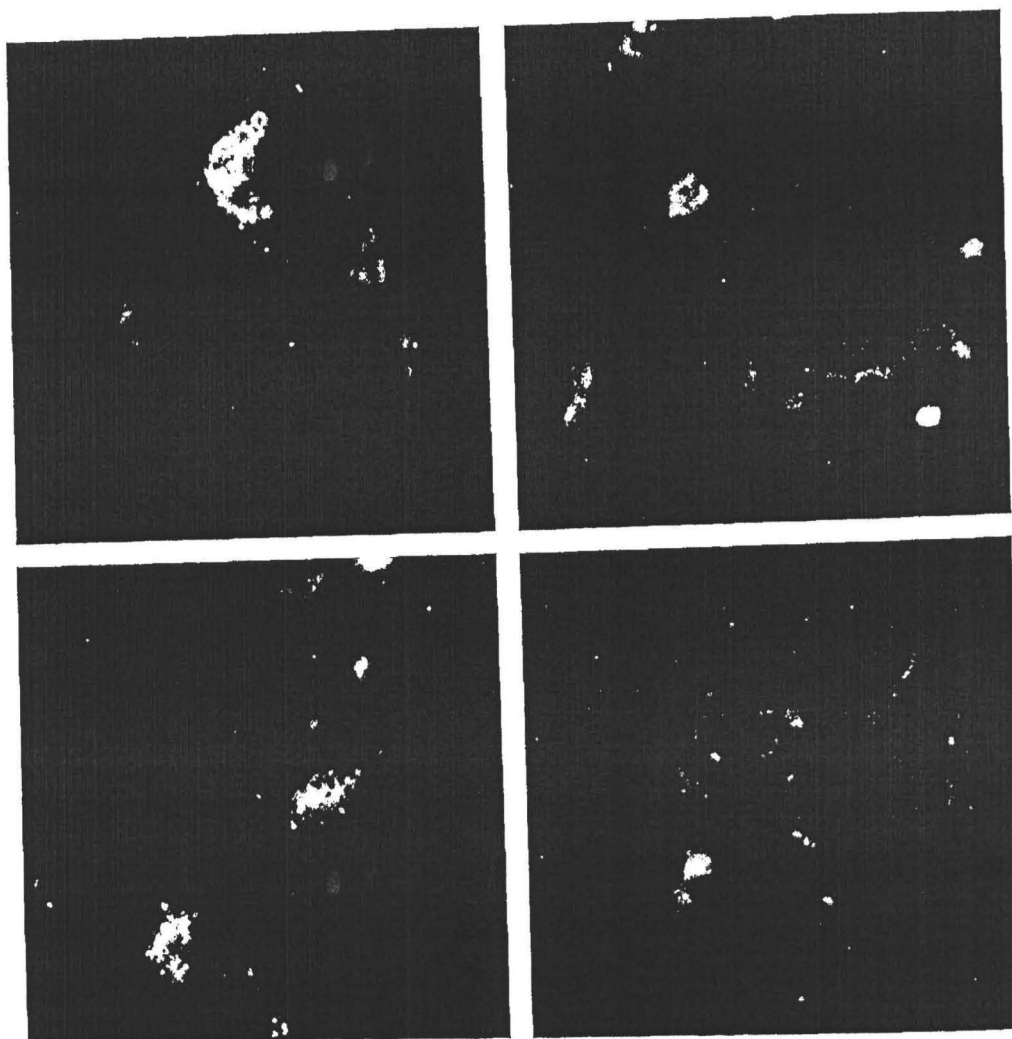
Fig. 1.   MHC class II antigens of the various rat islet cells detected by indirect immunofluorescence using the MonAb 0X6. Islets from normal CAP (RT1^c) rats: (a) An irregularly shaped dendriticlike cell (DC) and two small lymphocytes (LY). (b) An intensively fluorescent cell which resembles control macrophages (MΦ) is surrounded by vascular endothelium (VE). (c) Three more or less intensively fluorescent, irregularly shaped cells of dendriticlike appearance (DC) are embedded within the islet parenchyma. Islet from a pregnant LEW (RT1^l) rat: (d) the majority of islet parenchymal cells express class II antigens on their cell surfaces. (In control experiments with an anti HLA-DR MonAb as first reagent, immunofluorescence was always completely negative.) × 1750.

TABLE I. Quantification of Class II Antigen Expressing Macrophages, Dendriticlike Cells (MΦ-DC), and Lymphocytes (LY) Within Freshly Isolated Islets of Three Different Rat Strains Using Male Individuals and the MonAb OX6 (Control studies with female rats gave identical results)

Rat strain haplotype	No. of animals	Islets screened	MΦ-DC per islet	LY per islet	MΦ-DC:LY ratio
CAP:RT1^c	7	1045	19.6	2.22	9:1
DA:RT1^a	6	820	6.9	0.56	12:1
LEW:RT1^l	7	940	20.1	0.63	32:1

0X6 and a guinea pig antiporcine insulin antibody (which crossreacts with rat insulin and was coupled to anti-guinea-pig IgG·rhodamine), it became confirmed that it was the insulin-producing beta cells that expressed Ia. Consequently, experiments were planned to activate insulin production in vitro and in vivo in order to test for an increase of the expression of these antigenic determinants on the endocrine parenchymal cells.
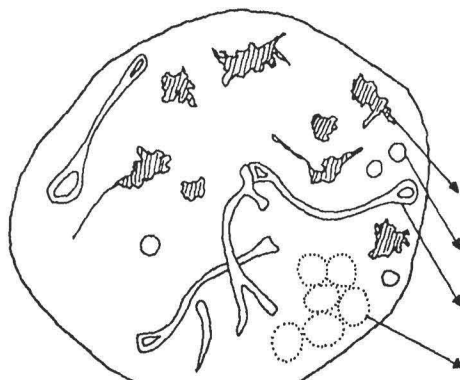
In vitro activation of islets. Attempts to activate isolated CAP, LEW, or DA islets in vitro by a high concentration of glucose in the CMRL medium (supplemented with 500 mg% glucose) for 24 or 48 hours led to a decrease rather than an increase of the amount of Ia antigen on the parenchymal cells as compared to fresh controls.

In vivo activation of islets. It was thought that the Ia antigen expression might be revealed following only in vivo activation of the endocrine cells. To test this conjecture, fresh islets from syngeneically mated pregnant CAP rats of varying gestation periods were studied, since it is known that pregnancy represents a strong, physiological activation stimulus for these cells (Hahn,

TABLE II. Antigenic Pattern of the Various Islet Cells as Evaluated by a Set of Five Monoclonal Antibodies and Two Polyclonal Antisera*

Cell type	Serum						
	OX2	OX6	OX8	OX19	W3/25	SAL	SAD
Macrophages, dendritic cells	−	++++	−	−	++	−	++++
Lymphocytes	−	++	++	++	(+)	++++	−
Vascular endothelial cells	+++	++	−	−	−	−	+++
Endocrine cells	−	+	−	−	−	−	−

*Grading of fluorescence: ++++, very strong; +++, strong; ++, medium; +, weak; (+), very weak; −, no fluorescence. N ≈ 200 islets in each antibody/antiserum experiment except for OX6, where n was 2,805.

TABLE III. Schematic Outline of Class II (OX6)-Reactive Cells Within Isolated Fresh or Cultured CAP Islets (Grading of fluorescence: see Table II; N = 5 experiments per day, each with 100–150 islets)

Antiserum	OX6 −	PBS + α mse. FITC	Control ascites + α mse. FITC	OX6 + antimouse IgG·FITC			
Days in culture	0	0	0	0	4	8	12
Cell type							
Macrophages, dendritic cells	−	−	−	++++	+++	++	+
Lymphocytes	−	−	−	++	+	(+)	(+)
Vascular endothelial cells	−	−	−	++	−	−	−
Endocrine cells	−	−	−	+	−	−	−

personal communication). Islets were incubated in OX6. On the 6th day of gestation about 35% of the beta cells expressed Ia antigens clearly on their cell surfaces, on the 12–14th day 55–65% did, and on the 18–20th day more than 85% did (grading: ++; Fig. 1d). The results show that this antigen expression increases with growing gestation time or with increasing amounts of fetal tissue (see Table IV). As in all previous experiments background fluorescence was completely negative. Here the double-staining experiments with OX6 and the antiinsulin antibody revealed even more impressively than in islets of normal animals that in addition to those Ia⁺ cells identified previously, it was the insulin-secreting cell that expressed Ia.

Long-Term In Vitro Culture of Islets

As it is widely believed that in vitro culture reduces islet immunogenicity and in particular the number of Ia⁺ PC [22,23]), immunofluorescence studies were carried out with cultured CAP and DA islets. These were kept up to 15 days in CMRL medium supplemented as described at 24°C and in 5% CO_2 in air, ie, following the proce-

dure established by Lacy [23]. The results for the various OX6⁺ islet cells are summarized in Table III and indicate the cell-dependent differences in Ia antigen expression and the decrease of this expression during long-term culture. All positive reactions are clearly contrasted by extensive control studies, which were always negative.

For the MΦ-DC population, which is thought to be particularly immunogenic [24], the results are presented in a more detailed form in Figure 2. While the total number of Ia⁺ CAP MΦ-DC decreases strongly from 19.6 to 7 cells per islet within the first 3 days in culture, it decreases only from 6.9 to 5 cells in DA islets. Islets cultured for 7 days still contain a distinct number of such cells, namely 5.5 in CAP and three in DA rats. Surprisingly, even after the long culture period of 15 days, these cells have not yet completely disappeared, as can also be seen from Figure 2. Control studies with SAD gave identical results.

The decrease in LY from cultured islets was more pronounced than that in the MΦ-DC population: from day +4 onwards an OX6-reactive or a SAL-positive cell could be detected only very rarely (Table III). Ia antigen

TABLE IV. Survey of Class II Antigen Expression of Beta Cells in Normal and Variously Activated Islets*

Status	Day	No. of islets screened	Percent positive beta cells	Fluorescence grading
Normal	0	1,045	~20	+
Cult. 500 mg% gluc.	1–2	270	0	—
Cult. 150 mg% gluc.	+1	220	0	—
Pregnant 6 days	0	570	~35	+/++
Pregnant 12–14 days	0	610	55–65	++
Pregnant 18–20 days	0	705	>85	++
Syngeneic nondiabetic	+1	1,050	40–55	+
Syngeneic diabetic	+1	840	85–95	++
Allogeneic nondiabetic	+1	790	80–95	+/++
Allogeneic diabetic	+1	810	90–100	++

*For key to grading of fluorescence, see Table II.

expression on islet VE disappeared rapidly during in vitro culture and was completely abolished on day +4. This may be due to the quick breakdown of the VE's three-dimensional structure (as early as day +1) which was tested with the SAD antiserum. With regard to beta cells, no 0X6-positive reaction could be detected beyond day 0.

Ia Expression in Islet Grafts From Diabetic and Nondiabetic, Syngeneic Hosts

In connection with our interest in islet transplantation and in consequence of the findings described above, the question arose whether the Ia antigen expression of the parenchymal islet cells could be increased by activating insulin production via streptozotocin-induced diabetes. Two hundred fresh LEW islets were transplanted under the kidney capsule of diabetic syngeneic recipients (blood glucose level above 400 mg%). In a second group, nondiabetic syngeneic recipients also received LEW islets to evaluate the traumatic effects of the transplantation procedure on the Ia expression of grafted islets. Twenty-four hours later, all animals from the nondiabetic group and those animals from the diabetic group, which showed a significantly decreased though still above-normal (200 mg%) blood glucose level, were killed and their grafts quantitatively removed with the help of a fine Pasteur pipette.

The most striking observation was the massive reduction in Ia⁺ MΦ-DC, LY, and VE in 5/5 grafts from syngeneic diabetic recipients and also in 6/6 grafts from syngeneic nondiabetic recipients after the short period of only 24 hr of in vivo exposure. About one to three Ia⁺ MΦ-DC per islet, very rarely an Ia⁺ LY, and only a few remnants of Ia⁺ VE were seen. Control studies with SAL and SAD confirmed these findings.

Compared to ungrafted control islets, beta cell Ia expression in grafted islets increased significantly (Table IV): from 20% to 40–55% in the syngeneic nondiabetic
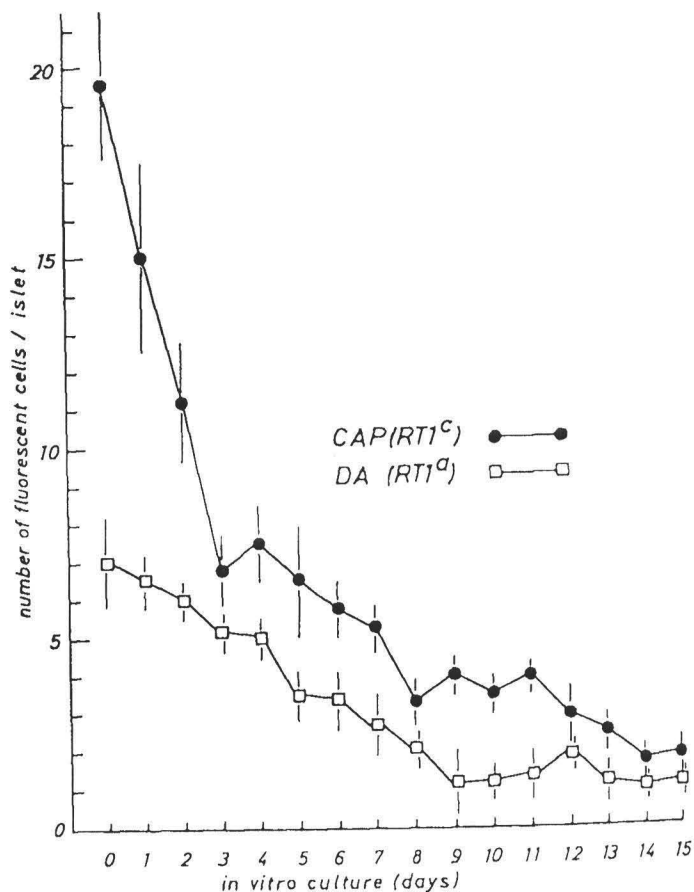

Fig. 2. Quantification of class II antigen expressing macrophages and dendriticlike cells during in vitro culture of isolated islets. Islets of two different rat strains were cultured in CMRL medium (supplemented with 150 mg% glucose) for 15 days at 24°C and 5% CO_2 in air. The medium was changed every second day. The number of 0X6-reactive cells was counted microscopically. N = 5 experiments per day; each with 100–150 islets.

group, obviously as a result of the traumatization due to transplantation, and to 85–95% in the syngeneic diabetic group, obviously as a result of the additional functional stimuli due to diabetes. Character and intensity of Ia expression were similar to that observed in pregnant rats (Fig. 1d).

Ia Expression in Islet Grafts From Nondiabetic Allogeneic Hosts

In addition to the above-mentioned stimuli, the effects of stimuli, which are attributable to the immunologic response to an allograft, were assayed in nondiabetic recipients in the MHC fully allogeneic strain combination CAP to LEW (n = 6). Again, 24 hr after transplantation, islets were removed and processed for immunofluorescence, as described above.

For the islet MΦ-DC, LY, and VE, the results were nearly identical to those reported for the syngeneic grafts, ie, a strong decrease in these cells after their short in vivo

exposure. In comparison to the syngeneic nondiabetic grafts, the Ia expression on beta cells increased significantly to 80–95% in the allogeneic nondiabetic group, obviously due to specific immunologic stimuli (Table IV).

DISCUSSION

In the present study, five monoclonal antibodies and two polyclonal antisera of defined specificity were used to characterize and quantify the cells bearing MHC class II antigens in normal and activated adult rat islets by indirect immunofluorescence. It is shown that not only passenger cells (PC), such as lymphocytes, macrophages, and dendriticlike cells, but also vascular endothelial and beta cells are capable of expressing MHC class II antigens (originally referred to as Ia). This expression is strongly influenced by in vitro culture, pregnancy, streptozotocin-induced diabetes, transplantation trauma, and alloantigenic stimuli.

The finding of distinct numbers of Ia$^+$ PC in the normal rat islet correlates well with similar observations in isolated mouse [14] and canine [15] islets and with our earlier findings in single-cell suspensions of rat islets [20]. Interestingly, the average number of PC per islet varies widely between the three species and appears to be particularly high in rats. Assuming that PC play a major role in tissue immunogenicity, their variation in different rat strains may explain why it is easier to achieve better islet transplantation results in some rat strain combinations than in others [25,26].

On the basis of literature, it was expected that rat islet PC would have completely disappeared following culture at 24°C: islets cultured for 7 days still contained 5 MΦ-DC per CAP islet, a number which is about the same in uncultured normal mouse and canine islets [14,15]. This may account for the poor survival rate when such cultured rat islets are transplanted without immunosuppression of the graft recipient [27, and own unpublished data]. In contrast to these in vitro data, the strong and rapid (within 1 day) in vivo reduction in PC from syngeneic and allogeneic grafts is impressive. The simple but effective diagnostic method presented here should help to quantify such cells under varying experimental conditions and may thus allow better understanding and control of islet transplantation results.

The reports on Ia expression on rat vascular endothelial cells are controversial [28–32]. In islets, a rare, more dotlike occurrence has been previously observed [32] which differs clearly from the even Ia expression all over the cell surfaces found here and may be due to the methodologic approach using electron microscopy. With respect to function, it has been claimed that a possible antigen presentation of this ubiquitous type of tissue should be taken seriously into consideration [33]. How-

ever, with regard to isolated islets, it may be mentioned that the VE's natural three-dimensional structure quickly breaks down after in vitro culture and transplantation, ie, after disconnection from circulation (recent unpublished observation). This obviously does not hold for the islets in pancreas organ grafts.

At present, it is generally believed that beta cells do not express Ia antigens [13,15,23,32,34–37]. The series of data presented here and obtained with the well-acknowledged antirat Ia antibody MRC-0X6 clearly contradicts this opinion. However, it may be mentioned that in our experience various batches may significantly vary in terms of antibody concentration requirements. Nevertheless, the most likely explanation for our observations may be related to the relatively rare and weak expression of Ia antigens on normal islets and corresponding methodologic limitations. Our approach differs from those of others in two respects: First, the newly devised procedure for three-dimensional evaluation may have accounted for a higher sensitivity. This view is supported by the observation that the "conventional" immunofluorescence technique using tissue sections did not allow to clearly detect the normal beta cell Ia antigen expression. Second, the inclusion of metabolically hyperactive islets, such as those from pregnant rats [38], in our study has allowed us to confirm and further extend the observation in question.

The finding of a variable Ia expression on islet parenchymal cells is not too surprising when we consider the increasing evidence for such an event in a variety of cells [4–6, 8–11]. The fact that beta cells can be induced to express Ia and/or increase this expression raises the possibility that they can develop immunologic potential following transplantation. This potential should be considered to be positively correlated to the density of Ia antigens expressed on the surface of the cells [39]. Thus, efforts to manipulate the inductive phase of the immune response, for which the costimulatory activity of an Ia$^+$ cell is believed to be obligatory [40], should not only focus on the manipulation of PC but also on this particular parenchymal cell. The passenger leukocyte concept, reviewed in detail elsewhere [41], may thus be too simple an approach toward such a complex problem as of islet immunogenicity.

The observation that the Ia expression of beta cells increases form 20 to 55% due to the traumatization effects of the transplantation procedure and to as much as 80% when transplantation is purposely performed badly (preliminary observation) indicates the possible importance of an optimized technique of islet grafting and possibly also of full-organ grafting. In other words, this nonspecific methodologic factor may indirectly obtain immunologic weight because of the initiation of thus-far unknown Ia expression. Further functional challenges in the form of streptozotocin-induced diabetes and the immunologic re-

sponse to transplantation alloantigens, both assayed separately in this study, obviously add to the above-mentioned traumatic effects. Their role in isolated pancreatic islet allotransplantation models of major immunogenicity are presently under further investigation.

ACKNOWLEDGMENTS

REFERENCES

1. Benacerraf B, Germain RN: The immune response genes of the major histocompatibility complex. Immunol Rev 38:70–119, 1978.
2. Steinman RM: Dendritic cells. Transplantation 31:151–155, 1981.
3. Unanue ER: Antigen-presenting function of the macrophage. Annu Rev Immunol 2:395–428, 1984.
4. Klareskog L, Forsum U, Peterson PA: Hormonal regulation of the expression of Ia antigens on mammary gland epithelium. Eur J Immunol 10:958–963, 1980.
5. Barclay N, Mason DW: Induction of Ia antigen in rat epidermal cells and gut epithelium by immunological stimuli. J Exp Med 156:1665–1676, 1982.
6. Lampert IA, Suitters AJ, Chrisholm PM: Expression of Ia antigen on epidermal keratinocytes in graft-versus-host disease. Nature 293:149–150, 1981.
7. Beller DI, Ho K: Regulation of macrophage populations: V. Evaluation of the control of macrophage Ia expression in vitro. J Immunol 129:971–976, 1982.
8. De Waal RMW, Bogman MJJ, Maass CN, Cornelissen LMH, Tax WJM, Koene RAP: Variable expression of Ia antigens on the vascular endothelium of mouse skin allografts. Nature 303:426–429, 1983.
9. Suitters AJ, Lampert IA: Class II antigen induction in the liver of rats with graft-versus-host disease. Transplantation 38:194–196, 1984.
10. Houghton AN, Thomson TM, Gross D, Oettgen HF, Old LJ: Surface antigens of melanoma and melanocytes: Specificity of induction of Ia antigens by human γ-interferon. J Exp Med 160:255–269, 1984.
11. Dallman MJ, Mason DW: Induction of Ia antigens on murine epidermal cells during the rejection of skin allografts. Transplantation 36:222–224, 1983.
12. Hart DNJ, Fabre JW: Demonstration and characterization of Ia-positive dendritic cells in the interstitial connective tissues of rat heart and other tissues, but not brain. J Exp Med 153:347–361, 1981.
13. Steiniger B, Klempnauer J, Wonigeit K: Phenotype and histological distribution of interstitial dendritic cells in the rat pancreas, liver, heart, and kidney. Transplantation 38:169–175, 1984.
14. Faustman D, Steinman R, Gebel H, Hauptfeld V, Davie J, Lacy P: Prevention of mouse islet allograft rejection by elimination of intra-islet dendritic cells. Transplant Proc 17:420–422, 1985.
15. Gebel HM, Yasunami Y, Diekgraefe B, Davie JM, Lacy PE: Ia-bearing cells within isolated canine islets. Transplantation 36:346–347, 1983.
16. Steiniger B, Klempnauer J, Wonigeit K: Effect of the rejection process on class I and class II major histocompatibility complex antigen expression in the rat pancreas. Transplant Proc 17:407–411, 1985.
17. Finke E, Lacy P, Ono J: Use of reflected green light for specific identification of islets in vitro after collagenase isolation. Diabetes 28:612–613, 1979.
18. Dallman MJ, Thomas ML, Green JR: MRC OX-19: A monoclonal antibody that labels rat T lymphocytes and augments in vitro proliferative responses. Eur J Immunol 14:260–267, 1984.
19. Müller-Ruchholtz W, Wottge HU, Müller-Hermelink HK: Bone marrow transplantation in rats across strong histocompatibility barriers by selective elimination of lymphoid cells in donor marrow. Transplant Proc 8:537–541, 1976.
20. Ulrichs K, Schang T, Keller R, Müller-Ruchholtz W: Reactivity of pancreas islet cells with antisera of known specificity. Transplant Proc 16:857–860, 1984.
21. Peters JH: Hybridomas of mouse dendritic cells (DC) expressing phenotypic markers of DC including growth-stimulatory action on T lymphocytes. In: Resch K and Kirchner H, eds. Mechanisms of Lymphocyte Activation. New York: Elsevier/North Holland Biomedical Press, pp 537–540, 1981.
22. Bowen KM, Lafferty KJ: Reversal of diabetes by allogeneic islet transplantation without immunosuppression. Aust J Exp Biol Med Sci 58:441–447, 1980.
23. Lacy PE, Davie JM: Transplantation of pancreatic islets. Annu Rev Immunol 2:183–198, 1984.
24. Klinkert WEF, La Bradie JH, Bowers WE: Accessory and stimulating properties of dendritic cells and macrophages isolated from various rat tissues. J Exp Med 156:1–19, 1982.
25. Reckard CR, Barker CF: Transplantation of isolated pancreatic islets across strong and weak histocompatibility barriers. Transplant Proc 5:761–763, 1973.
26. Reece-Smith H, McShane P, Morris PJ: Pretreatment of isolated adult islets with antibody. Effect on survival in allogeneic hosts. Transplantation 36:228–230, 1983.
27. Lacy PE, Davie JM, Finke EH: Induction of rejection of successful allografts of rat islets by donor peritoneal exudate cells. Transplantation 28:415–520, 1979.
28. Mayrhofer G, Schon-Hegrad MA: Ia antigens in rat kidney, with special reference to their expression in tubular epithelium. J Exp Med 157:2097–2109, 1983.
29. Paul LC, Paradysz JM, Milford EL, Kunz HW, Carpenter CB: Expression of RT1. A and RT1. B/D antigens on endothelium of rat kidneys. Transplantation 34:121–128, 1982.
30. Von Willebrand E, Parthenais E, Hayry P: Expression of the major AgB histocompatibility complex antigens on different structural components of rat kidney and heart. Cell Immunol 52:313–324, 1980.
31. Hart DNJ, Fabre JW: Endogenously produced Ia antigens within cells of convoluted tubules of rat kidney. J Immunol 126:2109–2113, 1981.
32. Rabinovitch A, Alejandro R, Noel J, Brunschwig JP, Ryan US: Tissue culture reduces Ia antigen-bearing cells in rat islets and prolongs islet allograft survival. Diabetes 31:48–54, 1982.
33. Burger DR, Vetto RM: Hypothesis: Vascular endothelium as a major participant in T-lymphocyte immunity. Cell Immunol 70:357–361, 1982.
34. Faustman D, Hauptfeld V, Davie J, Lacy P, Shreffler D: Murine pancreatic β-cells express H-2K and H-2D but not Ia antigens. J Exp Med 151:1563–1568, 1980.
35. Hart DNJ, Newton MR, Reece-Smith H, Fabre JW, Morris PJ: Major histocompatibility complex antigens in the rat pancreas, isolated pancreatic islets, thyroid and adrenal. Transplantation 36:431–435, 1983.

36. Parr EL, Lafferty KJ, Bowen KM, McKenzie IFC: H-2 complex and Ia antigens on cells dissociated from mouse thyroid glands and islets of Langerhans. Transplantation 30:142–148, 1980.

37. Baekkeskov S, Kanatsuna T, Klareskog L, Nielsen DA, Peterson PA, Rubenstein AH, Steiner DF, Lernmark A: Expression of major histocompatibility antigens on pancreatic islet cells. Proc Natl Acad Sci USA 78:6456–6460, 1981.

38. Hahn HJ, Ziegler B, Weiss I, Besch W, Heinke P: In vitro pancreatic B-cell response of hyperinsulinemic rats. Differences between pregnancy and VMH-lesions. Acta Diabetol Lat 20:231–240, 1983.

39. Janeway CA, Bottomly K, Babich J, Conrad P, Conzen S, Jones B, Kaye J, Katz M, McVay L, Murphy DB, Tite J: Quantitative variation in Ia antigen expression plays a central role in immune regulation. Immunol Today 5:99–105, 1984.

40. Lafferty KJ, Prowse SJ, Simeonovic CJ, Hegre O, Chase HP: Current status of experimental islet transplantation. Transplant Proc 16:813–819, 1984.

41. Lafferty KJ, Prowse SJ, Simeonovic CJ, Warren HS: Immunobiology of tissue transplantation: A return to the passenger leukocyte concept. Annu Rev Immunol 1:143–173, 1983.