Expression of major histocompatibility antigens on pancreatic islet cells

(major histocompatibility complex/transplantation antigens/monoclonal antibodies/pancreatic beta cells/insulin-dependent diabetes)

STEINUNN BAEKKESKOV*, TAKAHIRO KANATSUNA†, LARS KLARESKOG‡, DAVID A. NIELSEN†
PER A. PETERSON‡, ARTHUR H. RUBENSTEIN‡, DONALD F. STEINER†, AND ÅKE LERNMARK§

*Hagedorn Research Laboratory, DK-2800 Gentofte, Denmark; †Departments of Biochemistry and Medicine, University of Chicago, Chicago, Illinois 60637; and ‡Department of Cell Research, Wallenberg Laboratory, Uppsala, Sweden

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ABSTRACT Insulin-dependent diabetes mellitus is often accompanied by manifestations of autoimmune and is frequently associated with certain HLA haplotypes, predominantly DR3 and DR4. Because the major histocompatibility antigens are important determinants of the immune response in various tissues, we have investigated their expression on the pancreatic islet cells. Human, mouse, or rat islets of Langerhans, as well as lymphocytes or other differentiated cells, were biosynthetically labeled with radioactive amino acids, lysed in detergent, and immunoprecipitated with several antisera specific for major histocompatibility antigenic groups. The immunoprecipitates were analyzed by NaDodSO4/polyacrylamide gel electrophoresis under reducing conditions followed by autoradiography. The major histocompatibility antigens corresponding to the H-2 K, D molecules in mice, the H1-A in rats, and the HLA-A, -B, and -C in humans were precipitated from both islet and lymphocyte lysates and were accompanied by \( \beta_2 \)-microglobulin. Binding of H-2 antibodies to islet cells was also confirmed by a radioligand assay using \(^{125}\)I-labeled protein A and by indirect immunofluorescence. Analyses in the fluorescence-activated cell sorter revealed that >95% of the cells in the beta-cell-rich fraction were fluorescent, providing further evidence that the pancreatic beta cells express the major histocompatibility antigens. Monoclonal antibodies or mouse alloantisera against HLA-DR or Ia antigens did not react with labeled pancreatic islet cell proteins.

The development of insulin-dependent diabetes mellitus often follows pathological processes that result in destruction of the pancreatic beta cells. The pathogenic mechanism is not understood, but both virus infection and autoimmune appear to be important factors (1, 2). Patients with insulin-dependent diabetes of short duration frequently have insulitis or lymphocytic infiltration of their pancreatic islets (3) as well as a high incidence of organ-specific islet-cell antibodies (4-7). Tissue typing for factors of the HLA system, the major histocompatibility system of man, has shown that insulin-dependent diabetes is primarily associated with HLA-D/DR3 or DR4 alleles (1, 2, 8).

The major histocompatibility complex (MHC) contains several distinct loci that code for cell surface antigens that are important in the immune response. The antigens of the MHC that have been characterized thus far may be divided into major categories: (i) HLA-A, -B, and -C antigens in man (H-2K and D in mouse, H1-A in rat); and (ii) HLA-DR (Ia) antigens in man (Ia in rat and mouse) (9). The HLA-A, -B, and -C antigens and their equivalents in other species are target antigens in the transplantation reaction. The heavy chain (\( M_r, 44,000-47,000 \)) which carries the alloantigenic determinants is a glycoprotein that traverses the plasma membrane and is noncovalently associated with a light chain, \( \beta_2 \)-microglobulin (\( M_r, 12,000 \) at the cell surface) (10). This relatively invariant polypeptide is necessary for expression of the heavy chain on the cell surface (11, 12).

The heavy and light chain complexes appear to be the cellular receptor for virus (13) and bacteria (14). Cytotoxic T-lymphocyte recognition only occurs if the T cells and their target cells are compatible at the H-2K or D locus (15). There is controversy as to whether the major transplantation antigens are present on the pancreatic islet cells (16, 17).

The HLA-DR (Ia) antigens are believed to mediate certain cell-cell interactions essential for the function of the immune system (18). The Ia antigen is a complex of two noncovalently linked glycoproteins designated \( \alpha (M_r, 32,000-36,000) \) and \( \beta (M_r, 25,000-32,000) \) chains, both of which seem to span the plasma membrane (19). The Ia antigens appear to be restricted in their tissue distribution and are expressed on cells belonging to the immune system (B lymphocytes and certain subsets of macrophages or T lymphocytes) (19), on Langerhans cells of the epidermis (20), on sperm (21), and on a number of epithelial cells (22-26). Mouse Ia alloantisera do not convey complement-dependent cytotoxicity to mouse islet cells (17).

Because immunopathological reactions, somehow related to the HLA system, may be involved in the pathogenesis of insulin-dependent diabetes, we have tested whether the pancreatic islet cells express MHC antigens.

MATERIALS AND METHODS

Sources of Pancreatic Islets. A.TL and A.TH mice were bred and maintained at the Hagedorn Research Laboratory. CBA/H mice were either a gift from H. Wiggell (University of Uppsala, Sweden) or obtained from Bantin and Kingman (Hull, England). Male Sprague-Dawley or Lewis rats were of commercially available inbred strains. Insulin-producing tumors were from an x-ray-induced insulina (27) maintained in NEDH rats. The pancreas from four cadaver kidney donors were from the Departments of Surgery at the Rigshospitalet and Herlev Hospital, Copenhagen (consent was obtained from the patients' relatives prior to removal of the organ).

Islet Isolation. Pancreatic islets were prepared from 5- to 6-month-old male mice by several collagenase digestions followed by discontinuous Percoll (Pharmacia, Uppsala, Sweden) gradient centrifugation (28). Rat islets were isolated by collagenase digestion followed by a Ficoll (Pharmacia) gradient centrifugation (29). Human islets were obtained by collagenase digestion of 1- to 2-g pieces of pancreas (30).

Abbreviations: MHC, major histocompatibility complex; SAc, Cowan I strain of Staphylococcus aureus

†To whom reprint requests should be addressed.

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Lymphocyte Isolation. Lymphocytes were isolated from the spleens of mice or rats by Ficoll-Paque (Pharmacia) centrifugation (31). Human lymphocytes were similarly isolated from peripheral blood of healthy individuals (31).

Other Cells. Single-cell suspensions of epithelial cells were prepared from rat intestines (32). Cells were also prepared from the transplantable rat insulinoma (27) and kept in Swins S-77 medium supplemented with 4% bovine serum albumin (6).

Antiseria. The following antiseria were used: (i) H-2 K,D antigen antiserum, raised in rabbits against highly purified H-2K,D antigens (33) [an anti-β2-microglobulin antiserum (33) was also used]; (ii) W6/32 (Sera Laboratories, Sussex, England), a monoclonal antibody that recognizes a determinant present on all HLA-A, -B, and -C heavy chains when they are associated with β2-microglobulin (34, 35); (iii) OX4 (Sera Laboratories, Sussex, England), a monoclonal antibody that recognizes Ia antigens from all strains of rats tested so far (36). (iv) NE1-011 (New England Nuclear); a monoclonal antibody against human Ia. (v) Ia 4, +, a mouse alloantiserum against the Ia haplotype (anti-A.TH). (vi) Ia 2, +, and (vii) Ia 1, 2, 3, 7, + [εi and εii are mouse alloantiserum against the Ia haplotype (anti-A.TL)].

Labeling with [35S]Met. Lymphocytes and mouse and rat islets were labeled immediately after isolation. Human islets were maintained in culture 3–21 days before labeling to monitor the release of insulin (30). Islets or lymphocytes were incubated in leucine-free minimal essential medium with Earle’s salts supplemented with [35S]methionine-[>600 Ci/mmol; 1 Ci = 3.7 × 1010 becquerels; Radiochemical Centre, Amersham] at 100 μCi/ml. After labeling, the islets and lymphocytes were washed by centrifugation and lysed in 1% Triton X-100. Nonsolubilized material was removed by centrifugation for 30 min at 100,000 × g, and the supernatant was used immediately for immunoprecipitation.

Labeling with [3H]Leucine. Rat islets, suspensions of spleen, and insulinoma cells were incubated in leucine-free Eagle’s minimal essential medium with Hanks’ salts and 0.5 mCi of L-[3,4,5-3H(N)]leucine (118 Ci/mmol; New England Nuclear) per ml.

Immunoprecipitation. Aliquots of cell lysates were incubated with nonimmune sera followed by adsorption to formalin-fixed heat-killed Staphylococcus aureus (Cowman strain, SAC) (37). After centrifugation the supernatant fluid was incubated with antiserum and immunoprecipitation was carried out with SAC. Incubation with rabbit anti-mouse IgG (Dako-immunoglobulins a/s, Copenhagen, Denmark) was used before addition of SAC, for the monoclonal antibody OX4.

Polyacrylamide Gel Electrophoresis. Immunoprecipitates and aliquots of a cell lysates were boiled for 3 min in sample buffer [80 mM Tris-HCL, pH 6.8/2% NaDodSO4/10% (vol/vol) glycerol/0.001% bromphenol blue/5% (vol/vol) 2-mercaptoethanol]. The immunoprecipitates were centrifuged to remove SAC and the supernatant and cell lysate aliquots were analyzed by NaDodSO4/polyacrylamide gel electrophoresis with the discontinuous buffer system of Laemmli (38) on 10% slab gels or 10% tube gels (32) with a 5% stacking gel.

The slab gels were stained with 0.1% Coomassie brilliant blue in 50% methanol/10% acetic acid, destained in 33% methanol/2% acetic acid, and treated with the autoradiographic enhancer EN3HANCE (New England Nuclear). After drying, the gels were exposed to Kodak X-Omat R film at ~80°C. M, standards (Pharmacia) used were phosphorylase b (94,000), bovine serum albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), trypsin inhibitor (20,100), and lactalbumin (14,400). Tube gels were sliced and the slices were assayed for radioactivity in Aquasol (New England Nuclear).

Determination of Cell Surface-Bound Antibodies. Antibodies bound to dispersed rat islet, exocrine pancreatic, spleen, and intestinal cells were measured in a radioassay using 125I-labeled protein A (39). Suspensions of rat pancreatic islet and spleen cells were also used for immunofluorescence (39) with the rabbit H-2 K,D or β2-microglobulin antiserum and the OX4 monoclonal antibody. The cells were washed by centrifugation in Hanks’ buffer supplemented with 4% (wt/vol) bovine serum albumin and resuspended in buffer containing fluorescein isothiocyanate-conjugated goat antirabbit IgG (final dilution, 1:15). Washed cells were subjected to analysis in a fluorescence-activated cell sorter (FACS III Cell, Becton Dickinson FACS Systems, Mountain View, CA). Cells were identified by light scattering as described (40), and total cells and the beta-cell enriched fraction were analyzed for fluorescence intensity.

RESULTS

Bioisotopic Labeling of Islet and Lymphocyte Proteins. The autoradiogram of the gel electrophoresis profile of solubilized [35S]methionine-labeled proteins from A.TH mouse islets and lymphocytes is shown in Fig. 1. The proinsulin and insulin bands were characteristic of the islets. Some prominent bands in lymphocytes were not detected in islets. Similarly, different patterns for islet and lymphocyte proteins from rat and human material were observed (not shown). The insulin release in the human islet cultures was 2.1–4.4 ng of insulin per islet per day.

Immunoprecipitation with H-2 K,D and HLA-ABC Antiserum. The rabbit H-2 K,D antiserum, but not normal rabbit serum, precipitated major protein bands at M, 45,000–47,000 and M, 12,000 from both A.TH islet and A.TH lymphocyte lysates (Fig. 2). Some additional bands (M, 76,000 and 80,000) were precipitated from islets by this antiserum but not by normal rabbit serum. Similar results were obtained with A.TL and CBA/H mice. The binding of 125I-labeled protein A to suspensions of pancreatic islet, pancreatic exocrine, intestinal, and spleen cells incubated with the rabbit H-2 K,D antiserum was increased by a factor of 5–10 compared to normal rabbit serum (not shown).

The antiserum also crossreacted with the H1-A antigens in rats because components of M, 45,000 and 12,000 were precipitated from lysates of [3H]leucine-labeled rat islet and rat insulinoma cells as well as from rat lymphocytes (not shown.)

The monoclonal antibody W6/32, which recognizes a common determinant of HLA-ABC antigens, precipitated bands at M, 45,000 and 12,000 from both human islets and lymphocytes corresponding to HLA-ABC antigens and β2-microglobulin (Fig. 3). No additional bands were precipitated by this antiserum from human islets but in human lymphocytes an unknown high Mr band and a band at Mr, 44,000, probably representing actin (41), were precipitated by both W6/32 and a normal mouse serum. Identical results were obtained with the rabbit β2-microglobulin antiserum (not shown).

Immunoprecipitation with Ia Antiserum. The monoclonal antibody OX4 precipitated rat lymphocyte proteins of M, 32,000 and 26,000, corresponding to the rat Ia α and β chains, respectively (Fig. 4). Corresponding mouse Ia components of M, 34,000 and 27,000 were precipitated from lysates of A.TL mouse lymphocytes by mouse alloantiserum Ia 2 (not shown) and Ia 1, 2, 3, 7 (Fig. 4). Similarly, mouse alloantiserum Ia 4 precipitated bands at M, 32,000 and 25,000 from A.TH mouse lymphocytes (not shown). No proteins at these positions were precipitated from rat or mouse islets by any of these antisera. Despite preabsorption with normal mouse serum, a heavy band at M, 55,000, which may represent tubulin, was precipitated from rat islets by OX4 and normal mouse serum.
lymphocyte (lane B) proteins. The cells were washed twice by centrifugation before labeling in methionine-free Eagle's minimal essential medium (pH 7.4) with Earle's salts, sodium bicarbonate at 1.0 g/liter, 2.5% fetal calf serum, penicillin (100 units/ml), streptomycin (100 μg/ml), and glucose at 3 g/liter. Islets (200/ml) or lymphocytes (2 x 10^9/ml) were labeled for 16 hr at 37°C in the methionine-free medium supplemented with [35S]methionine. The islets and lymphocytes were then washed twice by centrifugation in medium containing 5 mM methionine and finally in 10 mM Hepes/150 mM NaCl/5 mM EDTA, pH 7.4, containing Trasylol (Bayer, Leverkusen, Federal Republic of Germany) at 100 kallikrein international units/ml. Lysates were prepared by incubating the islets (1000/ml) and lymphocytes (5 x 10^9/ml) in the same buffer containing 1% Triton X-100. Islets were frozen and thawed three times and kept on ice for an additional 30 min. Lymphocytes were incubated on ice for 30 min. After centrifugation at 150,000 x g for 30 min, the supernatant fluid was boiled in electrophoresis sample buffer and subjected to electrophoresis on a 10% NaDodSO4/polyacrylamide gel. Mr markers along with the positions for proinsulin (Pi) and insulin (i) are shown.

The monoclonal antibody NE1 011 recognizing human DR (Ia) antigens, precipitated bands at Mr 33,000 and 27,000 from lysates of human peripheral lymphocytes but not from lysates of human islets (Fig. 4).

Binding of H-2 Antibodies to Dispersed Cells. Flow cytofluorometry in the fluorescence-activated cell sorter of pancreatic islet cells incubated with rabbit H-2 K, D antiserum revealed that 96% of the total islet cell population had a fluorescence intensity higher than the mean fluorescence intensity with normal serum (Fig. 5) compared to 99% for the beta-cell enriched region within the islet cell peak. Similarly, spleen cells showed a homogenous population of fluorescent cells. The average fluorescence intensity was 3-8 times that of normal serum for the H-2 K, D and β2-microglobulin antisera.

**DISCUSSION**

Our results obtained by immunoprecipitation, protein A-binding, and immunofluorescence analysis with various antisera indicate that islet cells express the MHC antigens. In contrast, HLA-DR and Ia antigens were not detected in these experiments. The evidence suggesting that the antigens detected by immunoprecipitation of islet lysates originate from islet cells, and in particular the beta cells, includes the following. First, in our islet isolation procedure; care was taken to remove all exocrine tissue and lymph nodes by carefully selecting the islets under a dissecting microscope. The cultivation of human islets prior to biosynthetic labeling ensured not only detachment of nonislet cells, allowing a harvest of islet preparations free of contaminating extraneous cells, but also established the insulin-producing capacity of the beta cells. The fact that the pattern of labeled proteins in islets was clearly distinct from that of lymphocytes and the demonstration of Ia antigens in lymphocytes but not in islet lysates is strong evidence against the possibility
that lymphocytes within the islets were the source of the antigens detected in islet lysates.

Second, analyses in the fluorescence-activated cell sorter of islet cells stained with the rabbit H-2 K, D antiserum in the beta-cell-rich region (40) showed that >95% of the cells were labeled. These results allow us to conclude that the H-2 K, D antiserum binds to beta cells. Moreover, the major transplantation antigens also could be immunoprecipitated from labeled rat insulinoma cells.

These observations do not agree with the results of Parr (16) who was unable to detect immunoferritin labeling of trypsin-dissociated mouse islet cells incubated with an allo H-2 antisera and concluded that the beta cells lacked all H-2 antigens. However, the possibility exists that in Parr's experiment the H-2 antigens were digested by the trypsin used to disperse the cells. In cytotoxicity and absorption experiments using several H-2 alloantisera, Faustman et al. (17) found evidence for H-2 K, D antigens on the majority of islet cells dispersed by disperse, but only after a recovery period in culture.

The observations that beta cells express major transplantation antigens may be important in understanding possible mechanisms of beta-cell destruction. These antigens may act as cellular receptors for viruses (16) which may trigger the destruction of beta cells (42). They probably also can serve as target cellular antigens which, in combination with a foreign antigen (virus antigen or modified self antigen), may lead to recognition and lysis by cytotoxic T lymphocytes (15). The presence of the MHC antigens also accounts for the rapid rejection of allogeneic islet tissue in transplantation experiments.

Our failure to demonstrate any of the known Ia antigens in islet cell lysates suggests these are not expressed on pancreatic beta cells. This conclusion is consistent with the observation that specific mouse Ia alloantisera were not toxic to mouse islet cells (17). However, other products encoded by genes in or near the immune response region might be present on the beta cells. We have noted that islets transplanted between mice which differ only in certain genes located between the K and D regions are rejected (28). In interpreting these results we cannot rule out the possibility that small amounts of Ia antigens may be present on some or all of the beta cells but at a level 5% to 10% that on lymphocytes. Such low levels would escape detection by our immunoprecipitation or immunofluorescence procedures. It is also possible that the expression of Ia antigens is dependent on conditions that are not fulfilled by our in vitro incubation conditions. A more attractive possibility is that islet cells, and in particular the beta cells, may express certain specific antigens which are coded for by loci closely associated with the I, S, G, or Tla region on mice or HLA-D in man.

Recent experiments by Murphy et al. (43) suggest that one I locus in mice regulates the surface expression of the product of a second I locus. Such a gene–gene interaction in the immune response region, if applicable to man, could be involved in the association between disease susceptibility and HLA-DR haplotypes, either by affecting the immune response mechanism or through alterations of the antigenic properties of various

Fig. 3. NaDodSO₄ slab gel electrophoresis and autoradiography of immunoprecipitates of human islet (lanes A and B) and lymphocyte (lanes C and D) proteins with the monoclonal antibody W6/32 (lanes A and C) and normal mouse serum (lanes B and D). Preparation of lysates of islets and lymphocytes and subsequent immunoprecipitations were carried out as in Figs. 1 and 2, respectively.

Fig. 4. NaDodSO₄ slab gel electrophoresis and autoradiography of rat, mouse, and human lymphocyte and islet proteins immunoprecipitated with anti-Ia antisera and normal mouse serum. Lanes: A and B, rat lymphocyte proteins with OX4 (A) and normal mouse serum (B); C and D, rat islet proteins with OX4 (C) and normal mouse serum (D); E and F, ATL mouse lymphocyte proteins with Ia 1, 2, 3, 7 + (E) and normal mouse serum (F); G and H, ATL mouse islet proteins with Ia 1, 2, 3, 7 + (G) and normal mouse serum (H); I and J, human lymphocyte proteins with NE1 011 (I) and normal mouse serum (J); K and L, immunoprecipitation of human islet proteins with NE1 011 (K) and normal mouse serum (L). Preparation of lysates of islets and lymphocytes and subsequent immunoprecipitations were carried out as in Figs. 1 and 2, respectively. Samples with the OX4 antibody were subjected to an additional incubation with rabbit anti-mouse IgG before final immunoprecipitation.
differentiated cell types. The presence on beta cells of an antigen coded for by a region closely linked to or regulated by the HLA-D/DR region may explain the strong positive association between insulin-dependent diabetes and the HLA-D/DR3 and DR4 haplotypes (8). Such a DR-linked beta-cell-specific antigen could account for the selective destruction of the beta cells in insulin-dependent diabetes.

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