REGULATION OF ANTIGEN EXPRESSION

Genetically Determined Variation of Constitutive Major Histocompatibility Complex Class II Antigen Expression in Various Rat Strains and Cell Types

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MAJOR HISTOCOMPATIBILITY complex (MHC) class II (Ia) antigens are thought to act as major factors in initiating a T-dependent immune response. They are either expressed constitutively on the cell surface or variably following activation under appropriate influences.[1] In allotransplantation, immunogenicity is determined by the presence of these gene products on the grafted cells, ie, by the extent of their expression that may be manipulated by various protocols.[2–4] Constitutive expression in the mouse, rat, and dog is believed to be restricted to the marrow-derived macrophages, dendriticlike cells, B lymphocytes, and activated T lymphocytes, whereas in humans it includes the non–marrow-derived vascular endothelial cells.[5] In contrast to this widely held view, we recently found in rats constitutively expressed class II (I-A) antigens on pancreatic islet vascular endothelial and epithelial β cells.[6] The question arose whether this surprising finding would also hold for (1) the non–marrow-derived cells of other tissues, (2) rat strains, which so far were not included in our studies, and (3) the I-E subregion of the rat RT1 complex. This study was greatly facilitated by the availability of the high-titered anti–I-E monoclonal antibody (MoAb) 29A1 of our own production that we used in comparison with MRC-OX17 and MRC-OX6.

MATERIALS AND METHODS

Animals

Male LEW ($RT1^{l}$), AS ($RT1^{l}$), F344 ($RT1^{lv1}$), BN ($RT1^{n}$), DA ($RT1^{av1}$), CAP ($RT1^{c}$) and AVN ($RT1^{a}$) rats were bred at our department. AUG ($RT1^{c}$) rats ($RT1^{c}$) were obtained from OLAC, England; PUG ($RT1^{c}$) from Möllegaards Breeding Center, Denmark; and LEW.1C ($RT1^{c}$) from Institut Versuchstierkunde, Hannover, FRG. All rats were aged 2 to 3 months.

Islet Isolation

Islet isolation was performed by collagenase digestion according to Lacy and Kostianovsky.[7] Only clean, handpicked islets were used.

Tissue Section Preparation

The animals were killed under ether anesthesia. The spleen, thymus, liver, heart, kidney, pancreas and adrenal gland were removed immediately, placed into ice-cold phosphate-buffered saline, cut into small pieces, snap-frozen, and stored in N_2. Cryostat sections of 8 μm were cut, air-dried on gelatinized slides, fixed in acetone, and stored at −20 °C until used.

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MoAbs

MRC-OX6 (anti-rat I-A) and MRC-OX17 (anti-rat I-E) were obtained from Camon, Wiesbaden, FRG, as ascites fluids and used in dilutions 1:40 to 1:70. 29A1 (anti-rat I-E) was produced and described by us[8] and used as culture supernatant 1:1. Following careful recloning, hybridoma cells produced high avidity antibodies only during the early culture phase. Therefore, supernatants collected in the first 2 weeks are referred to as 29A1-HT (high titer), whereas later supernatants are called 29A1-LT (low titer). Secondary antibodies of standard quality were obtained from various companies.

Histological Techniques

The procedure for immunofluorescence studies with intact islets has been described elsewhere.[6] Briefly, islets were incubated with the appropriate MoAb in 10-mL plastic tubes, transferred onto glass slides (20 islets in 10 μL buffer), sealed with a cover slide, and screened microscopically for fluorescent cells under a Zeiss ICM 405. The immunoperoxidase technique was performed according to a standard procedure.

RESULTS

Immunofluorescence Studies of Intact Islets

Table 1 summarizes the results with islets of the various rat allotypes after staining with the four anti–class II MoAbs. When using the 29A1-HT, all four $RT1^c$ allotypes showed a significant MHC class II (I-E) antigen expression not only on macrophages, dendriticlike cells and lymphocytes, but also on vascular endothelial and epithelial β cells. (Evaluation of the relatively weaker antigen expression on the non–marrow-derived cells was facilitated by the fact that background staining in all experiments was definitely negative). By contrast, I-E antigen expression on islet cells of the non-$RT1^c$ group was clearly limited to the marrow-derived cells. When using the 29A1-LT, MRC-OX17, and MRC-OX6 MoAbs, islets of both groups showed identical patterns, ie, I-E and I-A antigens were detectable only on the marrow-derived cells.

Immunoperoxidase Studies of Nonlymphoid Tissues (Pancreas, Heart, Liver, Kidney, Adrenal Gland)

The results of the studies with frozen tissue sections are summarized in Table 2. As expected from the preceding studies with intact islets, the four MoAbs reacted uniformly positive with the marrow-derived cells in all five tissues investigated and in all rat strains, $RT1^c$, or non-$RT1^c$. The specification of these cells into cells of the macrophage/dendriticlike group and, occasionally, lymphocytes was not intended.

However, in studying non–marrow-derived cells, 29A1-HT clearly differentiated between the $RT1^c$ and the non-$RT1^c$ allotypes in contrast to the other MoAbs. In all five tissues, cells of the vascular endothelium (including venous, arterial, and capillary endothelial cells) stained positively for I-E antigens. This finding is documented for the sinusoids of the liver in Fig 1A. Other non–marrow-derived cells that by the use of 29A1-HT were detected to express I-E antigens in $RT1^c$ tissues are the following: pancreatic islet β cells (in confirmation of the aforementioned immunofluorescence data) as shown in Fig 1C, hepatocytes as shown in Fig 1A, and medullary epithelial cells of the adrenal gland. It may be pointed out that background staining of control sections was definitely negative.

Immunoperoxidase Studies of Lymphoid Tissues (Spleen, Thymus)

The results are given in Table 2. Like the nonlymphoid tissues, vascular endothelial cells of the various regions within the two lymphatic tissues stained positively only with the 29A1-HT and only in the $RT1^c$ group. In addition, cortical epithelial cells of the thymus that reacted similarly with 29A1-LT, MRC-OX17, and MRC-OX6 in both RT1 groups showed a clearly stronger staining pattern with 29A1-HT in the $RT1^c$ group.

Preliminary Studies With Human Islet Tissue

Of ten crude human islet preparations eight were investigated by immunofluorescence for a possibly variable DR expression on the various cell types within the preparation. Marrow-derived macrophages and dendriticlike cells (including a larger and a smaller

Table 1. MHC Class II Antigen Expression of Various Cells in Intact Islets Using Rat Strains of Different Allotypes (Immunofluorescence Studies)

MoAbs	Cell Type Within Isolated Islet	RT1^c Group				Non-RT1^c Group					
		CAP	AUG	PVG	LEW.1C	LEW	AS	F344	BN	DA	AVN
29A1-HT (I-E)	MØ-DC	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	LY	++	++	++	++	++	++	++	++	++	++
	VE	++	++	++	++	–	–	–	–	–	–
	β cells	++/+	++/+	++/+	++/+	–	–	–	–	–	–
29A1-LT (I-E)	MØ-DC	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
MRC-OX17 (I-E)	LY	++	++	++	++	++	++	++	++	++	++
MRC-OX6 (I-A)	VE	–	–	–	–	–	–	–	–	–	–
	β cells	–	–	–	–	–	–	–	–	–	–

Cell types: MØ-DC, macrophages and dendriticlike cells; LY, lymphocytes; VE, vascular endothelial cells (venae, arteriae, capillaries); β cells, endocrine β cells.

Fluorescence grading: ++++, very strong; +++, strong; ++, medium; +, weak; (+), very weak; –, no detectable fluorescence; N = 200 to 600 clean, hand-picked islets per strain per MoAb.

Table 2. MHC Class II Antigen Expression of Various Cells in Tissue Sections of Different Nonlymphoid and Lymphoid Organs Using Rat Strains of Different Allotypes (Immunoperoxidase Studies)

Tissue	RT1^c Group		Non-RT1^c Group	
	29A1-HT	29A1-LT MRC-OX17 MRC-OX6	29A1-HT	29A1-LT MRC-OX17 MRC-OX6
Nonlymphoid				
Pancreas				
Endocrine				
MØ-DC	+ + +	+ + +	+ + +	+ + +
VE	+ +	–	–	–
Epithelial cells	+ +/+	–	–	–
Exocrine				
MØ-DC	+ + +	+ + +	+ + +	+ + +
VE	+ +	–	–	–
Epithelial cells	–	–	–	–
Heart				
MØ-DC	+ + +	+ + +	+ + +	+ + +
VE	+ +	–	–	–
Myocardial cells	–	–	–	–
Liver				
MØ-DC	+ + +	+ + +	+ + +	+ + +
VE	+ +	–	–	–
Hepatocytes	+/+ +	–	–	–
Kidney				
MØ-DC	+ + +	+ + +	+ + +	+ + +
VE	+ +	–	–	–
Tubular epithelial cells	–	–	–	–
Adrenal gland				
Cortex				
MØ-DC	+ + +	+ + +	+ + +	+ + +
VE	+ +	–	–	–
Adrenocortical cells	–	–	–	–
Medulla				
MØ-DC	+ + +	+ + +	+ + +	+ + +
VE	+ +	–	–	–
Adrenomedullary cells	+/+ +	–	–	–
Lymphoid				
Spleen				
MØ-DC	+ + +	+ + +	+ + +	+ + +
Reticular cells (germinal center)	+	+	+	+
Reticular cells (PALS)	+ +	+ +	+ +	+ +
Marginal zone (LY)	+	+	+	+
VE	+ +	–	–	–
Thymus				
MØ-DC	+ + +	+ + +	+ + +	+ + +
Cortical epithelial cells	+ +	+	+	+
Medullary reticular cells	+ + +	+ + +	+ + +	+ + +
VE	+ +	–	–	–
Thymocytes	–	–	–	–

Cell type: PALS, periarterial lymphatic sheeth.

Peroxidase staining: + + + +, very strong; + + +, strong; + +, medium; +, weak; (+), very weak; –, no detectable staining; N = 3 experiments per tissue per strain per MoAb.

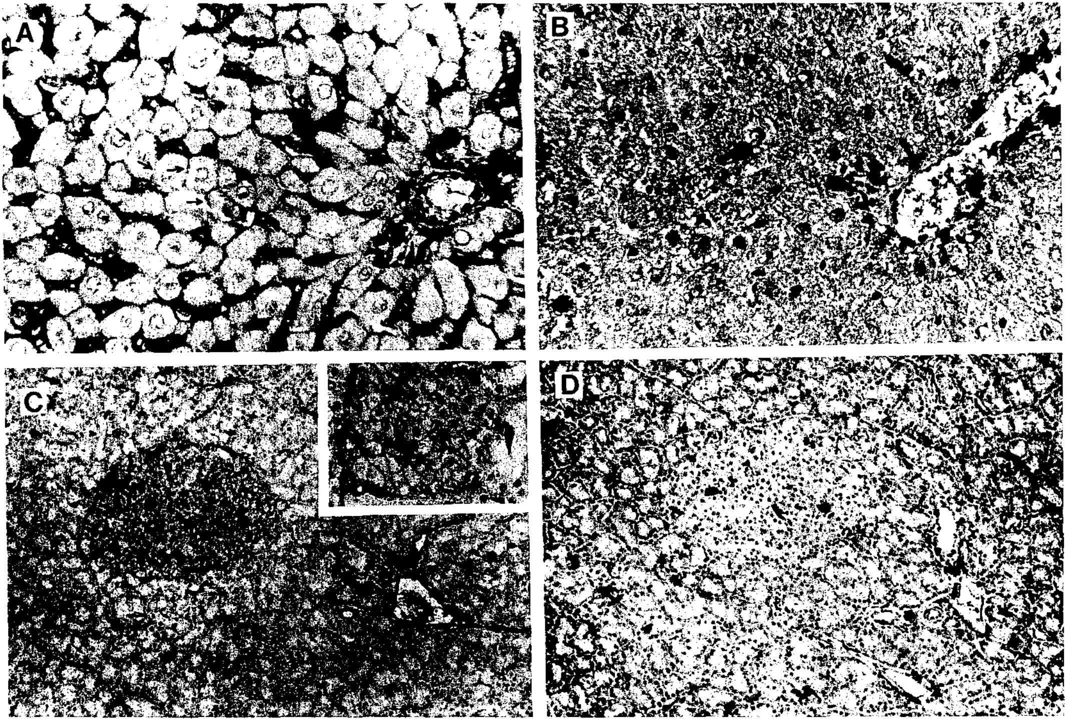

Fig 1. Immunoperoxidase staining of nonlymphoid $RT1^c$ tissues with anti-MHC class II MoAbs. (A) Liver with 29A1-HT, original magnification ×250 (arrows, positively stained hepatocytes); (B) liver with 29A1-LT, MRC-OX17, and MRC-OX6 original magnification ×250; (C) exocrine and endocrine pancreas with 29A1-HT, original magnification ×100 (inset, positively stained endocrine epithelial cells, original magnification ×250); (D) exocrine and endocrine pancreas with 29A1-LT, MRC-OX17, and MRC-OX6, original magnification ×100.

subtype) expressed MHC class II antigens uniformly very strongly, whereas non–marrow-derived endocrine epithelial cells did not express this class of antigens in either one of the preparations. However, a variation was observed with non–marrow-derived islet vascular endothelial and exocrine epithelial cells: In four of eight preparations only a very limited amount (10% to 30%) of the total capillary net expressed class II antigens strongly, whereas in the remaining four of eight preparations, this strong expression could be detected on 80% to 90% of the endothelial cell mass; with regard to exocrine epithelial cells, in three of eight preparations they definitely expressed class II antigens, though weakly; in four of eight preparations this expression was reduced to very weak, and in one of eight preparations it remained undetectable. These preliminary results with human islet preparations show a variably determined MHC class II expression also in human tissue; however, a close correlation of this variability with HLA-DR tissue type, sex, or age of the islet donor could not be demonstrated thus far.

DISCUSSION

The results of our studies with rats, newly obtained from intentionally varied sources, indicate a genetically variably determined constitutive expression of the MHC class II (I-E) antigen on non–marrow-derived cells in various rat organs. These findings are dependent on the particular reactivity of 29A1-HT. This antibody is clearly of monoclonal origin and anti–RT1/I-E, as revealed by compara-

tive serology, affinity chromatography, and immunoprecipitation/gel electrophoresis.[8] One possible explanation for the drop of antibody avidity, which takes place when the hybridoma cells are cultured over a long period of time, may be seen in chromosomal rearrangements.[9] The fact that neither high concentrations of purified 29A1-LT protein nor high antibody concentrations in ascites fluid from 29A1-LT cells were able to react demonstrably with the antigen on non–marrow-derived cells in RT1^c tissues supports this hypothesis.

The question arises whether the low-level expression of the I-E determinant that remains undetectable by other antibodies is of functional relevance in terms of allograft immunogenicity. Our preliminary answer to this question is yes because of the following observations: (1) In MLC-like T lymphocyte proliferation assays the stimulator CAP pancreatic islets provided reduced stimulation upon preincubation with 29A1-LT, but they ceased to stimulate following preincubation with 29A1-HT (which, presumably, provides additional blockage of the I-E epitopes on RT1^c vascular endothelial and β cells) (manuscript in preparation). (2) In allografting experiments with 29A1-LT (plus complement)-pretreated islets, CAP (RT1^c) grafts were more rapidly rejected (3 to 4 days) than BN (non-RT1^c) grafts (6 to 9 days), which presumably do not express I-E on the many present endothelial and β cells. Both donors do not differ significantly from each other in class I antigens (manuscript in preparation).

From the latter consideration it follows that for successful transplantation in different strain combinations it may be advantageous to take the variably determined MHC class II antigen expression into account. This implies that better defined manipulation protocols can be planned to obtain satisfying grafting results. Many problems remain open, not the least is the question of an analogue in the human, which we are presently looking for.

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