

**MURINE PANCREATIC  $\beta$ -CELLS EXPRESS  
H-2K AND H-2D BUT NOT Ia ANTIGENS\***

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Allograft survival is markedly prolonged by a variety of pretreatment regimens such as lymphocytotoxic drugs, irradiation, and/or culture of donor tissue. These procedures, initially applied to the thyroid (1, 2) and subsequently to islets of Langerhans, stress the role of passenger lymphoid cells in allograft rejection. Marked prolongation of islet allograft survival (>100 d) across a major histocompatibility barrier has recently been accomplished in rats by in vitro culture of islets at 24°C for 7 d before transplantation in conjunction with a single injection of rabbit anti-rat lymphocyte serum into the recipient (3, 4). Successfully transplanted islets were promptly rejected after injection of donor lymphoid cells (5). Thus, cultured islets, although deficient in the capacity to stimulate allograft rejection, are capable of serving as the targets of an established response.

A recent study with immunoferritin labeling of fixed and trypsin-dispersed mouse islet cells concluded that these cells lack all H-2 antigens (6). Because only brain and ova of adult animals have been shown to lack H-2 antigens completely, we have repeated these studies with a more conventional assay for histocompatibility antigens. 20 anti-H-2 and anti-Ia sera have been tested on islet cells from mice of three different H-2 haplotypes by direct microcytotoxicity and by absorption. Contrary to the results of Parr (6), we find both H-2K and H-2D, but not Ia, antigens on the majority of islet cells.

**Materials and Methods**

*Animals.* A/J and B10.AKM mice of either sex were obtained from The Jackson Laboratory, Bar Harbor, Maine. BALB/c mice were obtained from The Jewish Hospital of St. Louis, Mo.

*Islet Isolation and Culture.* Islets were isolated by the collagenase technique (7). The method was modified for mouse islets by directly injecting the pancreas at numerous sites with Hanks' balanced salt solution (HBSS). 10–15 mice were used for each isolation, with a yield of ~50 islets/mouse. Isolated islets were separated on a Ficoll (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N. J.) gradient (8). To distinguish islets from lymph nodes and other contaminating structures, the reflected green light technique (9) was used, and islets were hand picked with a dissecting microscope. They were then maintained in culture medium (8) in 5% CO<sub>2</sub> for 1 or 2 d before being pooled on the 3rd d for dispersion.

*Dispase Dispersion of Islets.* Following exactly the procedure as described by Ono et al. (10), with Dispase (Godo Shusei, Ltd., Tokyo, Japan), ~400 viable cells/islet were obtained, and the aldehyde-fuchsin stain (11) revealed that 85% of the cells were  $\beta$ -cells. The dispersed cells were maintained for 6 or 24 h at 24° or 37°C before the microcytotoxicity test.

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*Mechanical Dispersion of Islets.* Pooled islets were collected by centrifugation and treated 3 min with 1 ml of 0.04% EDTA at room temperature. The islets were centrifuged at high speed for 1 min and resuspended into  $\text{Ca}^{+2}$ - and  $\text{Mg}^{+2}$ -free balanced salt solution for mechanical disruption into a single-cell suspension. This was accomplished by gentle agitation with a Pasteur pipet under a dissection microscope. The agitation was stopped when islets were no longer visible.

*Microcytotoxicity Test.* A two-stage microcytotoxicity test (12, 13), modified for islet cells, was used to assess H-2 and Ia antigens on enzymatically dispersed islet cells. The incubation with antiserum was shortened to 10 min. For complement, a mixture of 3% agarose-absorbed rabbit serum and 20% guinea pig serum (pooled, fresh frozen, Grand Island Biological Co., Grand Island, N. Y.) was used.

*Antisera for Microcytotoxicity Test.* Two anti-H-2 antisera (D13 and D30) were supplied by the Transplantation and Immunology Branch, National Institute of Allergy and Infectious Diseases, Bethesda Md. All other anti-H-2 and anti-Ia antisera were produced in the Department of Genetics, Washington University School of Medicine, St. Louis, Mo. Each antiserum has been tested in the microcytotoxicity test against lymphocytes from a panel of strains representing 30 independent and recombinant H-2 haplotypes to determine the H-2 or Ia specificities detected (Miroslav Hauptfeld. Personal communication.).

*Absorptions.* Antisera were absorbed with both enzymatically and mechanically dispersed islets. The antisera were diluted in HBSS cytotoxicity test medium to a dilution twofold less than that at which a decline in the titration curve began.  $2.5 \times 10^5$  enzymatically dispersed islet cells (or  $2.5\text{--}30 \times 10^5$  splenocytes as positive absorption controls) were added to 10  $\mu\text{l}$  of this diluted antiserum, and the suspension was incubated 1 h at room temperature with frequent shaking. Alternatively,  $2.5 \times 10^5$  mechanically dispersed islet cells were incubated first with 10  $\mu\text{l}$  of diluted anti-H-2 serum for 30 min. The cells were removed by centrifugation, and the absorbed serum was tested in the microcytotoxicity assay. Control sera were treated similarly, but without addition of cells.

## Results

Initial results of microcytotoxicity tests with BALB/c islet cells cultured for 6 h at 24°C after dispersion are presented in Table I. Antisera directed against the H-2D<sup>d</sup> antigen killed the majority of the cells. Cross-reactive antisera, detecting public H-2D<sup>d</sup> specificities, gave lower but significant killing. Antisera reactive with the H-2K<sup>d</sup> antigen effected a very weak killing (18–25% cell death), whereas anti-Ia sera gave no killing above background, which indicated an absence from islet cells of determinants controlled by either the I-A or I-E/C subregions.

To evaluate the possibility that the islet cells failed to express H-2K<sup>d</sup> and Ia antigens because of degradation by Dispase, splenocytes were treated with Dispase under conditions identical to those used for islets, then incubated at room temperature for 2, 4, 5, and 6 h and compared with untreated splenocytes in the microcytotoxicity assay. No difference in expression of H-2K or H-2D antigens was found between treated and control groups after any time interval, but Ia antigen expression on treated splenocytes was initially low and did not return to control levels until 5 h after treatment (data not shown).

These results suggested that the failure to detect H-2K<sup>d</sup> and Ia antigens on BALB/c islet cells could be a result of the Dispase treatment and that the 6-h recovery period at room temperature might be insufficient for full H-2 and Ia antigen expression on islet cells. Consequently, the effect of incubation at 37°C for 6 h was evaluated (Table II). It is clear that BALB/c islet cells incubated at 37°C for 6 h were more susceptible to killing by anti-H-2K<sup>d</sup> sera than those held at room temperature (Table II A). However, tests with anti-Ia serum remained negative. The higher temperature of incubation thus enhanced H-2K<sup>d</sup> but not Ia expression.

TABLE I  
Results of Microcytotoxicity Assays of BALB/c Islet Cells

Antiserum	Detects	Percent dead cells at anti-serum dilution*		
		1:2	1:4	1:8
(SJL × B10) anti-B10.S(24R)	H-2D <sup>d</sup>	68	65	45
(B10 × LP.R.III) anti-B10.A(5R)	H-2D <sup>d</sup>	80	70	70
(SJL × B6) anti-B10.S(24R)	H-2D <sup>d</sup>	70	70	65
(A.SW × B10.AKM) anti-A.TH	H-2D <sup>d</sup>	70	70	60
C3H.B10 anti-B10.D2	H-2D <sup>d</sup> + H-2K <sup>d</sup>	80	78	60
C3H anti-A.SW	H-2D <sup>d</sup> (public)	35	30	22
B10.OH anti-A.SW	H-2D <sup>d</sup> (public)	50	50	40
(A × B10) anti-B10.D2	H-2K <sup>d</sup> + I <sup>d</sup>	18	18	15
(A × B10.A) anti-B10.D2	H-2K <sup>d</sup> + I <sup>d</sup>	20	10	10
(C3H.B10 × B10.A) anti-B10.D2	H-2K <sup>d</sup> + I <sup>d</sup>	25	25	20
(B10.LG × A.TFR4) anti-B10.D2	I <sup>d</sup>	12	12	12
(B10.LG × B10.M[11R]) anti-B10.D2	I <sup>d</sup>	12	12	12
(B10 × D2.GD) anti-B10.BDR1	I-E/C <sup>d</sup>	15	20	20
(A × B10.A) anti-B10.A(5R)	I-A <sup>d</sup>	18	12	15
(AKR.M × C3H.B10) anti-C3H.Q	I-A <sup>d</sup>	12	12	12

\* Background (normal mouse serum plus complement) was 10–15% dead cells. Each percentage value is the mean of two to three experiments. Variations in kill from one experiment to another did not exceed 5%.

TABLE II  
Results of Microcytotoxicity Assays of Islet Cells Allowed to Recover for 6 h at Two Different Temperatures After Dispersion

Antiserum	Detects	Percent dead cells after recovery at*					
		24°C			37°C		
		1:2	1:4	1:8	1:2	1:4	1:8
		%			%		
<b>A. BALB/c islets (H-2<sup>d</sup>)</b>							
(SJL × B10) anti-B10.S(24R)	H-2D <sup>d</sup>	80	65	65	95	85	75
C3H anti-A.SW	H-2D <sup>d</sup> (public)	48	30	18	48	30	20
(A × B10) anti-B10.D2	H-2K <sup>d</sup> + I <sup>d</sup>	25	25	18	65	60	55
(A × B10.A) anti-B10.D2	H-2K <sup>d</sup> + I <sup>d</sup>	20	22	18	45	35	30
(C3H.B10 × B10.A) anti-B10.D2	H-2K <sup>d</sup> + I <sup>d</sup>	52	42	20	68	50	22
(B10.LG × A.TFR4) anti-B10.D2	I <sup>d</sup>	12	12	12	18	18	18
<b>B. A/J islets (H-2<sup>a</sup>)</b>							
(SJL × B10) anti-B10.S(24R)	H-2D <sup>d</sup>	80	80	80	85	82	85
(B10 × CR) anti-B10.K	H-2K <sup>k</sup> + I <sup>k</sup>	75	78	68	75	75	70
(129 × A.TL) anti-A.AL	H-2K <sup>k</sup>	75	70	70	75	80	80
(B10.D2 × HTC) anti-B10	H-2K <sup>k</sup> (public)	45	45	40	70	68	60
A.TH anti-A.TL	I <sup>k</sup>	15	15	15	12	15	12
<b>C. B10.AKM islets (H-2<sup>m</sup>)</b>							
(B10.A × LP.R.III) anti-B10.AKM	H-2D <sup>a</sup>	85	85	85	90	90	90
(B10.A × LP.R.III) anti-B10.A(5R)	H-2D <sup>a</sup> (public)	80	80	70	95	90	90
(B10 × CR) anti-B10.K	H-2K <sup>k</sup> + I <sup>k</sup>	65	70	65	90	90	80
(129 × A.TL) anti-A.AL	H-2K <sup>k</sup>	70	60	30	90	90	85
A.TH anti-A.TL	I <sup>k</sup>	20	20	20	20	20	20

\* Background (normal mouse serum plus complement) was 15–20% dead cells. Each percentage value is the mean of two experiments.

Cells from two more inbred strains were then assayed with several additional antisera. Both A/J and B10.AKM islet cells, cultured for 6 h at either 24° or 37°C were killed by both the anti-H-2D and the anti-H2K sera (Table II B and C). Again, somewhat greater killing was seen after a 37°C incubation, and no reactivity with

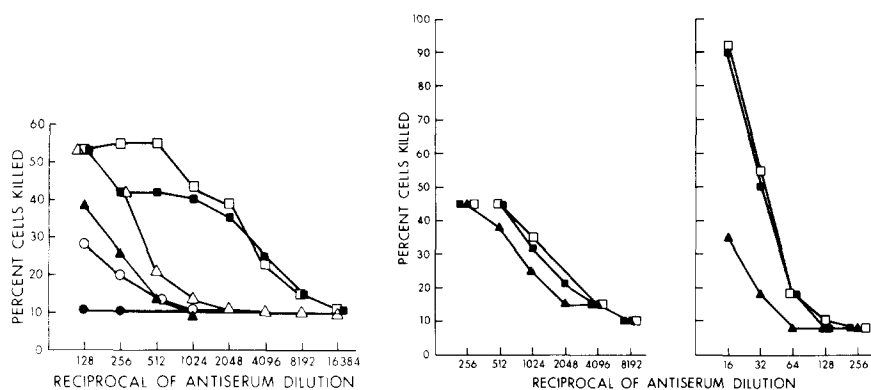


FIG. 1. (Left) Reactivity of anti-Ia serum (A.TH anti-A.TL) after absorptions with Dispase-dispersed islet cells and spleen cells from B10.A mice. Residual activity was assayed by microcytotoxicity against B10.A spleen cells. (□) Unabsorbed control; (■) absorbed with  $2.5 \times 10^5$  islet cells; (△) absorbed with  $2.5 \times 10^5$  spleen cells; (▲) absorbed with  $5 \times 10^5$  spleen cells; (○) absorbed with  $10^6$  spleen cells; (●) absorbed with  $3 \times 10^6$  spleen cells.

FIG. 2. (Right) Reactivity of anti-Ia serum (A.TH anti-A.TL) (left panel) and of anti-H-2D<sup>d</sup> serum [(S)JL × B10] anti-B10.S(24R)] (right panel) after absorption with  $2.5 \times 10^5$  mechanically dispersed B10.A islet cells. (▲) Absorbed with islets; (□) unabsorbed, incubated without cells; (■) unabsorbed, freshly diluted.

anti-Ia sera was detected. Even longer recovery periods (24 h at 37°C) did not result in killing above background by anti-Ia sera (data not shown).

Finally, two absorption analyses, one with Dispase-dispersed islets and one with mechanically dispersed islets were conducted. Dispase-dispersed B10.A islet cells failed to absorb the Ia antiserum, whereas the same or larger numbers of B10.A splenocytes absorbed anti-Ia activity for B10.A splenocytes (Fig. 1). To test the possibility that islet cells express Ia antigens that are stripped by Dispase and not restored to detectable levels under our treatment conditions, mechanically dispersed A/J islet cells were consecutively incubated with anti-Ia serum and then with anti-H-2D<sup>d</sup> serum. These cells did not absorb activity from the anti-Ia serum, but did significantly reduce the ability of the anti-H-2D<sup>d</sup> serum to kill B10.A splenocytes (Fig. 2). Both absorption experiments thus confirm the microcytotoxicity test results.

### Discussion

By direct microcytotoxicity testing, we have demonstrated the presence of H-2K and H-2D antigens on dispersed  $\beta$ -cells from murine islets of Langerhans, but have failed to detect Ia antigens. Our data indicate that Dispase treatment, necessary for dispersion of the cells, significantly reduces their H-2 reactivity and that an incubation period of 6 h at 37°C is necessary for reexpression of the antigens. This raises the possibility that Ia antigens are also present on islet cells before dispersion, but are stripped by the treatment and are restored more slowly than H-2 antigens. Both prolonged recovery periods and absorption with mechanically dispersed islets still failed to show I-region products. Clearly, islet cells lack detectable levels of Ia antigens.

These results are in disagreement with a recent publication (6), which reported the absence of H-2 antigens on  $\beta$ -cells. In that study, trypsin-dispersed islets were tested with only a single alloantiserum potentially reactive with products of the entire H-2

complex. The cells were examined only by electron microscopy after immunoferritin labeling. The failure to detect H-2 antigens could have been a result of the loss of antigen after treatment of islets with trypsin, as we have observed with Dispase treatment. Because the alloantiserum was diluted in normal rabbit serum (a source of complement), it is also possible that the more strongly H-2-positive cells were lysed and eliminated before examination.

The ease with which islets of Langerhans can be transplanted across major histocompatibility barriers without rejection thus cannot be explained by the absence of major transplantation antigens. This was predicted by the prompt rejection of established allografts upon injection of peritoneal exudate cells (5). Whereas H-2 antigens are present on the surfaces of nearly all adult nucleated cells (14), Ia antigens have been found to be expressed only on T and B lymphocytes (15), macrophages (16, 17), epidermal cells (16, 18), spermatozoa (16), intestinal epithelial cells (19, 20), and various products released by T cells (21). Cells bearing Ia antigens have been directly implicated in a variety of allograft responses, including stimulation of the mixed-lymphocyte reaction and the graft-versus-host reaction (14, 22). It may be that culture of islets at 24°C removes cells capable of stimulating an allograft rejection response. Future experiments will determine whether those cells bear Ia determinants.

### Summary

Direct microcytotoxicity testing and absorption analyses were employed to determine whether H-2K, H-2D, and Ia antigens are present on murine islet of Langerhans cells. Products of the *H-2K* and *H-2D* loci were found on  $\beta$ -cells from three different mouse strains, but *I*-region (Ia) antigens were not detected. The absence of Ia gene products from islet cells may be of importance in explaining the survival of islet allografts across major histocompatibility barriers.

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