GLUCOSE INDUCES INTRACISTERNAL TYPE A RETROVIRAL GENE TRANSCRIPTION AND TRANSLATION IN PANCREATIC BETA CELLS

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In a study (1) analyzing the response of different inbred strains of mice to the autosomal recessive obesity mutation, "diabetes" (*db*), we found that only those inbred strains constitutively expressing in beta cells an endogenous retrovirus, the intracisternal type A particle (IAP),[1] developed severe diabetes. The beta cell was the only islet endocrine cell type observed to express IAP; cell necrosis was limited to this cell type as well. In chronically hyperglycemic *db/db* mice, prenecrotic beta cells showed increased numbers of morphologically identifiable IAP, as well as increased immunoreactivity against a rabbit antiserum detecting both a 73 kD IAP group-specific internal structural protein (p73) and higher-molecular mass proteins containing p73 determinants (1–3). The glucose-dependent nature of this induction was demonstrated in vitro using cultures of normal beta cells from diabetes-susceptible inbred strains, such as C57BL/KsJ (BKs) or CBA/LtJ. Beta cell cultures established from diabetes-resistant strains such as C57BL/6J (B6) whose beta cells did not contain morphologically identifiable IAP in situ could not be induced by glucose to express these particles in vitro (1). The mRNA for p73 is transcribed from the full-length genetic unit (7.3 kb) as colinear 7.2 kb transcripts (4). A subgenomic 5.4 kb mRNA encodes a family of higher-molecular mass peptides (114–120 kD), which are structurally related to p73, but which apparently are not major precursors (5). In the B6 thymus, this novel class of IAP-related polypeptides (p114–p120) can be expressed independently of p73, which is not expressed (5). In this study, we asked whether glucose might exert differential control of expression of IAP genomes in beta cells of diabetes-susceptible vs. -resistant inbred strains of mice. Glucose is the primary physiologic regulator of insulin biosynthesis and secretion by the mouse pancreatic beta cell, stimulating insulin gene transcription and translation (6, 7), beta cell membrane depolarization (8), and insulin secretion (9). Conceivably, hyperglycemia associated with establishment of a diabetic condition could elicit synthesis and possibly secretion of proteins that are not produced at appreciable levels in the normoglycemic state. If glucose were capable of stimulating abnormally high levels of transcription and translation of an IAP gene(s),

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[1] *Abbreviations used in this paper:* dFBS, dialyzed FBS; IAP, intracisternal type A particle; LTR, long terminal repeat.

and if the retroviral proteins normally sequestered within beta cells at low levels (neoantigens?) were expressed at the beta cell surface, then autoimmune elimination of the glucose-stressed beta cells might result.

Materials and Methods

Mice. Mice were obtained from the Animal Resources Unit of The Jackson Laboratory, and were maintained on a custom diet (diet 96W; Emory Morse Co., Guilford, CN). Four week-old B6 and BKs males homozygous for the recessive obesity mutation, "diabetes" (*db*) on chromosome 4, were kindly supplied from the research colony of Dr. D. L. Coleman, The Jackson Laboratory.

Islet Culture. Islets from 8–10 month-old CBA/J male mice were isolated from minced pancreas by manual shaking for 10 min in collagenase solution (type IV; 300 U/ml, Worthington Biochemicals, Freehold, NJ) exactly as described elsewhere (10). This procedure was modified for islet isolation from C57BL/6J (B6) and C57BL/KsJ (BKs) males in that two or three month-old males were used, and a different collagenase preparation (Serva; 694 U/ml; Accurate Chemicals, Westbury, NY) and digestion time (6 min) were employed. These modifications were necessitated by inbred strain differences in pancreatic digestibility by collagenase. Following purification of free islets from exocrine tissue and ducts by Ficoll gradient centrifugation (10) and hand-picking under a dissecting microscope, 2,000 islets were obtained from the digest of 15 CBA/J pancreases, whereas 40–44 B6 or BKs pancreases had to be digested to achieve the same yield. Matched batches of 800–1,000 islets from each inbred strain were transferred by micropipette into sterile plastic 60 × 15-mm bacteriological dishes (1007; Falcon Labware, Oxnard, CA) which did not permit islet attachment to the substratum. Islets were incubated for 48 h at 36°C in 5 ml of a modified Dulbecco's minimal essential medium (DMEM) containing 15 mM HEPES, 0.2% BSA, 50 mg/l gentamicin sulfate, MEM nonessential amino acids, and 10% heat-inactivated, dialyzed fetal bovine serum (dFBS). All culture reagents were obtained from Gibco Laboratories, Grand Island, NY). The glucose concentration of this culture initiation medium was either 5.5 mM (low glucose) or 16.5 mM (high glucose) as indicated in the text.

Radiolabeling of Beta Cell and Thymocyte Proteins. The synthesis of IAP-related proteins and insulin II was analyzed by immunoprecipitation from extracts of pancreatic islets metabolically labeled in vitro. In the pancreas of normal mice, insulin II represents no more than 35% of the total content (11). Insulin II contains a single methionine in the B chain, whereas (processed) insulin I contains no methionine in either the A or B chains, thus allowing discrimination of insulin II in [^{35}S]methionine-labeled preparations. After 43 h of incubation, the islets were collected with a siliconized pipette into a sterile 12 x 75 mm plastic centrifuge tube (Falcon Labware) and sedimented at 60 *g* for 1 min. The pelleted islets were washed in 4 ml of methionine-free MEM containing 5.5 mM glucose and 10% dFBS, pelleted, and resuspended in 2 ml of this medium containing 500 μCi [^{35}S]methionine (sp act 1,320 Ci/mmole; Amersham Corp., Arlington Heights, IL). The glucose concentration of the labeling medium (either 5.5 mM or 16.5 mM) for a given batch of islets was identical to that of the initiation medium. At the end of a 4 h labeling period, the labeling media containing secreted insulins were removed, and insulin (both unlabeled insulin I and [^{35}S]methionine-labeled insulin II) was purified by acid/ethanol extraction of a washed TCA pellet as described previously (12). The islets, pelleted by centrifuging for 2 min at 500 *g*, were washed and recentrifuged three times with 4-ml volumes of methionine-containing DMEM (5.5 mM glucose) plus 10% dFBS. The islets, resuspended in 1 ml, were transferred into 1.5 ml polypropylene centrifuge tubes, pelleted, and then lysed in 0.5 ml of lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 8.5% sucrose, 0.6% Triton X-100, 2 mM PMSF, and 100 U/ml apoprotinin). Each tube was vortexed for 3 min and centrifuged at 800 *g* for 10 min at 4°C. The supernatant fraction was used for immunoprecipitation (see below). Freshly-isolated thymocyte suspensions were prepared from normal (+/+) and genetically diabetic (*db/db*) males at 5 weeks of age, and labeled for 4 h with [^{35}S]methionine, as described

elsewhere (5), in DMEM containing high (25 mM) glucose. [^{35}S]-labeled insulin II and IAP-associated proteins were analyzed as described below.

Immunoprecipitation of Labeled Proteins. For analysis of [^{35}S]methionine-labeled IAP-related proteins, 100 μl volumes of islet or thymocyte extracts were immunoprecipitated with 3 μl of rabbit antiserum A1.3 prepared against electrophoretically-purified IAP structural protein p73, or a preimmune control serum (A1.0) from the same rabbit. Details of the immunoprecipitation procedure and acrylamide gel electrophoresis have been given elsewhere (5). Briefly, the extract proteins were treated at 100°C with 2-ME in the presence of SDS, after which the 2-ME was reacted with a slight excess of iodoacetamide. The samples were then diluted and the antisera added. After 15 min at 22°, the immune complexes were collected on *Staphylococcus aureus*, washed repeatedly with detergent-containing buffer, and then solubilized by boiling in SDS. The solubilized proteins were analyzed by electrophoresis in SDS gels containing 8.5% or 10% polyacrylamide, using the buffer system of Laemmli (13). In one experiment (shown in Fig. 4*B*), the procedure was varied in that control and specific immunoprecipitations were performed sequentially on the same sample of extract. The IAP-related proteins from islet extracts were identified by reference to those immunoprecipitated by antiserum 1.3 from cytoplasmic extracts of [^{35}S]methionine-labeled N4 neuroblastoma cells, which actively synthesize a variety of IAP gene products (5, 14).

Insulin that was released into the culture media during the labeling period was collected in an acid/ethanol extract. For analysis of insulin II in the media or contained within the islet lysates, 11 μl of neutralized acid/ethanol extract or 50 μl of islet lysate were immunoprecipitated with 5 μl of guinea pig antiinsulin serum and equal aliquots with a preimmunization control serum (both gifts from Dr. Jesse Roth, NCI). The insulin immunoprecipitates, prepared as described above, were analyzed by electrophoresis on SDS-containing 20% polyacrylamide gels. Methyl-[^{14}C]insulin (New England Nuclear, Boston, MA) was used as an electrophoretic standard.

RNA Analysis. Two groups of islets (1375/group) from CBA/J males were hand-picked and incubated for 48 h in DMEM initiation medium containing 5.5 and 16.5 mM glucose respectively. The islets from each group were pelleted and RNA extracted by a recently-described micromethod (15) involving homogenization in 500 μl of 8 M guanidine HCl, 0.01 M EDTA, and 1% *N*-lauroylsarcosine (Sarkosyl). For electrophoresis and dot-blot analysis, samples of RNA representing equal numbers of islets were denatured at 65°C in the presence of formaldehyde and formamide, and either analyzed by electrophoresis in formaldehyde-containing agarose gels (16), or serially diluted and applied as dots to nitrocellulose membrane. Samples of total cellular mRNA from BALB/cJ thymus or of polyadenylated RNA from purified myeloma IAP were used as standards for the electrophoretic and dot-blot analyses, respectively. The electrophoretically fractionated RNA were transferred by blotting to nitrocellulose membrane (Northern blot) in the presence of formaldehyde after a brief treatment with 50 mM sodium hydroxide to enhance recovery of high–molecular mass components from the gels. For detection of IAP-specific sequences in the Northern and dot-blots, a probe was prepared from a recombinant plasmid, pMIA1, that contained 5.2 kbp of internal sequence from a genomic IAP copy. The plasmid was labeled by nick translation with [^{32}P]dATP and [^{32}P]dCTP (each at 800 Ci/mmole; New England Nuclear) to a sp act of 2×10^8 cpm/μg DNA, and used at a concentration of 10^6 cpm/ml in the hybridization mixtures. All of the above procedures have been described in detail (5).

RNA samples to be used for dot blotting were treated with DNase I and recovered by alcohol precipitation after extraction with phenol/chloroform/isoamyl alcohol (50:49:1). Islet RNA samples containing 0.5 μg (low-glucose preparation) or 1 μg (high-glucose preparation) were digested for 30 min at 37°C in a mixture containing 40 mM Tris-HCl, pH 7.9, 10 mM sodium chloride, 6 mM magnesium chloride, and 1 U of highly purified DNase I (Promega Biotec, Madison, WI). These conditions were established in preliminary experiments as the conditions needed for elimination of contaminating DNA that could contribute to the hybridization signals by virtue of its high content of IAP sequences. The comparison between the DNase-treated and untreated IAP RNA series in Fig. 3*A* shows

that some RNA was lost under the conditions used. The sequence content of islet mRNA was determined by reference to the DNase-digested IAP-RNA dilution series.

For analysis of actin and insulin mRNA, the Northern blot was rehybridized after 4 wk with a mixture of probes prepared from plasmid-cDNA clones containing 1,600 bp of chicken β-actin sequence (17) and 440 bp of rat insulin I sequence (clone pcR1354, kindly furnished by Dr. M. A. Permutt, Washington University, St. Louis, MO), respectively. Each probe was used at a level of 10^6 cpm/ml in the hybridization mixture. The insulin probe was used singly for hybridization of an RNA dot-blot.

In a separate experiment, glucose stimulation of total RNA synthesis was assessed by incubation of batches of 250 CBA/J islets for 48 h in 3 ml of the low- and high-glucose DMEM media plus 30 μCi of [^{3}H]uridine (sp act, 43 Ci/mmol; Amersham Corp.) followed by extraction and counting of the labeled RNA.

Results

Glucose Induction of IAP Protein Synthesis in Cultured CBA/J Islets. The ability of medium with elevated (16.5 mM) glucose concentration to induce synthesis of IAP-related proteins in islets of a constitutively-expressing inbred strain (CBA/J) is shown in Fig. 1. In comparison to islets cultured at low glucose, from which only a small amount of labeled antigen was immunoprecipitated, islets cultured for 48 h in high-glucose medium showed strong induction of the synthesis of a high–molecular mass protein, p114, as well as of the retroviral group-specific core structural antigen, p73. The relative labeling of IAP-related proteins in the high- and low-glucose media could be calculated from the total

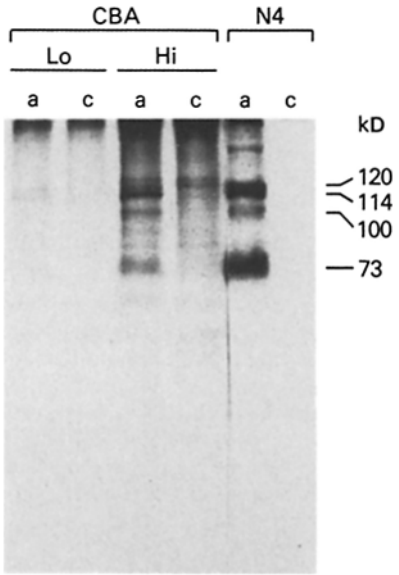

FIGURE 1. Effect of medium glucose concentration on labeling patterns of IAP-related proteins in CBA/J pancreatic islets. Equal volumes (100 μl) of [^{35}S]methionine-labeled extracts from islets cultured in 5.5 mM (*Lo*) or 16.5 mM (*Hi*) glucose were immunoprecipitated with either A1.3, an IAP specific antiserum (*a*), or A1.0, a control serum (*c*), as described in the text. A sample of identically labeled extract from N4 neuroblastoma cells was also immunoprecipitated to provide a reference pattern of IAP-related proteins. The samples of extract used for immunoprecipitation contained 0.5×10^6, 1.9×10^6, and 2.2×10^6 protein-associated cpm for CBA/J Lo, CBA/J Hi, and N4, respectively. Autoradiographs were exposed 26 d for the CBA lanes, and 18 h for N4.

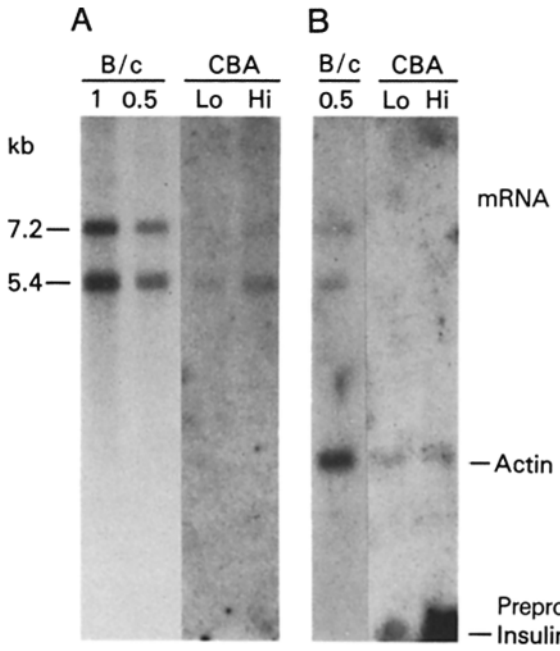

FIGURE 2. Glucose induction of IAP RNA in CBA/J pancreatic islets. *A* shows a Northern blot of RNA from CBA/J pancreatic islets, with a preparation of poly(A)$^+$ RNA from BALB/cJ (*B/c*) thymus serving as reference standard (5). The first two lanes contain 1 and 0.5 μg, respectively, of BALB/cJ thymus poly(A)$^+$ RNA. The third and fourth lanes contain 1 μg and 2 μg, respectively, of total RNA from CBA/J pancreatic islet cells cultured in either 5.5 mM (*Lo*) or 16.5 mM (*Hi*) glucose. Lanes containing CBA/J islet and BALB/cJ thymus RNA were autoradiographed for 10 d and 1 d, respectively. *B* represents the same blot rehybridized with a mixture of ^{32}P-labeled cDNA probes for rat insulin and chicken actin, as described in the text, and autoradiographed for 4 d.

protein counts used for immunoprecipitation (see legend, Fig. 1) and the exposure time required to reach similar band densities on the autoradiographs (exposure series not shown). On this basis, labeling of IAP-related protein components was increased ~20-fold in the high-glucose–cultured islets. Since incorporation into total cellular protein was also four times greater in the high-glucose medium, the specific induction of IAP protein synthesis in 16.5 mM glucose was approximately fivefold.

Glucose Induction of IAP RNA in CBA/J Islets. Culture of CBA/J islets in high-glucose stimulated the incorporation of [^{3}H]uridine into total RNA by fivefold (49,500 cpm/μg at 16.5 mM glucose vs. 9,600 cpm/μg at 5.5 mM), and twice as much total RNA was recovered from the islets maintained at the higher glucose level for 48 h (5 μg and 2.5 μg per 1,750 islets in high and low glucose, respectively). When samples of total RNA from equal numbers of islets were electrophoresed in agarose gel, blotted to nitrocellulose membrane, and hybridized with a [^{32}P]-labeled IAP-specific DNA probe, a higher level of IAP transcripts was found in the preparation from the high-glucose–treated islets (Fig. 2*A*). Reference lanes in Fig. 2 contain poly(A)$^+$ RNA from BALB/cJ thymus. The major transcripts of 7.2 and 5.4 kbp are known to code for p73 and the p114–120 polypeptides, respectively (5). Thus, the preponderance of 5.4 kbp transcript

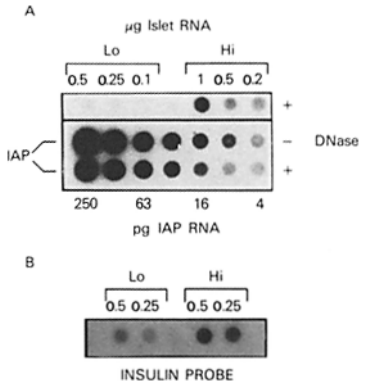

FIGURE 3. Coinduction by glucose of IAP and preproinsulin mRNA in CBA/J beta cells. *A*, top row, shows a dot-blot of three concentrations of DNase-treated RNA from equal numbers of islets maintained at either 5.5 mM (*Lo*) or 16.5 mM (*Hi*) glucose, and hybridized to an IAP-specific probe. The 0.5 μg sample in the Hi RNA series hybridized anomalously because of a trapped air bubble. The lower two rows show probe hybridization to a dilution series of authentic IAP-associated poly(A)$^+$ RNA with or without prior DNase treatment. *B* shows a dot-blot of the same islet RNA hybridized with probe made from a cloned 440 bp rat insulin I sequence.

in islet RNA was consistent with the higher synthesis rate of p117 as compared to p73 in these cells.

The IAP-specific RNA in cultured islets was quantified by dot-blot analysis (Fig. 3*A*). A sample representing 275 islets cultured at 16.5 mM glucose was calculated to contain ~30 pg of IAP sequence in 1 μg of total RNA, while the sample from 275 islets cultured in low-glucose medium contained some 2–3 pg in 0.5 μg total RNA. Therefore, glucose induction of IAP-specific RNA in CBA/J islets was 10–15-fold on a tissue basis and 5–7-fold in terms of total RNA. These values are consistent with the specific increase in IAP-related protein synthesis in islets maintained in 16.5 mM glucose, indicating that glucose induction of IAP protein expression was related to increased gene transcription rather than to some type of posttranscriptional regulation.

Glucose Induction of Preproinsulin mRNA in CBA/J Islets. The RNA blot shown in Fig. 2*A* was held for several weeks, and then hybridized with a mixture of ^{32}P-labeled cDNA probes for rat insulin and chicken actin. There was a marked increase in the mRNA for preproinsulin in the RNA of islets cultured in high glucose (Fig. 2*B*). mRNA for cytoplasmic actin, a general "housekeeping" protein, was poorly represented in the islet cells as compared to thymus, but showed an increased hybridization signal in the high-glucose lane commensurate with the twice greater amount of total RNA applied.

The relative levels of preproinsulin mRNA were quantified by dot-blot assay (Fig. 3*B*). Islets cultured at 16.5 mM glucose showed an eightfold higher level based on equal number of islets, and a fourfold increase in terms of total recovered RNA as compared to islets maintained in 5.5 mM glucose. The increase in insulin message was similar to that found for IAP-specific RNA in islets maintained in high glucose for 48 h.

Strain-related Differences in IAP Protein Synthesis in Cultured Islets. Sensitivity

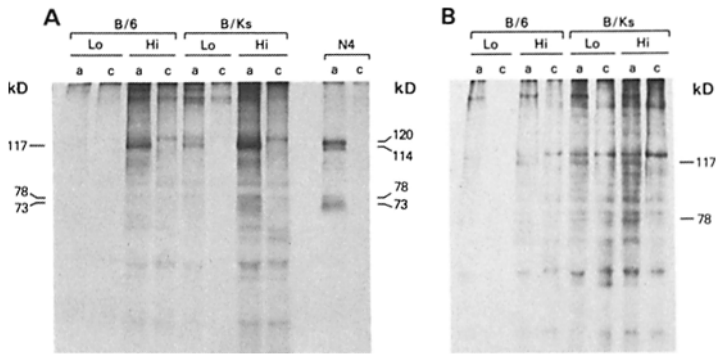

FIGURE 4. B6 and BKs strain differences in labeling patterns of IAP-related proteins from [^{35}S]methionine labeled islet extracts after culture in 5.5 mM (*Lo*) or 16.5 (*Hi*) glucose. *A* and *B* show the results of two separate experiments. Equal volumes of labeled islet extracts were immunoprecipitated with either A1.3, the IAP-specific antiserum (*a*) or A1.0 control (*c*) serum. A reference pattern from N4 neuroblastoma cells is included in *A*.

to glucose induction of synthesis of IAP-related proteins was dependent upon inbred strain background and correlated with constitutive ability to express IAP in beta cells in situ. Fig. 4 shows two separate experiments in which islets from B6 males (nonconstitutive for beta cell IAP in situ as judged by electron microscopy) were compared with those of BKs males (constitutive expression in beta cells in situ). In B6 islets, high glucose concentration in vitro induced synthesis of an IAP-related 117 kD protein (5), but there was little or no coordinate induction of p73, the major IAP structural protein (Fig. 4). This biochemical demonstration that glucose-stressed B6 beta cells are induced poorly or not at all for the IAP structural protein corroborates our previous report of the absence of morphologically identifiable IAP in B6 beta cells at the ultrastructural level (2). In contrast, BKs islets synthesized p117 and p78 (a variant form of the main structural protein), and incorporation into both of these components was stimulated at elevated glucose concentrations (Fig. 4). Again, this observation is consistent with our previous work showing increased numbers of IAP at the ultrastructural level in glucose-stressed BKs beta cells in culture (2). It is relevant to note here that p73 and the p114–120 proteins may differ markedly in turnover rate in the cultured beta cells. In the N4 neuroblastoma line, where this question has been studied, the half-lives of newly-synthesized p73 and the higher–molecular mass components was 24 h and 3–4 h, respectively (14, and J. Fewell and E. Kuff, unpublished observations). Therefore, the relatively minor labeling of p78 and p73 as compared to p117 after 4 h in both basal and glucose-stimulated BKs islets is compatible with the accumulation of the IAP structural protein in stable particulate form over longer time spans.

Glucose Induction of Insulin II Synthesis in Cultured B6 and BKs Islets. Measurement of insulin II labeling in the same cell lysates that were screened for IAP-related proteins showed that elevated glucose levels markedly enhanced [^{35}S]methionine incorporation into immunoprecipitable insulin II in cultured islets from both B6 and BKs mice. This was true with respect to both the intracellular pool of insulin II (Fig. 5*A*) and the secreted protein (Fig. 5*B*). Interestingly, in this experiment B6 and BKs islets showed a very different

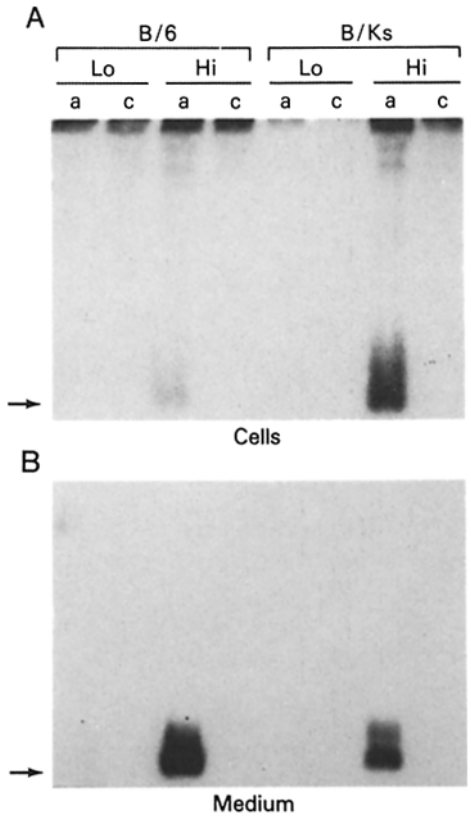

FIGURE 5. Glucose-stimulated insulin II biosynthesis and secretion in B6 and BKs pancreatic islets. Equivalent amounts of ^{35}S-labeled extracts were immunoprecipitated with either a guinea pig antiinsulin serum (*a*) or a control serum (*c*). *A* compares intracellular levels of newly-synthesized (pro)insulin II at Lo (5.5 mM) vs. Hi (16.5 mM) glucose, while *B* compares levels of newly-synthesized insulin II released by these islets into the culture media (acid/ethanol extracts). The arrow indicates the position of the insulin marker (not shown).

relationship between intracellular and secreted insulin II during the 4-h incorporation period in that BKs beta cells stored more and released less than did B6 beta cells. Although longer autoradiographic exposure times than shown in Fig. 5 revealed low levels of insulin II in both B6 and BKs islets maintained at 5.5 mM glucose, it is clear that the insulin II gene did not express strongly in the absence of glucose stimulation. The heightened synthesis of insulin II in the islets maintained at 16.5 mM glucose was not due to differential viability in the high- vs. low-glucose medium. Histological analysis of batches of 50 of these cultured islets after 48 h revealed a small amount of necrosis of cells in the center of the larger islets; however, most cells seemed morphologically intact, with no differences noted between cultures maintained at high- vs. low-glucose other than depletion of insulin storage (β-) granules in the former group.

Strain-specific Differences in Regulation of IAP Gene Activity in Thymocytes. A previous study (5) showed that the relative and absolute levels of IAP-related protein synthesis varied characteristically in the thymuses of several different inbred mouse strains. B6 thymocytes were found to synthesize a 117 kD com-

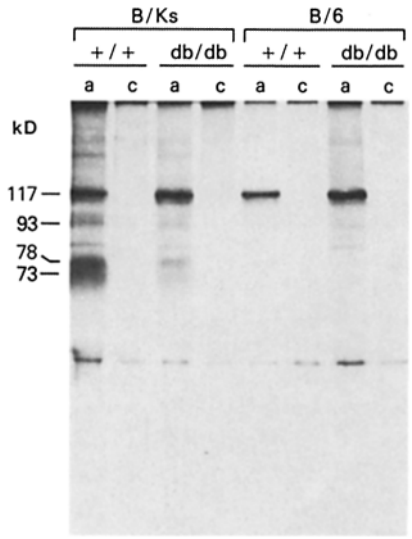

FIGURE 6. Labeling patterns of IAP-related proteins in [^{35}S]methionine labeled thymocytes from 4-wk-old B6 and BKs mice either normal (+/+) or homozygous for the *db* gene. The cells were labeled for 4 h in high glucose (25 mM) DMEM. Each lane represents the material immunoprecipitated with either IAP-specific antiserum (*a*) or control serum (*c*) from 250 μg of labeled thymocyte extract protein.

ponent (p117) to the exclusion of p73. Fig. 6 illustrates this fact, and also shows that the thymuses of young (4–5 wk) BKs +/+ mice supported significant synthesis of p78 and p73 together with a higher level of p117 labeling. These biochemical observations were consistent with ultrastructural analysis, since we routinely found IAP in BKs cortical thymocytes in situ, but not in the B6 thymocytes (our unpublished observations). The situation in the thymus of these two inbred strains was thus very similar to that found in their pancreatic beta cells, as if reflecting some fundamental strain-related differences in the regulation of IAP gene expression.

We have not yet succeeded in culturing thymocytes at high viability for 48 h in order to assess glucose inducibility of IAP gene function. However, because it is known that the thymic cortex of BKs-*db/db* mice undergoes accelerated atrophy compared to normal mice (18), and that blood glucose levels begin to rise at weaning, we also compared IAP expression in thymocyte suspensions prepared from BKs- and B6-*db/db* males (Fig. 6). Thymocytes from four week-old B6-*db/db* males showed an enhanced synthesis of p117 compared to thymocytes from B6 normal males, but no induction of p73. These changes parallel the high-glucose response seen in cultured pancreatic beta cells from B6 +/+ mice. On the other hand, the thymocytes from BKs-*db/db* males showed no change in labeling of p117 compared to BKs normal controls, but rather a sharply diminished incorporation into p73. The thymus of a 7-wk-old BKs-*db/db* male was found to be heavily infiltrated with fat (separable from the thymocytes by floatation), and the yield of thymus cell protein was sharply reduced. The surviving thymocytes incorporated label into total protein at the usual levels, but showed no p73 synthesis at all and showed a much-reduced incorporation into

p117 (not illustrated). Even at 4 wk, some selection may be operating in favor of cells with reduced synthesis of IAP-related proteins. The selective elimination of IAP protein–producing cells in the thymus and the pancreas of diabetes-susceptible BKs mice could have features in common (see Discussion).

Discussion

The present study shows a coordinate induction by glucose of both preproinsulin mRNA and IAP-associated mRNA in cultured islets that is also reflected at the translational level. The ability to express or not to express p73 (equivalent to the unprocessed *gag* polypeptides of conventional retroviruses) is under control of the background genome of the inbred strain. B6 mice are unable to express p73 in either beta cells or thymocytes, whereas this retroviral antigen is constitutively expressed in BKs and CBA/J. The latter two strains are very susceptible to the diabetogenic action of the *db* mutation, while the B6 strain is resistant (1). An interesting parallel is that the two p73-constitutive cell types analyzed in this study (pancreatic beta cell and cortical thymocyte) both undergo accelerated necrosis in BKs-*db/db* mice (1, 18). Cortical thymocytes expressing p73 in BKs-*db/db* mice may, like the beta cells in these animals, be selectively eliminated.

Recently in vitro methylation of a cloned IAP 5′ long terminal repeat (LTR) at specific positions upstream of the RNA start site was found to abolish the promoter activity of the LTR toward a coupled chloramphenicol acetyl transferase (CAT) gene (A. Feenstra, J. Fewell, K. Lueders, and E. Kuff, manuscript submitted for publication). Furthermore, the number of 5′ LTR that were not methylated at these same sites in the DNA of normal thymus and liver were found to be below the level of detection; i.e., <10–20 copies per genome equivalent. Therefore, it is likely that only a minute fraction of the many IAP genes can be active in normal mouse tissues, and that strain differences in IAP expression reflect the differential activities of but a few genes. The mouse has two nonallelic structural genes coding for preproinsulins I and II: insulin I on chromosome 6 and insulin II on chromosome 7 (19). The expression of these two genes appears to be independently regulated since insulin I predominates in both mouse and rat pancreas (11). Both promoter and enhancer elements in the 5′ flanking region of insulin genes have been described (20). Although alternative mechanisms can of course be suggested to account for the coordinate glucose induction of insulin and IAP protein synthesis in pancreatic beta cells, it is intriguing to speculate that in diabetes-susceptible strains, one or more IAP proviruses are positioned near a promoter/enhancer element upstream of an insulin gene, and that these copies are activated with the insulin gene response to glucose stimulation.

In a previous immunocytochemical study of IAP proteins in the beta cells of genetically diabetic (*db/db*) mice, a more widespread intracytoplasmic distribution of antigen(s) was observed than could be accounted for by particle-associated p73 (1). The antiserum used was the same rabbit anti-p73 antiserum employed in this study, thus crossreactive with the 114–120 kD p73-related polypeptides. The antigen(s) was primarily localized to nascent IAP in islets from the younger *db/db* mice. However, in the beta cells of older *db/db* mice, which had been chronically and severely hyperglycemic, a much more widespread, non–particle

associated distribution of antigen was observed. These normally sequestered molecules might, we suggested earlier (1), lead to autoimmune sensitization of the host, either by integration into the beta cell surface, or by secretion. We have begun preliminary screening of sera of normal and genetically diabetic BKs and B6 mice for antiislet reactivity. We are detecting, by immunofluorescence assay, spontaneous production of antibodies to islet cell cytoplasmic determinants in normal BKs, but not in B6 males; this incidence is increased by the hyperglycemic stress imposed by the *db* genes expressing in the BKs inbred background (21). Using ELISA technique, we are detecting IgM subclass antibodies reactive against porcine and bovine insulin and against purified p73 (21). Autoantibodies against insulin have been documented in diabetic humans and mice (22, 23), leading to the proposal that autoimmune reactions against beta cells are of major significance in the pathogenesis of diabetes. When such autoimmunity is engendered, it is unclear whether it is initiated by lymphocytes spontaneously autoreactive against organ-specific antigens, or whether the target cells themselves initiate expression of neoantigens that elicit autoreactivity from an otherwise normal immune system (24). In the BB rat, a lymphopenic animal that develops a spontaneous insulin-dependent diabetes with an autoimmune pathogenesis, evidence has been presented (25) which indicates that the beta cells do not initiate their own destruction by expression of neoantigens, but instead are victims of the aberrant immune system. On the other hand, these studies indicate that differential expression of a genetic repertoire of glucose-inducible beta cell gene products discriminates the islets of diabetes-susceptible inbred mouse strains such as BKs and CBA/J from a diabetes-resistant strain such as B6. Since all strains of inbred mice found to be susceptible to the diabetogenic action of the *db* mutation constitutively expressed IAP in beta cells, and since accelerated pathogenesis was obtained in a hybrid cross that effected an even greater repertoire of retroviral expression (IAP and C-type in the beta cell [26]) glucose induction of retroviral antigens may well be of pathogenic significance in mice.

Elevation of glucose to diabetogenic level (20 mM) is known to mark isolated islets in such a way as to cause preferential release of newly-synthesized insulin (27). Although fetal mouse beta cells cultured in high-glucose media remain viable and secrete more insulin than do those maintained at low glucose, those marked by the high-glucose media show impaired endocrine function upon grafting into chemically-induced syngeneic recipients (28). Indeed, when 50 freshly-isolated BKs islets were transplanted into the spleens of streptozotocin-diabetic BKs recipients, the number was suboptimal and hyperglycemia was not reversed. These islets were lost within 11 d.[2] In contrast, 50 B6 islets intrasplenically implanted into streptozotocin-diabetic B6 hosts, although still incapable of reversing hyperglycemia, were nevertheless retained in the spleen.[2] The differences between responsiveness of BKs and B6 beta cells to high-glucose challenge presented in this study provide insight as to how glucose may be effecting changes at the genetic level. Although these changes may not be of serious consequence in vitro, expression of increased levels of retroviral proteins could deleteriously mark beta cells in vivo.

[2] Leiter, E. H. Induction of autoreactivity against islets in C57BL/KsJ mice: use of a transplantation model. Manuscript submitted for publication.

Summary

C57BL/KsJ (BKs) and CBA/J, but not C57BL/6J (B6) mice are susceptible to the diabetogenic action of the obesity gene, "diabetes" (*db*). BKs and CBA/J, but not B6 mice, constitutively express intracisternal type A particles (IAP), an endogenous class of retrovirus, in beta cells and in cortical thymocytes. IAP genetic expression in these cell types included production of the group-specific antigen, p73, as well as higher–molecular mass p73-related antigens (p114–120). We used islet culture techniques to show that both transcription and translation of IAP genomes in beta cells is enhanced by glucose. Maintenance of CBA/J islets for 48 h in 16.5 mM glucose–containing medium effected a fivefold induction of IAP protein synthesis in comparison to islets cultured in low- (5.5 mM) glucose medium. Analysis of RNA from 16.5 mM glucose–cultured islets revealed induction of 7.2 and 5.4 kbp transcripts known to code for p73 and the p114–120 polypeptides, respectively. This induction in CBA/J islets was 10–15-fold on a tissue basis, and 5–7-fold on an RNA basis. Glucose induction of preproinsulin mRNA levels was also analyzed in the same samples. Islets cultured in 16.5 mM glucose showed an eightfold higher level on a tissue basis, and a fourfold increase in terms of total recovered RNA.

Comparison of these glucose-inducible parameters in islets isolated from the diabetes-susceptible BKs strain vs. the resistant B6 strain revealed that expression of the group-specific retroviral p73 antigen was limited to BKs beta cells. This inbred strain control of p73 expression was also found in cortical thymocytes, with B6 thymocytes producing a 117 kD component to the exclusion of p73, while both components were expressed in thymocytes from normal BKs mice. In comparison to normal BKs males, thymocytes from four week-old genetically diabetic (*db/db*) BKs males showed no change in labeling of p117, but showed a sharply diminished incorporation into p73. This suggested that accelerated thymic involution characteristic of *db/db* mice may entail selective elimination of p73-producing cells. The possibility that glucose-stressed BKs pancreatic beta cells are marked for autoimmune elimination by the elaboration of p73 or other IAP-related proteins is discussed.

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