tion in the human enhancer. Fourth, alteration of the spacing between the μB and μA elements affects enhancer function (*21*), which suggests the need for appropriate spatial configuration of these elements.

On the basis of in vitro binding, mRNA expression, and trans-activation experiments, we propose that μB and μA binding proteins are encoded by the *PU.1/Spi-1* and *ets*-1 proto-oncogenes, which implicates them in the activation of the *IgH* locus (*22*). Other early B cell–specific genes such as λ5, *V–pre-B*, and *mb-1* also have μB homologous sequences in their promoters (*23*), which suggests that PU.1 may be a general regulator of early B cell gene expression. Although a role for Ets-1 has been suggested in T cell receptor α enhancer activity (*24*) and in the regulation of several T lymphotropic viruses (*18, 25*), our results provide the first evidence of Ets-1 function in B lymphoid cells.

The expression of PU.1 and Ets-1 together in nonlymphoid cells is sufficient to activate the core μ enhancer, which suggests that no other lymphoid-specific components are required in COS cells to induce core enhancer activity. The requirement for both proteins for efficient trans-activation in COS cells may reflect a necessity for occupancy of both μA and μB sites for enhancer function (*26*). Such a complex may provide a joint activation domain for the basal transcription machinery or may recruit (*27*) a different activator protein for this purpose. Further, the coordinate requirement for two different ETS-domain proteins, with overlapping expression patterns, may be an example of regulatory specificity achieved by the combinatorial use of factors with differing properties. Identification of the sequence motifs and the gene products that govern the activity of a B cell–specific domain of the μ enhancer provides a starting point for the biochemical reconstruction of the multiple DNA-protein interactions that constitute a functional enhancer.

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Increased Activity of L-Type Ca^{2+} Channels Exposed to Serum from Patients with Type I Diabetes

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Type I diabetes [insulin-dependent diabetes mellitus (IDDM)] is an autoimmune disease associated with the destruction of pancreatic β cells. Serum from patients with IDDM increased L-type calcium channel activity of insulin-producing cells and of GH_3 cells derived from a pituitary tumor. The subsequent increase in the concentration of free cytoplasmic Ca^{2+} ($[Ca^{2+}]_i$) was associated with DNA fragmentation typical of programmed cell death or apoptosis. These effects of the serum were prevented by adding a blocker of voltage-activated L-type Ca^{2+} channels. When the serum was depleted of immunoglobulin M (IgM), it no longer affected $[Ca^{2+}]_i$. An IgM-mediated increase in Ca^{2+} influx may thus be part of the autoimmune reaction associated with IDDM and contribute to the destruction of β cells in vivo.

Insulin-dependent diabetes mellitus (IDDM) is an autoimmune disease associated with massive lymphocytic infiltration of the endocrine pancreas, reaction of circulating autoantibodies with autoantigens on the insulin-producing β cells, and destruction of the β cells (*1–4*). The latter effect is believed to precede the onset of IDDM. In serum from children with newly diagnosed IDDM, antibodies to the islet cell surface affect glucose-induced insulin release (*2*). Voltage-dependent Ca^{2+} channels participate in various cellular processes, including the stimulus-secretion coupling in the β cell. We were interested in clarifying a possible effect of IDDM serum on voltage-activated L-type Ca^{2+} channels, and the molecular consequences, in insulin-secreting cells.

Normal pancreatic β cells from mice and clonal insulin-producing RINm5F cells (5), incubated in the presence of serum from newly diagnosed IDDM subjects (IDDM serum), exhibited increased Ca^{2+} current amplitudes as compared with the current amplitudes observed in cells incubated with serum from healthy individuals (Fig. 1, A and B). Whereas mouse β cells contain exclusively L-type Ca^{2+} channels, in rat β cells a sizeable fraction of the inward Ca^{2+} current flows through T-type Ca^{2+} channels (6). These two types of channels, T- and L-type Ca^{2+} channels, differ with regard to their sensitivities to Cd^{2+}, voltage dependence of activation, and steady-state inactivation properties (*7–10*). Our data suggests that IDDM serum acts selectively on L-type Ca^{2+} channels because (i) the effect was maximal around 0 mV (Fig. 1, A and B), which roughly coincides with the membrane potential at which L-type Ca^{2+} currents are maximal (*11*); (ii) no effect was obtained at membrane potentials more negative than −20 mV (Fig. 1B), which corresponds to membrane potentials at which T-type Ca^{2+} channels predominate; and (iii) no stimulatory effect was observable after blocking L-type Ca^{2+} channels with Cd^{2+} (Fig. 1B). Prolactin-secreting pituitary tumor cells (GH_3 cells), incubated in the presence of IDDM serum, also demonstrated an increased voltage-dependent Ca^{2+} current (Fig. 1E). The effect was most pronounced at voltages around 0 mV and remained observable when the cell was held at −50 mV, suggesting that the serum affects L-type Ca^{2+} channels in this cell type as well.

The most direct approach to demonstrate that L-type Ca^{2+} channels are affected by serum from patients with IDDM is to record currents from single Ca^{2+} channels. Selective blockers of T-type Ca^{2+} channels are not available. However, the steady-state inactivation properties of the T-type Ca^{2+} channels (holding potential, V_h = −66 ± 2 mV; n = 5) are such that the portion of current flowing through them is almost completely inactivated (>90%) at −50 mV, whereas most of the L-type Ca^{2+} current is activated (Fig. 1C) (*12*). Single channel measurements with 110 mM Ba^{2+} as the charge carrier revealed that T-type Ca^{2+} channels opened when the cell membrane was depolarized from −100 to −30 mV. These openings ceased when the holding potential was changed to −50 mV (Fig. 2A). Single channel openings during depolarization to 0 mV, elicited from a holding potential of −50 mV, occurred throughout the depolarization and had an amplitude of ≈1 pA (Fig. 2A). Moreover, applying the L-type Ca^{2+} channel agonist BAY K 8644 (5 μM) evoked a transition from short to long openings (*13*) and produced a threefold increase in the associated mean current compared with the control (mean ± SEM, 3.0 ± 0.6; n = 6; $P < 0.005$, Student's t test for paired data) (Fig. 2B). Some of the openings extended beyond the end of the depolarization. These biophysical and pharmacological properties are those expected for L-type Ca^{2+} channels. Thus, we con-

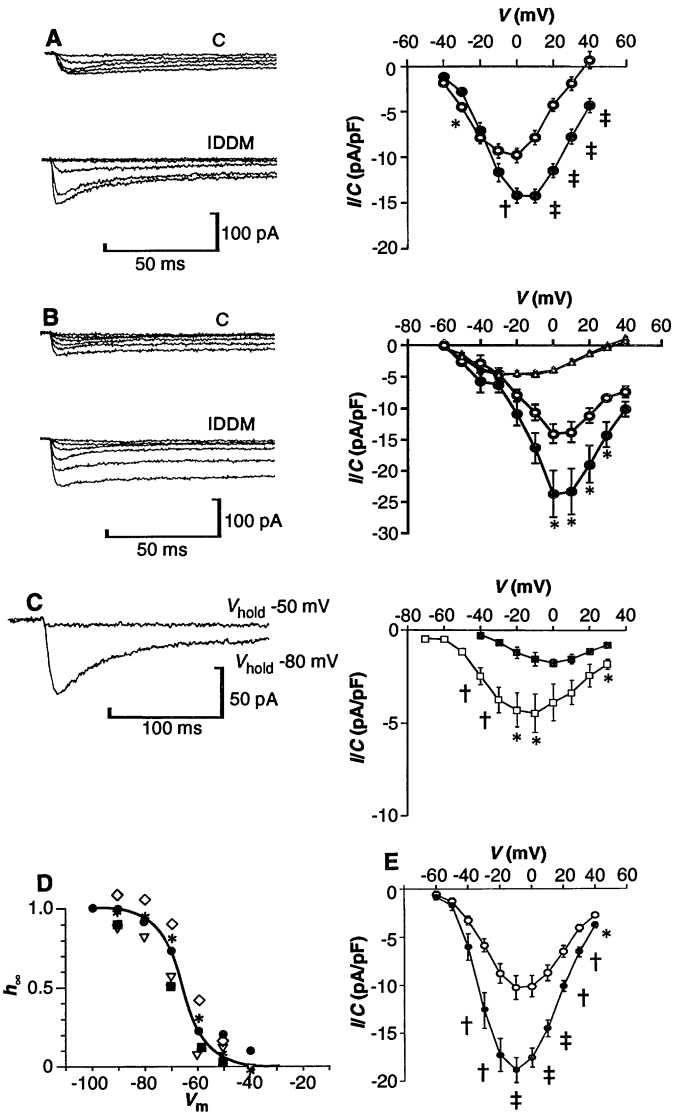

Fig. 1. Effects of diabetic serum on whole cell Ca^{2+} currents. Membrane currents (left) were recorded in normal pancreatic β cells (**A**) and RINm5F cells (**B**) during depolarizing voltage steps to membrane potentials between −40 and +40 mV (A) and −50 and +40 mV (B) (only the falling part of the current-voltage (*I-V*) relation is shown for the current traces). The holding potential was −50 mV in (A) and −80 mV in (B). To the right, the complete *I-V* relation is shown (mean ± SEM of 8 to 14 experiments). To compensate for variation in cell size, currents were expressed as current densities (*I/C*), which we obtained by normalizing the current amplitude (*I*) to cell capacitance (*C*). Open circles represent control cells and filled circles represent cells pretreated with IDDM serum. In (**B**) peak currents observed in the presence of 20 μM Cd^{2+} after exposure to control (C) serum (open triangles) and after pretreatment with IDDM serum (filled triangles) are also displayed (data points superimpose). (**C**) Membrane currents from RINm5F cells in the presence of 20 μM Cd^{2+} during a depolarizing step to −30 mV [to selectively activate T-type Ca^{2+} channels (*6*); compare with (B) (open triangles)] from holding potentials of −50 mV and −80 mV. Peak current *I-V* relation (right) obtained from holding potentials of −80 mV (open squares) and −50 mV (filled squares). In (**D**) the steady-state inactivation of T-type Ca^{2+} currents is shown. A standard test pulse to −20 mV (80 ms) was preceded by a pulse going to potentials between −100 and −40 mV (10 s). The current size evoked subsequent to a prepulse to −100 mV was taken as unity. The relative peak amplitude (h_∞) was plotted against the respective conditioning membrane potentials (V_m) (*26*). The different symbols correspond to the individual experiments performed. Some data points are superimposed. We evaluated statistical significances by comparing control and IDDM serum at each voltage using Student's t test for unpaired data. (**E**) Effect of diabetic serum on whole cell Ca^{2+} currents in GH_3 cells. Membrane currents were recorded during depolarizing voltage steps to membrane potentials between −60 and +40 mV from a holding potential of −80 mV. Open circles represent cells treated with control serum and filled circles represent cells treated with IDDM serum. We evaluated statistical significances by comparing control and IDDM sera at each voltage using Student's t test for unpaired data. *$P < 0.05$, †$P < 0.01$, and ‡$P < 0.001$.

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cluded that the channel activity we observed was attributable primarily to L-type Ca^{2+} channels.

We recorded Ba^{2+} currents through L-type Ca^{2+} channels in RINm5F cells treated with control serum (Fig. 2C) and IDDM serum (Fig. 2D). Under control conditions, channel openings were short and separated by long closures, which yielded a small ensemble current (0.02 ± 0.01 pA; n = 6). In cells treated with IDDM serum, Ca^{2+} channel activity was more intense, and there was a tenfold increase in the associated mean current (0.20 ± 0.05 pA; n = 6; $P < 0.01$, Student's t test for unpaired data). Analysis of single channel kinetics indicated that this tenfold increase in mean current was the result of not only longer open times, but also shorter closed times. The time constants for the distribution of the open times averaged 0.7 ± 0.1 ms and 1.2 ± 0.2 ms ($P < 0.025$, Student's t test for unpaired data) after treatment with control serum and IDDM serum, respectively. Analysis at the single channel level, omitting sections containing superimpositions, showed that the mean closed time was reduced from 14.0 ± 1.8 ms in cells exposed to control serum to 3.9 ± 0.4 ms in cells exposed to the IDDM serum ($P < 0.001$, Student's t test for unpaired data). This result along with the values we obtained for open times account for most of the increase of the associated mean current.

The stimulatory effect of the serum from patients with IDDM was more pronounced in the intact cell (cell-attached recordings) than in the whole cell recordings (exchange of the cell interior), suggesting the involvement of a soluble, cytosolic constituent in mediating the response to the IDDM serum. To exclude a direct effect on the Ca^{2+} channel protein, we added IDDM and control sera to RINm5F cells just prior to measuring the current in the whole cell configuration of the patch-clamp technique. Under these experimental conditions, there was no effect of IDDM serum on Ca^{2+} channel activity (*14*).

Increases in free cytoplasmic Ca^{2+} ($[Ca^{2+}]_i$) were observed when RINm5F cells, normal mouse β cells, and GH_3 cells were depolarized after the extracellular K^+ concentration was increased to 30 mM (Fig. 3). Cells treated with IDDM serum displayed greater $[Ca^{2+}]_i$ transients than their respective controls. In individual experiments, sera from four different subjects with IDDM (Table 1) all potentiated the K^+-induced increase in $[Ca^{2+}]_i$ compared with the K^+ response obtained with control serum. The $[Ca^{2+}]_i$ increase was expressed as the difference in the 340 nm/380 nm fluorescence ratio obtained before and after K^+ depolarization (Δ340/380 ratio) and corresponded to (mean ± SEM) 0.90 ±

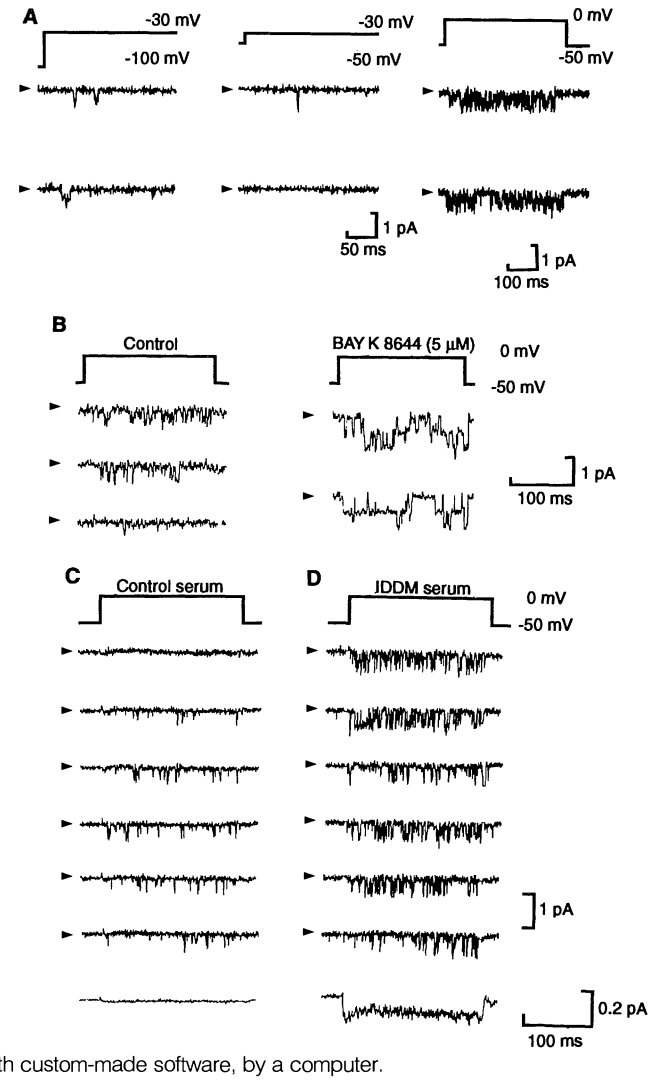


Fig. 2. Increased activity of single Ca^{2+} channel currents in cells treated with IDDM serum. (**A**) Examples of single channel currents recorded from a cell-attached patch on a RINm5F cell when depolarized as indicated in the figure. All traces are from the same cell. Note the difference in time scale. (**B**) Examples of single channel currents recorded from the same cell-attached patch on a RINm5F cell in the absence and presence of BAY K 8644 (5 μM). (**C** and **D**) Examples of cell-attached, single channel recordings from RINm5F cells treated with normal and IDDM serum, respectively. The bottom traces represent the mean currents obtained by averaging 125 (C) and 90 (D) traces. Arrowheads indicate current level when the channel is closed. The pipette solution contained 110 mM $BaCl_2$, 10 mM TEA-Cl, and 5 mM Hepes-$Ba(OH)_2$ (pH 7.4). To control membrane potential, we bathed cells in a depolarizing solution containing 125 mM KCl, 30 mM KOH, 10 mM EGTA, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM Hepes-KOH (pH 7.15), and 10 mM glucose. Pulses were applied at a frequency of 0.5 Hz. Currents were filtered at 1 kHz (8-pole Bessel filter, −3-dB point) and digitized at 4 kHz and analyzed with custom-made software, by a computer.

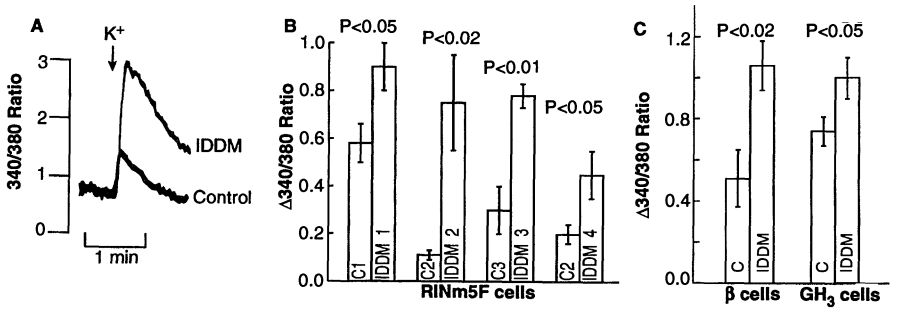
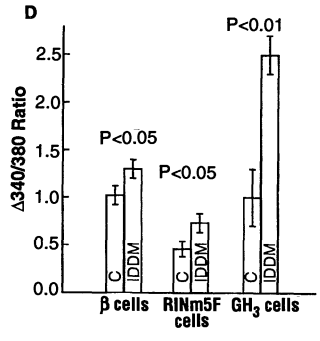


Fig. 3. Effect of IDDM serum on $[Ca^{2+}]_i$. (**A**) Representative trace of effects of K^+ depolarization on $[Ca^{2+}]_i$ in RINm5F cells incubated with IDDM or control serum. (**B** and **C**) Effect of K^+ depolarization on $[Ca^{2+}]_i$ in RINm5F cells (B), normal β cells, and GH_3 cells (C) treated with control (C) or IDDM serum. (**D**) Effect of K^+ depolarization on $[Ca^{2+}]_i$ in β cells, RINm5F cells, and GH_3 cells treated with IDDM serum 1 and control serum 1 after ammonium sulfate precipitation. Mean ± SEM (n = 4 to 10) increase in $[Ca^{2+}]_i$ is expressed as the change in ratio of fura-2 fluorescence at 340 nm and 380 nm (Δ340/380 ratio). For the $[Ca^{2+}]_i$ measurements, cells were seeded onto plastic cover slips and cultured overnight in RPMI 1640 medium supplemented with serum (10%) from normal subjects or patients with IDDM. Changes in $[Ca^{2+}]_i$ were measured as described (*27*).



0.10 (n = 6), 0.75 ± 0.22 (n = 4), 0.78 ± 0.05 (n = 4), and 0.45 ± 0.11 (n = 5) for IDDM serum and 0.58 ± 0.09 (n = 5), 0.11 ± 0.02 (n = 5), 0.30 ± 0.12 (n = 3), and 0.20 ± 0.04 (n = 6) for control serum. Because the various cell preparations demonstrated a varying K^+ response when treated with control sera, we had to have a corresponding control for each experiment performed with IDDM serum. When cells were incubated with IDDM and control sera subjected to ammonium sulfate precipitation (Fig. 3D), the differences remained. These findings indicate that an immunoglobulin may participate in the stimulatory action of IDDM serum on $[Ca^{2+}]_i$. As above, IDDM serum that passed through a protein A column retained its activity. The Δ340/380 ratio corresponded to 0.6 ± 0.1, n = 15, and 0.4 ± 0.1, n = 14 ($P < 0.02$, Student's t test for unpaired data) for RINm5F cells subjected to IDDM serum and control serum, respectively. This suggests that immunoglobulin G (IgG) is not the factor mediating the increase in $[Ca^{2+}]_i$. By contrast, IDDM serum depleted of immunoglobulin M (IgM), by passage over an anti-IgM Sepharose column, was without stimulatory effect on $[Ca^{2+}]_i$. In this case the Δ340/380 ratio corresponded to 1.1 ± 0.1, n = 15, and 1.0 ± 0.1, n = 15 (not significant), for normal β cells treated with IDDM serum devoid of IgM and control serum, respectively. The corresponding values for this particular IDDM serum, before it was depleted of IgM, and control serum were 1.3 ± 0.1, n = 16, and 1.0 ± 0.1, n = 16, respectively ($P < 0.05$, Student's t test for unpaired data). In the presence of D-600 (50 μM), which blocks voltage-activated L-type Ca^{2+} channels, the K^+-induced increase in $[Ca^{2+}]_i$ was inhibited in cells treated with either IDDM or control serum (*15*).

Increases in $[Ca^{2+}]_i$ have been linked to cell death in a number of experimental systems (*16–18*). Internucleosomal DNA cleavage by a Ca^{2+}- and Mg^{2+}-dependent endonuclease, activated by intranuclear Ca^{2+} uptake (*19, 20*), is a characteristic marker of programmed cell death (*21*). We measured DNA fragmentation to clarify whether DNA damage was induced by increased L-type Ca^{2+} channel activity and the resulting increase in $[Ca^{2+}]_i$, which we observed in RINm5F cells incubated in the presence of IDDM serum. Incubated under such conditions, RINm5F cells exhibited DNA fragmentation after 24 hours (Fig. 4A). To investigate the pattern of DNA fragmentation, DNA extracted from RINm5F cells that had been incubated for 48 hours with IDDM serum was analyzed by agarose gel electrophoresis. We observed a DNA fragmentation pattern typical of endonuclease-mediated DNA cleavage. Fragmentation was maximal at 48 hours, when 40% of the DNA was cleaved. When RINm5F cells were exposed to IDDM serum in the presence of the L-type Ca^{2+} channel blocker verapamil, to prohibit Ca^{2+} from

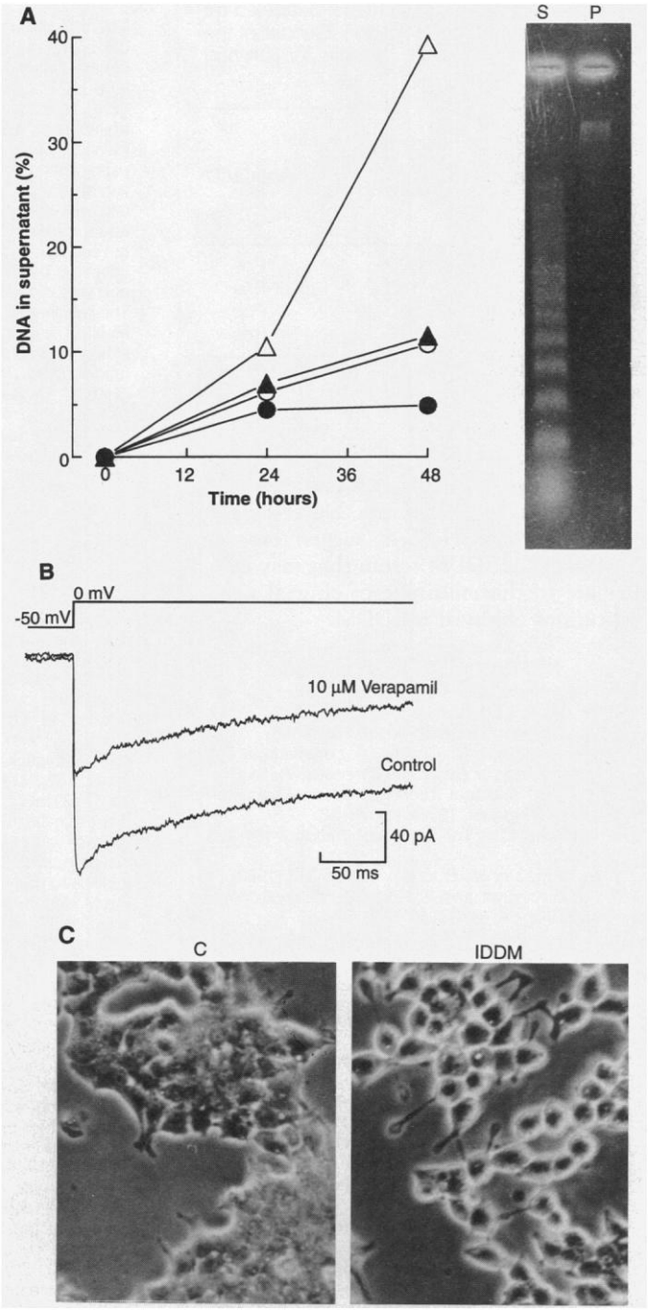


Fig. 4. The effects of serum from normal and diabetic individuals on DNA fragmentation (A), whole cell Ca^{2+} currents (B), and morphology of RINm5F cells (C). (**A**) Normal serum (○); IDDM serum (△); IDDM serum and verapamil (10 μM) (▲); normal serum and verapamil (10 μM) (●). For quantitation of DNA fragmentation 9 × 10^4 cells were seeded in a 12-well plate (4 cm^2 of growth area per well) in RPMI 1640 medium supplemented with serum (10%) as detailed below. Cells were incubated with [^{14}C]thymidine (0.03 μCi/ml) for two generations and then for 24 hours in RPMI 1640 medium supplemented with fetal calf serum (10%). Thereafter, cells were washed with RPMI 1640 devoid of serum and then exposed to medium supplemented with IDDM or control serum (10% v/v) for up to 48 hours. At the times indicated cells were collected, washed free of medium, and treated with 450 μl of cold lysis buffer containing 20 mM EDTA, Triton X-100 (0.5% v/v), and 5 mM tris-Cl (pH 8.0) overnight at 4°C. Cells were then centrifuged for 15 min at 13,000*g* in a microfuge (Biofuge A, Heraeus, Sepotech, Germany) to separate intact chromatin (pellet) from DNA fragments (supernatant). Sedimented material was resuspended in 0.5 ml of buffer containing 10 mM tris-HCl and 1 mM EDTA (pH 8.0). The graph shows DNA fragmentation as the percentage of the total radioactivity (in the supernatant and sedimented fractions) that was present in the supernatant. To clarify the nature of DNA degradation, and thereby verify the existence of the typical ladder pattern of the internucleosomal cleavage by an endonuclease associated with apoptosis, we performed an agarose gel electrophoresis (*28*) on DNA from cells treated with IDDM serum from supernatant (S) or sedimented (P) fractions. Because cells treated with control serum did not demonstrate DNA fragmentation, only the lanes of the gel corresponding to cells treated with IDDM serum are shown. (**B**) Whole-cell Ca^{2+} currents recorded from intact RINm5F cells in the presence and absence of verapamil (10 μM). Currents were recorded during voltage steps to 0 mV from a holding potential of −50 mV, using the perforated-patch whole cell technique (amphotericin B). The pipette solution consisted of 76 mM Cs_2SO_4, 1 mM $MgCl_2$, 10 mM NaCl, 10 mM KCl, and 5 mM Hepes (pH 7.35 with CsOH). (**C**) Phase contrast photographs of RINm5F cells treated with normal serum or IDDM serum for 48 hours. Photographs were taken with a Nikon F301 camera attached to a Nikon PFx microscope. Magnification ×250.

Table 1. Characterization of the IDDM patients. The amount of antibodies to islet cells (ICA) was determined by an indirect immunofluorescence technique, using unfixed cryostat sections of human pancreas. The assay was calibrated against the Juvenile Diabetes Foundation (JDF) standard serum to permit conversion of the titers to JDF units. In the fifth Immunology of Diabetes Workshop ICA Proficiency Test, the assay obtained a 93% validity, 100% specificity, and 88% sensitivity. Antibodies to GAD (anti-GAD) were detected by immunoprecipitation of ^{35}S-labeled rat islet proteins (*29*). The diabetic sera contained normal concentrations of glucagon. Gender of the patients is designated as F, female, and M, male. Healthy blood donors, all negative for ICA and anti-GAD, served as sources for control sera.

Patient	Sex	Age (years)	Duration of IDDM since diagnosis (weeks)	Medication	ICA (JDF units)	Anti-GAD
1	F	46	<2	No*	320	Pos
2	F	25	1	No*	160	Pos
3	F	70	78	Yes†	320	Pos
4	M	15	<1	No*	20	Pos

*Insulin was the only medication administered. †The patient was treated with insulin, 36 immunizing units per day; glibenclamide, 3.5 mg/day; levothyroxine, 0.1 mg/day; and spironolactone, 50 mg/day.

entering the cell, DNA fragmentation was prevented (Fig. 4A). Verapamil also blocked Ca^{2+} influx in RINm5F cells (Fig. 4B). The progression of DNA fragmentation was associated with morphological changes of the cells (Fig. 4C). After 48 hours, cells treated with IDDM serum assumed a spherical appearance and began to lose adherence to the culture dishes. Membrane permeabilization, measured as leakage of lactate dehydrogenase, invariably followed at later stages. These changes were also prevented by verapamil. These findings suggest that alteration in Ca^{2+} channel activity is associated with programmed cell death in insulin-producing cells.

We identified one of the pancreatic β cell autoantigens, with a molecular size of 64 kD, as glutamic acid decarboxylase (GAD) (*22*). Autoantibodies to GAD were not restricted to pancreatic β cells but were also found in stiff-man syndrome (*22*, *23*). However, we could not detect GAD in our RINm5F cells by immunofluorescence or immunoprecipitation of ^{35}S-labeled proteins with several different IDDM sera (*24*). Moreover, no other band was detected in immunoprecipitation experiments or by protein immunoblotting with IDDM serum. These results, together with the fact that purified IgG did not affect $[Ca^{2+}]_i$, makes it unlikely that the autoantibody to the 64-kD autoantigen accounts for our results. Instead, proteins in the IgM fraction of IDDM serum may indirectly affect the voltage-dependent L-type Ca^{2+} channels and thereby increase $[Ca^{2+}]_i$, resulting in apoptosis. Hence, Ca^{2+}-induced apoptosis may be involved in the complex autoimmune reaction associated with IDDM. Although a number of cells express voltage-activated L-type Ca^{2+} channels, the regulation of those channels at the molecular level may be different from that in the β cell, making these cells less vulnerable to the cytotoxic effect of IDDM serum. Nevertheless, our results in GH_3 cells suggest broader effects of the IDDM serum that may contribute to the multitude of clinical complications observed in IDDM.

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26. The data were fitted by the Hodgkin-Huxley equation

$$h_\infty(V_m) = \frac{1}{1 + e^{(V_h - V)/k_h}}$$

where V_h is the membrane potential at which h_∞ = 0.5 and k_h is the steepness coefficient. In five separate experiments, V_h and k_h corresponded to −66 ± 2 mV and −5.2 ± 0.3 mV (mean values ± SEM), respectively Sera from diabetic and control subjects were collected, sterile-processed in identical ways, and stored frozen at −20°C until used. The sera were heat-inactivated by incubation at 56°C for 30 min. We obtained immunoglobulin fractions by incubating the different sera with equal volumes of a saturated solution of ammonium sulfate on ice overnight. The precipitates were collected by centrifugation at 10,000*g* for 20 min and washed twice with 1.6 M ammonium sulfate. The resulting precipitates were dissolved in 0.15 M NaCl and then dialyzed extensively against sterile phosphate-buffered saline (Dulbecco's modification) (PBS-D). One part of the immunoglobulin fractions was passed over a protein A–Sepharose 4B column (Pharmacia, Uppsala, Sweden). A new column was used for each serum. The flow-through fractions were collected, and bound IgG was eluted from the column with 0.2 M citrate-HCl buffer (pH 3.0); the fractions were collected in tubes containing 1 M tris-HCl (pH 8.0). All fractions were again dialyzed extensively against sterile PBS-D We depleted immunoglobulin fractions of IgM by passing them over anti-IgM Sepharose columns. We prepared these columns by coupling an immunoglobulin fraction of a sheep antiserum specific for human IgM (DAKO-immunoglobulins A/S, Glostrup, Denmark) to Sepharose 4B (Pharmacia). Cells were maintained overnight in tissue culture medium RPMI 1640, containing 5 mM glucose and supplemented with 10% serum (normal or diabetic) and antibiotics. The extracellular solution contained 138 mM NaCl, 5.6 mM KCl, 1.2 mM $MgCl_2$, 10.2 mM $CaCl_2$, 10 mM tetraethylammonium chloride (TEA-Cl), 5 mM Hepes-NaOH (pH 7.4), 5 mM glucose, and tetrodotoxin (1 μg/ml). The pipette solution consisted of 150 mM *N*-methyl-D-glucamine, 110 mM HCl, 10 mM EGTA, 1 mM $MgCl_2$, 5 mM Hepes-NaOH (pH 7.15), and 3 mM Mg^{2+} adenosine triphosphate. Pulses were applied at a frequency of 0.5 Hz. Currents were recorded with a List Electronic EPC 7 or an Axopatch 200 patch-clamp amplifier and filtered at 1 kHz. All experiments were performed at room temperature.
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28. In 100-mm Costar dishes 1 × 10^6 cells were seeded and exposed to normal or IDDM sera, as described for the studies of DNA fragmentation. After 48 hours cells were lysed and centrifuged to separate DNA fragments (S) from intact chromatin (P). DNA was precipitated overnight with cold absolute ethanol and 0.17 M NaCl. Samples were then extracted and DNA was precipitated and separated by agarose gel electrophoresis (1% gels) (*25*). DNA was visualized by ultraviolet fluorescence after the gels had been stained with ethidium bromide (6 μg/ml). This experimental protocol was repeated four times.
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