Functional Alterations in Pancreatic β Cells as a Factor in Virus-induced Hyperglycemia in Mice*

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Alterations in the functional capacity of pancreatic β cells appear to contribute to coxsackievirus B4-induced, long-term hyperglycemia in mice. Mice infected with prototype B4 or its diabetogenic E2 variant were monitored for abnormalities in sugar metabolism (by the glucose tolerance test), for total protein and insulin synthesis in intact β cells, for alterations in β cell proteins, and for virus replication. The infected mice were hypoglycemic at 72 h postinfection and hyperglycemic at 6 weeks. At 8 weeks postinfection, few of the prototype- but most of the E2-infected mice remained hyperglycemic. Total protein and synthesis of immunoprecipitable insulin decreased during early infection. At 8 weeks postinfection, insulin synthesis in the prototype-infected mice increased almost to the level of control mice. Although insulin synthesis increased likewise in the E2-infected mice, it remained well below the control level. Two-dimensional gel electrophoresis revealed the disappearance of many cellular proteins in β cells from E2-infected mice but of very few in cells from prototype-infected mice at 72 h postinfection. Many of the disappearing proteins reappeared gradually in the E2-infected group. Infectious virus was recovered from the infected β cells only at 72 h postinfection. Functional impairment in these cells appears to be a factor in virus-induced hyperglycemia.

Insulin-dependent, type 1 diabetes mellitus, also called juvenile-onset diabetes, is caused by insulin deficiency in susceptible individuals (1–3). Epidemiologic observations (4, 5) and studies of animals and human patients (6–9) have implicated several viruses in the etiology of the disease. A recent report (10) indicates that persistent viral infection in mouse pancreatic β cells may also be associated with aberrations in blood chemistry similar to those found in the early stages of adult-onset diabetes.

One of these agents is coxsackievirus B4, a member of the family Picornaviridae (11, 12). Infections caused by pancreatic tropic strains of this virus may be etiologically related to insulin-dependent diabetes in humans and to diabetes-like disease in mice (5, 13–16). The tropism for the insulin-producing β cells can be enhanced by serial passage of unadapted virus in β cell cultures.

Infection of mice susceptible to diabetes (e.g., strains SJL, SWR, CD1, and DBA) with the diabetogenic strains of coxsackievirus B4 or certain other picornaviruses results in decreased immunoreactive insulin concentrations in the pancreas and serum, increased blood glucose concentrations, and impaired glucose tolerance. However, the mechanism by which this virus infection decreases the insulin concentration has not been established. Histopathologic investigations (14, 15, 17–20) indicate that β cell destruction may be partly responsible for the decrease in pancreatic insulin content. Neurologically regulated hormones may also contribute to some extent to the abnormalities in glucose homeostasis.

The present study was initiated to see whether coxsackievirus B4-induced alterations in the functional capacity of the β cells contribute to the long-term hyperglycemic state in CD1 mice. Changes in total protein synthesis and insulin synthesis in intact β cells during early and late infection were correlated with abnormalities in sugar metabolism. In addition, changes in the cellular proteins of β cells were monitored at successive times after infection to assess cell damage and repair.

The results demonstrate that although the infected β cells sustain severe damage, they continue to synthesize insulin but at a reduced capacity. The damage appears to be reversible with the passage of time. Thus, functional impairment of the infected β cells may be responsible for virus-induced hyperglycemia.

EXPERIMENTAL PROCEDURES

RESULTS

Perturbation of Glucose Metabolism Associated with Virus Infection—Mice infected with either the prototype or E2 strain of coxsackievirus B4 exhibited hypoglycemia at 72 h postinfection (Table I), possibly due to a rapid release of insulin into the blood from infected pancreatic β cells. Blood glucose then began to increase. The concentrations were maximum at 6 weeks postinfection and much higher in the E2-infected mice than in the controls. At 8 weeks postinfection, blood glucose concentrations in both infected groups were still above the controls and remained much higher in the E2-infected group as before. Blood glucose values higher than the mean + 3 S.E. of the uninfected animals (158 mg/dl) were considered abnormal (19). By this criterion, we detected hyperglycemia in approximately 76% of the E2-infected mice and about 9% of the prototype-infected mice at 8 weeks postinfection.

Portions of this paper (including "Experimental Procedures," Table II, and Figs. 2 and 3) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. M5-0370, cite the authors, and include a check or money order for $3.20 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
Previous studies on coxsackievirus- and encephalomyocarditis virus-induced diabetes in mice have shown that the development of the disease depends on the genetic background of the host and that of the virus (8, 14, 27). The genetic factors controlling susceptibility operate at the level of β cells, and infections caused by pancreateotropic strains of the virus produce the disease. To ensure that infection of the pancreas produced hyperglycemia in our study, we conducted a control experiment infecting CD1 mice with mouse hepatitis virus A59 (about 10⁶ plaque-forming units/mouse intraperitoneally). This virus has been reported to infect various organs such as lung and liver but not pancreas (28). We detected infectious A59 virus in the lung and liver but not pancreas at 7 days postinfection, however, the infection did not produce hyperglycemia at 6 weeks postinfection (data not shown).

**Protein and Insulin Synthesis**—To see whether the differences in glucose metabolism were related to the actual synthesis and secretion of insulin, enriched β cell preparations from the infected and control groups of mice were examined for protein synthesis at various times after infection.

Total protein synthesis was lower in the infected groups than in the controls (Fig. 1A). The extent of reduction was about the same in prototype- and E2-infected mice at 72 h (about 40%) and 6 weeks (about 48%) postinfection. No further drop in protein synthesis was evident in the prototype-infected group at 8 weeks postinfection, but in the E2-infected group synthesis had dropped to about 57%.

Insulin synthesis (released plus retained) also decreased in the infected groups compared to the controls at 72 h and 6 weeks postinfection (Fig. 1, B and C). The decrease was slight (about 8%) in the prototype-infected mice but much more (about 17% at 72 h and 12% at 6 weeks postinfection) in the E2-infected mice. At 8 weeks postinfection, insulin synthesis increased in both infected groups. Compared to the controls (about 27%), it became almost normal (about 26%) in the prototype-infected mice but remained below normal (about 18%) in the E2-infected mice.

The chromatographic profiles of the immunoprecipitates are indicated in Fig. 2 (see Miniprint). When [14C]-labeled insulin and unlabeled bovine insulin were chromatographed together, the material separated as a major insulin peak and a minor peak, probably insulin precursor (Fig. 2A). The immunoprecipitates retained (Fig. 2B) and released (Fig. 2C) from protein synthesis experiments on uninfected control mice also separated as two peaks, insulin and a larger molecule, presumably its precursor. Similar profiles were obtained for immunoprecipitates from virus-infected mice (not shown).

Virus-induced hyperglycemia may be related to the actual synthesis of insulin. For example, at 8 weeks postinfection, E2-infected mice, which exhibited hyperglycemia, also synthesized less insulin than the controls. In contrast, blood glucose had returned close to normal in the prototype-infected group, which synthesized almost as much insulin as the controls. At 72 h postinfection, however, this relationship between glucose concentration and insulin synthesis could not be detected, possibly due to a rapid release of insulin into the blood from damaged β cells. Moderate to severe β cell damage has been reported during early virus infection (14, 19).

**Nature of Proteins**—The foregoing results showed that virus infection inhibited total protein synthesis in β cells and that insulin synthesis, which was inhibited initially, began to rise during late infection. To examine whether the virus-induced abnormalities in sugar metabolism might be related to β cell protein changes, we analyzed the distribution of the cellular proteins.

The gel electrophoretic patterns of newly synthesized, cell-retained total proteins after protein synthesis in β cells purified from mice at 72 h postinfection are shown in Fig. 3 (see Miniprint). Synthesis of proteins of various molecular weights (5,600–69,000), including insulin and its precursor, was evident in cells from uninfected control mice (Fig. 3A). Prototype- or E2-virus infection inhibited the synthesis of both high- and low-molecular-weight proteins, including insulin.
(Fig. 3, B and C). During late infection, synthesis of insulin and its precursor increased, but synthesis of high-molecular-weight proteins (25,000–69,000) remained inhibited in the E2-infected mice (data not shown).

From these and the preceding results, it appears that virus-induced inhibition of insulin synthesis can reverse by itself, almost totally (in prototype-infected mice) or partially (in E2-infected mice), with time.

The compositions of cellular proteins in β cells from uninfected and prototype-infected mice, after separation in two-dimensional gels and subsequent Coomassie Brilliant Blue staining, are shown in Fig. 4. Numerous proteins of heterogeneous molecular weights (15,000 to >92,500) were visible in cells from control mice (Fig. 4A). Many of these proteins were slightly acidic (pI 5), although several neutral to slightly alkaline proteins were also visible. Insulin (M, 5,600) migrated very close to the dye front and therefore was not resolved in the two-dimensional gel system.

Most of the prominent proteins in the control cells (Fig. 4A) were of intermediate size (estimated Mr 35,000–68,000), with few as large as 92,500 (Table II, see Miniprint). Several pairs of proteins showed similar molecular weights (e.g., spots 16 and 17, spots 22 and 23, and spots 24–26), but their pI values were different.

The composition of cellular proteins was considerably altered in β cells from virus-infected mice. In the E2-infected group, many of these proteins had disappeared or were present in undetectable amounts at 72 h postinfection (Fig. 5A), but some reappeared at 6 weeks postinfection (Fig. 5B) and still more at 8 weeks postinfection (Fig. 5C). Several of these proteins (spots 3, 4, 9, 16, 18, 20, 21, 22, and 23) that reappeared at 8 weeks postinfection are identified in Fig. 5C, which also shows at least two new proteins (spots A and B, Mr > 71,000) not detected in the control group.

In the prototype-infected group (Fig. 4B) at 72 h postinfection, very few proteins (e.g., spots 1, 13, and 20) were missing, and several (e.g., spots 2–5 and 9) were present in lesser amounts than in the controls. The protein profile of prototype-infected cells became normal at 8 weeks postinfection (data not shown).

Virus infection thus alters the composition of β cell proteins during the initial stages of infection. This alteration may make the infected β cells less efficient in protein synthesis and sugar metabolism.

**Virus Replication**—To determine whether the virus was replicating in the infected pancreas and β cells, extracts from whole pancreases or purified β cells at various times after infection were plaque-assayed. The yields (total plaque-forming units) of infectious virus per 4 × 10⁷ β cells at 72 h postinfection were 3.7 × 10⁷ for prototype and 0.4 × 10⁷ for E2. Thus, over nine times more prototype virus was recovered from the infected β cells. About four times more prototype than E2 virus was recovered from the infected pancreas at 72 h postinfection. No infectious virus was detected in the pancreas or β cells from mice at 6 or 8 weeks postinfection (limit of detection, 10⁷ plaque-forming units).

**DISCUSSION**

The results of this study demonstrate that coxsackievirus infection decreases protein and insulin synthesis in mouse β cells and alters the composition of β cell proteins, apparently leading to the development of hyperglycemia or diabetes. Thus, our findings strongly suggest that virus-induced alterations in the functional capacity of the β cells contribute to the long-term hyperglycemic state. The study by Oldstone et al. (10) has also implied that subclinical impairment of cells of the islets of Langerhans may cause virus-induced diabetes.

The capacity of coxsackievirus B4 to induce diabetes depends on the strain of the virus. The results show that E2 virus is clearly diabetogenic in CD1 mice. The E2 strain is a plaque-purified virion of the Edwards’ isolate of B4. Edwards’ isolate was isolated from myocardial tissue of an infant (Edwards) with generalized coxsackievirus infection, focal necrosis, and inflammation of the pancreas (22). Both the Edwards’ isolate and the E2 strain caused abnormalities in sugar metabolism in strains of inbred mice (16, 20). The prototype strain, Van Barscholten, does not appear to induce overt diabetes, although the infection creates dysfunction in sugar metabolism in a small percentage of mice. Other studies have also described diabetogenic and nondiabetogenic strains of coxsackieviruses (13, 14, 27).

Hyperglycemia in E2-infected mice occurred after considerable alteration in β cell protein composition and after detectable infectious virus had disappeared from the pancreas. Thus, the direct initial assault of virus infection on healthy β
cells may be partly responsible for the development of diabetes, which is consistent with other observations based on histopathologic changes in the pancreas (14, 20, 29). A new finding in our study is that the initial assault on β cells appears to be at least partly reversible. This is supported by finding in our study that the initial assault on β cells is that the damage cells are repaired or replaced with both early and late infection. Although the infected decreases in late infection. Perhaps the virus-induced lesion is in early infection, an increasing number reappear during late infection. Perhaps the virus-induced lesion is the functional impairment in these cells, rather than cell death.

Precisely how insulin synthesis is reduced is unknown, but three mechanisms seem feasible. 1) A reduction in the level of translatable mRNA: virus infection could have reduced transcription of insulin mRNA or accelerated its degradation. Degradation of mRNA by 2-5A-dependent RNase (2-5A synthetase is interferon-induced) is well known (for a review, see Ref. 30). Interferon induction in mice by encephalomyocarditis virus, another picornavirus, has also been reported (27). 2) A defect in mRNA translation: poliovirus, also a picornavirus, inhibits host protein synthesis by causing a defect in host mRNA translation (31, 32). Such a mechanism could also operate in coxsackievirus-infected β cells. 3) Accelerated degradation of intracellular insulin, possibly by lysosomal granulolysis (33, 34) or by dissolution of the insulin crystal in the β granule and subsequent proteolysis (24): the newly synthesized insulin in the virus-infected β cells may thus be degraded by proteolysis before it is transferred to the β granule. Experiments are in progress to examine these possibilities.

Our results support the hypothesis that coxsackievirus B4 may be one cause of diabetes. The virus replicates in β cells during early infection and may persist in these cells in late infection without killing them. Alterations in the functional capacity of the infected β cells appear to contribute to the hyperglycemic state. Long-term follow-up investigations of patients with laboratory-confirmed coxsackievirus infection or recent-onset diabetes mellitus are needed to ascertain the mechanisms of a viral etiology in diabetes.

Acknowledgments—We thank Dr. S. R. Webb for the gift of E2 virus and Buffalo green monkey cells and for instructions in experimental procedures. We also thank Dr. Lawrence Sturman of the New York State Health Department for supplying the A59 virus, for technical instructions, and for performing the plaque assay in tissue extracts.

REFERENCES

FIG. 5. Separation of cellular proteins of β cells from E2-infected mice by two-dimensional gel electrophoresis. Sonicated proteins of β cell preparations at 72 h (A), 6 weeks (B), and 8 weeks (C) postinfection were analyzed as described in the legend to Fig. 4.
Experimental Procedures

Virus and Cells

Prototype paracoccidioides brasilensis were obtained from the New York State Health Department virus repository. The virus was grown in HeLa cells and plaque assayed in Vero cells according to the procedures described previously (24). The diabetogenic E2 strain was obtained from Dr. S. R. Webb’s laboratory (Department of Biology, Virginia Commonwealth University, Richmond, VA 23284). The isolation and characterization of the virus has been described elsewhere (24).

Animals, Virus Infection, and Experimental Design

CD1 male mice, 5- to 6 wk old, were obtained from Charles River Laboratory, Wilmington, MA. The mice were inoculated with about 2 x 10³ plaque-forming units (PFU) of either virus by the intraperitoneal route. Uninfected animals of the same strain, age, and sex were used as controls. At various times postinfection (p.i.) 10-20 randomly selected mice from each group were assayed by glucose tolerance test (GTT) and then sacrificed. Pancreatic beta cells from these animals were purified and stored for studies on protein synthesis, cellular protein analysis, and virus isolation. The experiments were repeated 2 to 4 times with replications.

Blood Glucose Assay

For the GTT each animal received an intraperitoneal injection of 2 mg of aqueous glucose solution (5%) per body weight and was bled from the tail 60 min later under general anesthesia with ether. Blood glucose in whole blood was measured by the glucose oxidase method (10).

Preparation of Cultures Enriched in Beta Cells

The procedure for isolation and purification of beta cells by a series of digestions with enzymes (collagenase, alpha-chymotrypsin, DNase I) and centrifugation through a ficoll gradient has been described (24). The purified beta cells were suspended in Medium 199 (Grand Island Biological) containing 10% fetal bovine serum and 16.7 mM glucose and 1% penicillin/streptomycin (GIBCO) containing 10% fetal bovine serum and 16.7 mM glucose and 1% penicillin/streptomycin (GIBCO).

Isotopic Labeling of Intact Beta Cells for Protein and Insulin Synthesis

Cultures enriched for beta cells were washed with warm (37°C), amino-acids-free Medium 199 containing 20 mM glucose and 100 U/ml insulin. Washed beta cells (about 2 x 10⁷/10 ml) were then suspended in 0.5 ml of this medium containing 100 μCi of high-specific-activity ³⁵S-labeled amino acids (Amersham Corporation) and incubated for 24 h at 37°C in a 5% CO2 incubator. All aliquots of the cell suspension (about 0.5 x 10⁶ cells) were assayed for total trichloroacetic acid-soluble radioactivity to represent total protein synthesis. Cells in the remaining suspension were pelleted by low-speed centrifugation, and the supernatant was assayed for radioactivity. The pellet was immediately electrophoresed in cylindrical isoelectric focusing gels. Markers (glycine/BSA buffer, glycine/BSA buffer, glycine/BSA buffer, glycine/BSA buffer, glycine/BSA buffer, glycine/BSA buffer) were run on the same gel. Glycine/BSA buffer was used as a 5-min brown line and diabase (28,000), lysozyme (14,400), and insulin (5,600). The gels were stained with Coomassie blue R-250 and then photographed as dried gels and photographs containing the marker proteins were used to estimate the molecular weights of beta cell proteins.

Supplementary Material:

Functional Alterations in Pancreatic Beta Cells as a Factor in Virus-Induced Hyperglycemia

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Virus-induced Hyperglycemia in Mice


Virus-induced Hyperglycemia in Mice


TABLE II

Estimated molecular weights of prominent proteins in beta cells from control CD1 mice

<table>
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<th>Spot no.</th>
<th>Molecular weight ( \times 10^3 )</th>
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<tr>
<td>1</td>
<td>&gt;92.6</td>
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<tr>
<td>2</td>
<td>73 ( \pm ) 3.22</td>
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<tr>
<td>3</td>
<td>71 ( \pm ) 3.89</td>
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<td>4</td>
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<td>68 ( \pm ) 2.89</td>
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<tr>
<td>6</td>
<td>62 ( \pm ) 2.65</td>
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<td>60.7 ( \pm ) 2.03</td>
</tr>
<tr>
<td>8</td>
<td>59 ( \pm ) 2.31</td>
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<tr>
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<tr>
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</tr>
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<td>15.2 ( \pm ) 0.30</td>
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Fig. 2. Column chromatographic analysis of the immunoprecipitated, released or retained insulin.

Labeled, immunoprecipitated material using guinea pig anti-bovine insulin serum from the experiments of Fig. 3 was chromatographed in a Sephadex G-50 column. Aliquots of column fractions were assayed for radioactivity and, in A, were used for \(^{14}C\)-measurements.

\(^{14}C\)-labeled insulin and unlabeled bovine insulin were cochromatographed. B. retained and C. released insulin were determined after protein synthesis in beta cells from uninfected control mice.

Fig. 3. Cylindrical gel electrophoresis of cell-retained total proteins labeled with \(^{14}C\)-amino acids in beta cells.

Beta cells from (A) uninfected, (B) prototype B4-infected, and (C) B5-infected mice at 72 h p.i., were labeled with amino acids for 24 h and then pelleted by centrifugation. Proteins in the cell pellet sonicates were electrophoresed along with \(^{14}C\)-labeled marker proteins (arrows) in a parallel gel. Radioactivity in 1-mm slices was plotted.