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Major Histocompatibility Complex Restriction of T-Lymphocyte Responses to Islet Cell Antigens in IDDM Rats

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SUMMARY

BBUF rats, derived from BB rats, spontaneously develop a form of insulin-dependent diabetes mellitus (IDDM) associated with infiltration of the islets of Langerhans by lymphocytes (insulitis). BBUF rats bear the RT1^u major histocompatibility complex (MHC) haplotype that we have shown to be necessary for the expression of this form of IDDM. A T-lymphocyte line obtained from the pancreas of a diabetic rat (UPCC.5) and three T-lymphocyte hybridomas derived by fusing T-lymphocytes of BBUF rats (MUS1.2, MUS1.13, and MUP3.21) respond to islet cell antigens in an MHC-restricted way. UPCC.5 responds to a combination of islet cell antigens (ICAg) and antigen-presenting cells by proliferation, whereas the T-hybridoma responses are detected on the basis of IL-2 production in a similar assay. This study reveals that an antiserum against μ-haplotype MHC antigens or a monoclonal antibody against the product of the D class II subregion of the rat MHC could inhibit ICAg recognition. A monoclonal antibody against the product of the B class II MHC subregion of the rat was not inhibitory. These results suggest that RT1.D antigens (analogous to human DR and mouse I-E) restrict islet cell recognition in this rat model of spontaneous IDDM. *Diabetes* 36:237–39, 1987

The BB rat spontaneously develops insulin-dependent diabetes mellitus (IDDM). Genetic studies reveal that the major histocompatibility complex (MHC) restricts the expression of disease in hybrid rats derived by crossing BB rats with non–diabetes-prone rat strains (1). Thus, only rats that are homozygous or heterozygous for RT1^u develop IDDM. Furthermore, only the class II subregions of the RT1^u complex appear to be necessary for disease expression (2). Because no recombinant MHC haplotypes separating the B^u and D^u class II loci are currently available, genetic studies cannot establish the exact MHC subregion restricting the expression of IDDM in BB rats. We studied the responses of a T-lymphocyte line obtained from the pancreas of a diabetic rat and three T-lymphocyte hybridomas derived from diabetic rats that all respond when stimulated by islet cell antigens (ICAg) and antigen-presenting cells. Antisera and monoclonal antibodies reacting against rat MHC antigens were added to the culture system. Results show that antisera against the entire RT1^u complex inhibited the response of these T-lymphocytes. Moreover, a monoclonal antibody against RT1.D class II antigens (analogous to HLA-DR in the human and I-E in the mouse) also inhibited proliferation, whereas a monoclonal antibody against RT1.B antigens (analogous to HLA-DQ in the human and I-A in the mouse) had no inhibitory effect. These results suggest that the RT1.D class II antigens of the rat MHC play a critical role in presenting antigen to autoreactive T-lymphocytes in rat IDDM.

MATERIALS AND METHODS

Rats. The diabetes-prone rats used in these experiments were originally derived by crossing the BB rat strain with the Buffalo (BBUF) strain. BBUF rats are homozygous for the RT1^u MHC haplotype and have an incidence of spontaneous IDDM of ~60%. The characteristics of these rats have been previously described (2,3).

Antibodies. Antisera against an entire MHC difference, i.e., against the RT1^u or RT1^a haplotypes, were prepared as previously described (4) and assayed in a microcytotoxicity assay. Monoclonal antibody 14.4.4s is a mouse antibody that binds to the mouse public determinant Ia.7, found on mouse I-E, and cross-reacts with a monomorphic determinant on rat RT1.D (5). Monoclonal antibody OX6 (Serotec, Sussex, UK) is also of mouse origin and reacts against a monomorphic determinant of the RT1.B class II MHC antigen. Monoclonal antibody 79.7.1 is a rat monoclonal antibody that reacts against a polymorphic determinant of RT1.B^a (4).

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TABLE 1
MHC restriction of pancreas-derived T-lymphocyte line UPCC.5

Blocking antibodies*	UPCC.5†	UPCC.5 plus ICAg‡
	899 ± 237§	8364 ± 1139
ANTI-RT1^u(serum)	391 ± 4	802 ± 249
ANTI-RT1^a(serum)	335 ± 62	4086 ± 238
Normal rat serum	402 ± 37	4436 ± 605
14.4.4s (MoAb anti-D)	279 ± 41	1184 ± 138
OX6 (MoAb anti-B)	529 ± 73	35,227 ± 4714

*Sera or monoclonal antibodies (MoAb) were added to cultures at a concentration of 10% vol/vol.
†10^5 UPCC.5 cells per well with 5×10^5 irradiated Wistar-Furth spleen cells.
‡20 μg/ml of islet cell–derived membrane-bound protein.
§[^{3}H]thymidine incorporation expressed as counts per minute ± SE on day 3 of culture. The results of a representative experiment are demonstrated. Three separate experiments gave similar results.

Monoclonal antibodies were prepared by saturated ammonium sulfate precipitation followed by purification on a protein A column. Antibodies were dialyzed extensively with phosphate-buffered saline and then with RPMI-1640. Monoclonal antibodies were then adjusted to a concentration of 1 mg/ml.

Preparation of T-lymphocytes. T-lymphocyte line UPCC.5 was derived from the pancreas of a newly diabetic rat by a method previously described (6). Briefly, islets of Langerhans obtained <24 h after the onset of overt IDDM in a rat were isolated by digestion of pancreatic tissue with collagenase type V (Sigma, St. Louis, MO) and separated from exocrine pancreatic tissue on a Percoll (Pharmacia, Uppsala, Sweden) density gradient as described by Buitrago et al. (7). Islet cell monolayers, which include accompanying T-lymphocytes that are infiltrating islets, were prepared by digestion with trypsin (0.25%). Pancreatic cells at a concentration of 10^5 cells per milliliter of medium were incubated in a medium supplemented with 15% T-cell growth factor (TCGF) (6) and contained 5×10^5 irradiated (3000 rads) autologous splenic filler cells per milliliter. Dead cells were removed after 6 days on a Ficoll-Hypaque density gradient. After 10 days the growth of T-lymphocytes (as determined by immunofluorescence) became apparent. These T-lymphocytes were maintained in culture with 15% TCGF and fed three times a week.

T-lymphocyte hybrids MUS1.13 and MUS1.2 were constructed and tested as previously described (8). Briefly, concanavalin A (ConA)–activated T-lymphoblasts obtained from the spleens of newly diabetic rats were grown in medium supplemented with interleukin 2 (IL-2) for 3–5 days. These T-lymphocytes were fused with the BW5147 mouse hypoxantine, aminopterin, thymidine (HAT)-sensitive thymoma line. Fusion was performed at a ratio of 10 ConA blasts per BW5147 cell with 35% polyethylene glycol. Cells were seeded in microwells (96-well plates) with 5×10^5 irradiated splenic filler cells per well. Hybridomas were selected in HAT medium, and after 3–6 wk hybridoma colonies appeared. MUP3.21 was derived by fusing pancreas-derived T-lymphoblasts of diabetic BBUF rat origin with BW5147 with the protocol described above. MUS1.2, MUS1.13, and MUP3.21 were screened on the basis of their ability to specifically secrete IL-2 in the combined presence of irradiated RT1^u antigen-presenting cells (APC) and membrane-bound ICAg (8). All three hybridomas were subcloned in agar.

Preparation of membrane-bound islet cell antigens. Islet cell monolayers were prepared as described above. Islet cell membrane fragments were extracted by lysis of cells in hyposmotic borate/EDTA, followed by differential centrifugation and collection of membranes on sucrose gradient as described by Thom et al. (9). The amount of membrane-bound protein extracted was determined by the Bio-Rad assay method (Mississauga, Canada).

Assay of T-lymphocyte responses to islet cell–derived antigens. The ability of UPCC.5 cells to respond against islet cell antigens was tested as previously described (6). Briefly, 10^5 UPCC.5 cells were added to 5×10^5 irradiated (3000 rads) Wistar-Furth (WF, RT1$^{u/u}$) spleen cells and 5–20 μg/ml of islet cell–derived membrane-bound protein in flat-bottomed 96-well tissue culture plates (Linbro, McLean, VA) in medium consisting of RPMI-1640, 10% fetal calf serum (Gibco, Grand Island, NY), 2 mM L-glutamine, 25 mM Hepes (Gibco), 50 μM 2-mercaptoethanol, and penicillin (50 U/ml)–streptomycin (50 μg/ml) mixture (Flow, McLean, VA). The plates were incubated at 37°C in a humidified atmosphere and 5% CO_2. On the 3rd day of culture, proliferation was assessed on the basis of [^{3}H]thymidine incorporation, measured after a 16-h pulse with 1 μCi [^{3}H]thymidine per well.

To assay T-lymphocyte hybridoma responses, IL-2 production was measured (8). We added 10^5 T-lymphocyte hybridoma cells to 5×10^5 irradiated WF spleen cells and 5–20 μg/ml of islet cell–derived membrane-bound protein in 96-well tissue culture plates. After 24 h of culture, supernatant were collected and assayed for IL-2 content. Where indicated, monoclonal antibodies or antisera were added to T-lymphocyte/APC/ICAg cultures at a concentration of 10% vol/vol.

IL-2 assay. IL-2 was measured with the IL-2–dependent CTLL-2 cell line as described (10). One unit of IL-2 per milliliter was defined as 10-fold the reciprocal of the dilution of cell culture supernatant that induced 50% of maximum proliferation ([^{3}H]thymidine uptake) of CTLL-2 cells (10^4 cells/200 μl) after 24 h of culture.

RESULTS

The results shown in Table 1 demonstrate that UPCC.5 responds by proliferation in the combined presence of RT1^u

TABLE 2
RT1.D restriction of three T-lymphocyte hybridomas*

Blocking antibodies†	Secretion of IL-2 (U/ml)		
	MUS1.13	MUS1.2	MUP3.21
None	50	102	32
14.4.4s (anti-D)	<1	<1	<1
OX6 (anti-B)	25	71	36
79.7.1‡	32	32	43

*10^5 T-hybridoma cells were added to cultures consisting of 5×10^5 irradiated Wistar-Furth spleen cells and 20 μg/ml of islet cell–derived membrane-bound protein. Supernatants were collected after 24 h of culture and assayed for IL-2 content with the IL-2–dependent CTLL-2 line.
†Monoclonal antibodies were added to cultures at a concentration of 10% vol/vol.
‡79.7.1 is a monoclonal antibody that reacts against RT1.B of the *a* haplotype.

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APC and islet cell membrane fragments. We have previously demonstrated that it is the MHC haplotype of the APC and not the source of the ICAg that restricts this response (6,8). A polyclonal antisera reacting against antigens of the whole $RT1^u$ complex inhibits responses against islet cell antigens almost completely, whereas an antiserum against the $RT1^a$ haplotype has only a mild inhibitory effect that is also seen when normal rat serum is added to the culture system (Table 1). Furthermore, a monoclonal antibody (14.4.4s) that reacts against RT1.D MHC class II antigens can also inhibit proliferation, whereas a monoclonal antibody against RT1.B (OX6) has no inhibitory effect. In fact, OX6 enhanced the response of UPCC.5 to ICAg. The reason for this effect of OX6 is unknown, but this phenomenon was not observed when ICAg-specific T-lymphocyte hybridomas were tested.

Table 2 shows that MUS1.13, MUS1.2, and MUP3.21 respond to a combination of APC/ICAg by secreting IL-2. This secretion of IL-2 is inhibited by 14.4.4s, but not by OX6 or 79.7.1 monoclonal antibodies. These results are similar to those obtained with the UPCC.5 pancreas-derived T-lymphocyte line.

DISCUSSION

The class II MHC restriction of T-lymphocyte responses in vitro to ICAg are concordant with our previous genetic studies in rat IDDM. Based on an extensive series of experiments where BB rats were crossed with other strains, we found that only animals homozygous or heterozygous for $RT1^u$ develop IDDM (1). Moreover, other investigators have shown that in passive-transfer experiments, ConA-stimulated T-lymphoblasts obtained from diabetic BB rats can only transfer IDDM to recipient rats that express the $RT1^u$ haplotype (11–14).

By crossing $RT1^{u/u}$ diabetes-prone rats with PVG.R8 rats that are *a* at the class I A locus and *u* at the class II B and D loci, we have identified offspring homozygous for the PVG.R8-derived haplotype that developed IDDM (2). This result suggests that class II antigens are probably the critical element contributed by the *u* haplotype. A potential role for the class I C locus is not excluded by these experiments, and additional studies are presently being performed to address this question. Because no recombinant haplotypes are available separating the B^u and D^u loci, genetic experiments cannot determine which class II locus is involved in IDDM. The results of our in vitro experiments with T-lymphocytes specific for ICAg strongly implicate $RT1.D^u$ as the restricting MHC antigen in this rat model of IDDM. Interestingly, the in vivo injection of anti-D^u but not anti-B^u monoclonal antibodies reduces the incidence of IDDM in BB rats (15). In fact, anti-$RT1.B^u$ antibodies appear to increase the incidence of IDDM. In our study, OX6 enhanced the response of the UPCC.5 cell line. The results of the in vivo experiments of Boitard et al. (15) are thus similar to the results of our in vitro studies with islet cell–specific T-lymphocyte lines.

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