

MURINE MACROPHAGES AND PANCREATIC β CELLS
Chemotactic Properties of Insulin and β -Cytostatic
Action of Interleukin 1

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Interactions between leukocytes and pancreatic β cells are of particular interest because many forms of insulin-dependent diabetes have an autoimmune etiology (1). Attention has been concentrated on T lymphocytes because of their ability to adoptively transfer diabetes in the BioBreeding (BB) rat (2) and nonobese diabetic (NOD) mouse (3). There is indirect evidence that cells of the reticuloendothelial system (RES), particularly macrophages ($M\phi$) may also play an important role in induction of diabetes. For example, depression of RES function in normal rats by colloidal carbon blockage improved glucose tolerance and insulin sensitivity (4). Peripheral blood monocytes bind and degrade considerably more insulin than do lymphocytes (5, 6), such that monocytes could regulate levels of circulating insulin. $M\phi$ could potentially interact with β cells more directly. $M\phi$ appear very early in the development of insulinitis in BB rats, and administration of silica, a macrophage toxin, protects these animals against diabetes development (7). We have previously shown that $M\phi$ from normal CBA/J mice develop spontaneous cytotoxicity against syngeneic pancreatic β cells in vitro (8). More recently, IL-1, a $M\phi$ -secreted monokine, was found to be cytotoxic to cultured rat islets (9, 10). In the present study, we use mouse islet cells in culture to elucidate further the potential for interaction between $M\phi$ and β cells. We demonstrate that both intact islets and soluble insulin are $M\phi$ chemoattractants, and that IL-1, while not cytotoxic to mouse islet monolayers, inhibits insulin release and alters β cell ultrastructure.

Materials and Methods

Peritoneal $M\phi$. CBA/J mice were obtained from the Animal Resources Unit of The Jackson Laboratory. Peritoneal $M\phi$ were harvested from 8-wk-old males 3 d after injection of 2 ml i.p. of 3% thioglycollate. The peritoneal cellular exudate was harvested in 5 ml of HBSS, pelleted, rinsed three times in HBSS, and the cells were counted in a hemacytometer. The cells (>95% $M\phi$ as determined by ability to bind and phagocytose mouse antibody-coated sheep erythrocytes [EA]) were adjusted to a concentration of 4×10^7 cells/ml in DMEM modified and supplemented with 10% heat-inactivated dialyzed FCS as described previously (8).

$M\phi$ Migration Assay. $M\phi$ migration from a 2- μ l agarose droplet was analyzed as described by Harrington and Stastny (11). The microdroplets containing 8×10^5 cells were placed in 8-well microculture chamber/slides and cooled in a refrigerator for 8 min before addition of medium containing either pancreatic islets or islet hormones. Cultures

This work was supported by grants DK 27722 and 36175 from the National Institutes of Diabetes and Digestive and Kidney Diseases.

were maintained in DMEM in a CO₂ incubator at 35°C. The circular area occupied by the droplet (A1) and the area encompassed by the M ϕ migration front (A2) were determined at 24, 48, and 72 h using an image analyzer (Optomax, Hollis, NH). A migration index (MI) for each test condition (done in quadruplicate sets of droplets) was calculated by dividing A2 by A1 for each of the three timepoints studied.

Pancreatic Islets and Islet Hormones. The agarose/M ϕ droplets were incubated with groups of 20 hand-picked islets isolated from 8-mo-old CBA/J males by collagenase digestion (12), or with pancreatic islet cell hormones added either individually or in combinations to final concentrations as follows: bovine insulin (20 ng/ml; Sigma Chemical Co., St. Louis, MO), somatostatin (5 ng/ml; Beckman Scientific Co., Palo Alto, CA), porcine glucagon (10 ng/ml); bovine pancreatic polypeptide (10 ng/ml). The latter peptides were generous gifts of the Eli Lilly Company (Indianapolis, IN). Controls were agarose/M ϕ droplets in modified DMEM only.

Effects of IL-1 on Structure and Function of Cultured CBA/J Islets. Monolayer cultures of CBA/J islets were established in 24-well plastic culture dishes in modified DMEM (16.5 mM glucose) as described previously (8). At day 14 of culture, quadruplicate wells were refed with modified DMEM containing 0, 5, or 25 U/ml of mouse rIL-1 α (Hoffmann-LaRoche Inc., Nutley, NJ), and were maintained in these media for 3–9-d periods, with media changes at 3-d intervals. Both control and IL-1-supplemented medium contained 500 U/ml aprotinin (Sigma Chemical Co., St. Louis, MO) to prevent degradation of IL-1 and of secreted insulin. Insulin/proinsulin biosynthesis in the cultures was measured in isoleucine-free DMEM by incorporation of [³H]isoleucine (10 μ Ci/ml) for the last 24 h of a 3-d exposure period. Insulin concentration in the acid/ethanol extracts (cells and medium) were determined by RIA, and 10- μ U amounts were immunoprecipitated as described previously (13).

To assess whether rIL-1 was unique in its action, human rTNF (a generous gift of Dr. K. Manogue, The Rockefeller Univ., New York) was tested at a concentration range between 0 and 100 ng/ml (100 ng/ml producing ~50% necrosis of mouse L cell monolayers). Also, lymphotoxin from Con A-stimulated T cell clone 153.E11 (a kind gift of Dr. N. Ruddle, Yale Univ., New Haven, CT) was tested between 0 and 30 U/ml (10 U effecting 30% killing in our mouse L cell monolayer cultures). rIL-2 (Genzyme, Boston, MA) was also studied at concentrations of 100 and 500 U/ml.

Methods for assessment of islet morphology, for radioimmunoassay of insulin content in acid/ethanol-extracted cells and media, and for insulin secreted in response to a challenge of 16.5 mM glucose plus 5 mM theophylline (after establishment of a baseline response to 5.5 mM glucose) have been described in detail previously (8). Student's *t* test was used for statistical comparisons of treatments.

Results

Cells migrating from the agarose droplets were identified as M ϕ by ability to phagocytose EA; the photomicrographs shown in Fig. 1 illustrate the potent chemotactic effect of either intact islets or of insulin-supplemented DMEM. Insulin-supplemented medium stimulated uniform radial outgrowth whereas when adherent islets were present, M ϕ migration was not uniform, but concentrated toward the foci of islet cells. Although M ϕ migration in response to either islets or to insulin was not significantly different after 72 h exposure, the initial (24 h) response was significantly greater in the presence of islets (Fig. 2). Radioimmunoassay of insulin secreted into the spent 3-d culture medium from the wells containing 25 islets plus M ϕ showed an average of 13 ng/ml, a value comparable to the insulin-supplemented medium (20 ng/ml). The stronger stimulus produced by intact islets suggested that other islet cell products were also chemoattractants. Fig. 2 shows that pharmacologic concentrations of glucagon, somatostatin, and pancreatic polypeptide were without effect when tested

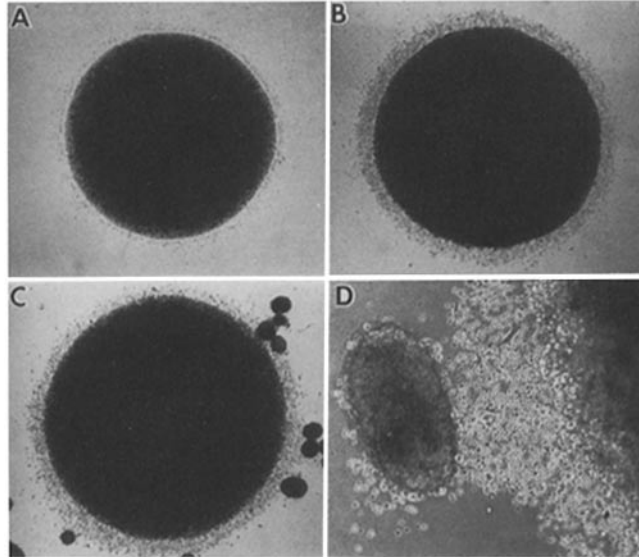


FIGURE 1. Photomicrographs illustrating stimulation of M ϕ migration from an agarose droplet (dark object in center) after a 72-h culture period in the presence of islets or insulin. (A) M ϕ only; (B) M ϕ + insulin; (C) M ϕ + islets; (D) Higher magnification of C showing directed M ϕ migration to islet cells.

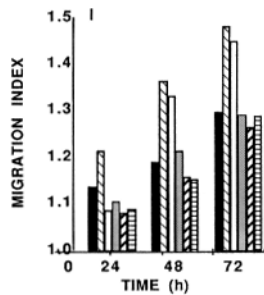


FIGURE 2. M ϕ migration stimulated by intact cultured islets vs. pancreatic islet hormones. Migration index (mean for two experiments; points in quadruplicate) is the ratio of the migration area to the microdroplet area at each time point. Controls are microdroplet cultures in hormone-free medium. Only migration in the presence of islets or insulin was stimulated in relation to control cultures ($p \leq 0.01$); the greater stimulation by islets at 48 h was also significant ($p \leq 0.01$). ■, Control; ▨, islets; □, insulin; ▤, glucagon; ▥, pancreatic polypeptide; ▧, somatostatin.

individually for stimulation of M ϕ migration. Thus, of the four islet hormones tested, only insulin alone was capable of stimulating M ϕ migration.

Continuous exposure of islet monolayers to concentrations of 25 U/ml rIL-1 for periods of up to 9 d did not alter the morphology of the cells or induce cytolysis. Nevertheless, as shown in Fig. 3, a β cell-specific ultrastructural effect of chronic rIL-1 treatment was observed. In comparison to the well-granulated β cells observed in control cultures (Fig. 3A), β cells treated with 25 U/ml rIL-1 exhibited a marked decrease in both the size and numbers of β granules (Fig. 3B). This degranulation was specific for β cells; glucagon-containing α cells, somatostatin-containing α cells, and pancreatic polypeptide-containing cells remained heavily granulated. Data in Table I show a ~30% decrease in insulin content of cells and culture media after chronic IL-1 exposure. Yet the specific radioactivity of [3 H]isoleucine incorporated into immunoprecipitated (pro)insulin was increased two-fold in rIL-1-treated cultures, indicating that biosynthesis of noninsulin proteins, possibly involving insulin processing and secretion (including the secretory granule membrane proteins) was being inhibited to a greater extent than insulin biosynthesis itself.

CBA/J islet cells incubated in DMEM containing 5, 10, or 25 U/ml rIL-1 for 3 d, and then switched to IL-1-free DMEM containing 5.5 mM glucose for a further 3-d culture period did not show cytopathic changes at the light micro-

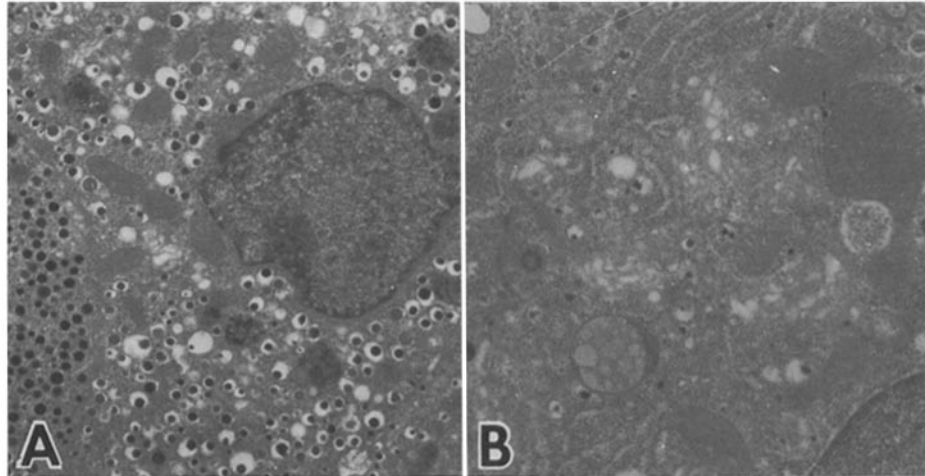


FIGURE 3. Ultrastructural contrast between (A) well-granulated β cell cultured for 4 d in the absence of IL-1 vs. (B) degranulated β cell cultured in 25 U/ml IL-1 and characterized by β granules much reduced in size and in matrix content, as well as by prominent rough endoplasmic reticulum. (A) \times 5,800; (B) \times 10,000.

TABLE I
Effect of Chronic IL-1 Incubation on Cultured β Cells

IL-1	Insulin content			Specific activity	
	Media	Intracellular	Percent decrease	Media	Cells
<i>U/ml</i>		<i>μU/well</i>		<i>μCi/μg insulin</i>	
0	1,125 \pm 130	451 \pm 63	—	0.43 \pm 0.06	1.93 \pm 0.62
4	833 \pm 116	279 \pm 45*	29.2	—	—
25	76 \pm 70*	292 \pm 39*	33.1	2.00 \pm 0.91	3.48 \pm 0.71

Insulin content in culture media and cells were determined over a 9-d incubation with or without IL-1. Percent decrease in total insulin production (medium + cells) as compared to control (0 U/ml IL-1) is shown. Specific radioactivity in immunoprecipitable insulin was determined in a separate set of cultures treated 3 d with or without IL-1, with [3 H]isoleucine present during the last 24 h.

* $p < 0.025$ from t test comparison to control islets cultured without IL-1.

scopic level. However, insulin secretory responsiveness of the IL-1-pretreated β cells to 16.5 mM glucose plus 5 mM theophylline challenge was abolished, despite the 3-d recovery period (data not shown).

The ability of IL-1 to diminish β cell secretory responsiveness to glucose and theophylline was unique among the factors tested. IL-2, TNF, and lymphotoxin were all without effect after 6-d exposure periods (data not presented).

Discussion

This study has used cell culture to explore the potential interactions between M ϕ and pancreatic β cells. M ϕ contained inside agarose microdroplets were strongly chemoattracted to pancreatic islets. Insulin is the major secretory product of the islets, and may achieve high local concentration in the interstitium of the islet. Culture medium supplemented with insulin, but not glucagon, somatostatin, or pancreatic polypeptide simulated the effect of whole islets on M ϕ migration. Intact islets, in addition to the peptide hormones tested, also

secrete products of prohormone processing (e.g., insulin C peptide), which could be chemoattractive. In addition, CBA/J islets cultured in high-glucose media produce intracisternal type A retroviral proteins (14), and further, may release proteins exhibiting advanced glycosylation end products for which M ϕ have high-affinity receptors (15). Local concentrations of all of these factors would be expected to rise in the interstitial spaces around islets during the early phases of β cell necrosis in diabetes, and may account for M ϕ being among the first leukocytic cells observed infiltrating islets containing necrotic β cells (16).

Although insulin may be a physiologic M ϕ chemoattractant, the consequence of M ϕ migration to the islets was a reduction in insulin secretion. Because insulin has been reported to depress, and glucagon and somatostatin to stimulate M ϕ phagocytosis (17), the IL-1-induced decline in amount of insulin secreted may permit activation of M ϕ cytotoxic activity. In a previous analysis of M ϕ -islet interaction *in vitro*, we observed that the inhibitory effect on glucose-stimulated insulin secretion preceded β cell lysis, the latter phenomenon requiring contact between the M ϕ and islet cells, whereas inhibition of secretory responsiveness occurred before physical invasion by M ϕ of the islet monolayers (8).

A major distinction between the published studies describing the effects of human IL-1 on free-floating rat islets in culture (10, 11) versus the present studies using mouse IL-1 and islet cells in monolayer is that cytolysis was not produced in the mouse monolayers. Nevertheless, the dearth of granules in mouse β cells treated for 4 d with 25 U/ml rIL-1, coupled with the reduced intracellular insulin levels, clearly demonstrated a potent monokine effect on β cell metabolism. This lack of a cytolytic effect of IL-1 in mouse β cells probably reflects differences in the culture systems (rat islets were maintained intact and free-floating; mouse islets were allowed to attach to substratum and form monolayers) rather than in species source of IL-1 or of β cells. The IL-1-induced degranulation was limited to β cells; non- β cell types in the monolayer remained heavily granulated. However, the fact that insulin/proinsulin-specific radioactivity actually increased indicated that IL-1 was not specifically inhibiting insulin biosynthesis, but rather was affecting insulin processing or granule membrane formation.

These results indicate the utility of the mouse islet cell monolayer culture system for the further analysis of islet cell interactions with macrophages and monokines.

Summary

This study has used *in vitro* techniques to investigate the potential interactions between mouse pancreatic islet cells and syngeneic macrophages (M ϕ). Islets strongly stimulated M ϕ migration from agarose microdroplets; insulin was the only one of four islet cell hormones tested that was effective individually. Chronic exposure of islet monolayers to recombinant mouse IL-1, an M ϕ secretory product, was not cytolytic, but inhibited insulin secretion, reduced intracellular insulin content, and produced β cell-specific degranulation. These effects were unique to IL-1; another monokine, tumor necrosis factor, as well as the lymphokine IL-2, and lymphotoxin were all without effect on insulin secretion or monolayer viability at the concentrations tested. The potential pathological

consequences of the chemoattractive action of insulin on $M\phi$, and the inhibitory effect of IL-1 on insulin secretion, are discussed.

I thank Connie Rowley, Greg Christianson, and Tracy Prosser for technical assistance.

Received for publication 8 June 1987 and in revised form 10 July 1987.

References

1. Eisenbarth, G. S. 1986. Type 1 diabetes mellitus: a chronic autoimmune disease. *N. Engl. J. Med.* 314:1360.
2. Koevary, S. B., D. E. Williams, R. M. Williams, and W. L. Chick. 1985. Passive transfer of diabetes from BB/W to Wistar-Furth rats. *J. Clin. Invest.* 75:1904.
3. Wicker, L. S., B. J. Miller, and Y. Mullen. 1986. Transfer of autoimmune diabetes mellitus with splenocytes from Nonobese Diabetic (NOD) mice. *Diabetes.* 35:855.
4. Filkins, J. P., and B. J. Buchanan. 1977. Depression of RES function and altered glucoregulation. *J. Reticuloendothel. Soc.* 21:391.
5. Schwartz, R. H., A. R. Bianco, B. S. Handwerker, and C. R. Kahn. 1975. Demonstration that monocytes rather than lymphocytes are the insulin-binding cells in preparations of human peripheral blood mononuclear leukocytes: implications for studies of insulin-resistant states in man. *Proc. Natl. Acad. Sci. USA.* 72:474.
6. Powers, A. C., S. S. Solomon, and W. C. Duckworth. 1980. Insulin degradation by mononuclear cells. *Diabetes.* 29:27.
7. Oschilewski, U., U. Kiesel, and H. Kolb. 1985. Administration of silica prevents diabetes in BB rats. *Diabetes.* 34:197.
8. Schwizer, R. W., E. H. Leiter, and R. Evans. 1984. Macrophage-mediated cytotoxicity against cultured pancreatic islet cells. *Transplantation (Baltimore).* 37:539.
9. Mandrup-Poulsen, T., K. Bendtzen, J. Nerup, J. Egeberg, and J. H. Nielsen. 1986. Mechanism of pancreatic islet cell destruction. Dose dependent cytotoxic effect of soluble blood mononuclear cell mediators on isolated islets of Langerhans. *Allergy (Copenh.).* 41:250.
10. Mandrup-Poulsen, T., K. Bendtzen, J. Nerup, C. A. Dinarello, M. Svenson, and J. H. Nielsen. 1986. Affinity purified human interleukin I is cytotoxic to isolated islets of Langerhans. *Diabetologia.* 29:63.
11. Harrington, J. T., and P. Stastny. 1973. Macrophage migration from an agarose droplet. Development of a micromethod for assay of delayed hypersensitivity. *J. Immunol.* 110:752.
12. Gotoh, M., T. Maki, T. Kiyozumi, S. Satomi, and A. P. Monaco. 1985. An improved method for isolation of mouse pancreatic islets. *Transplantation (Baltimore).* 40:437.
13. Leiter, E. H., D. L. Coleman, and C. Waymouth. 1974. Cell culture of the endocrine pancreas of the mouse in chemically defined medium. *In Vitro (Rockville).* 9:421.
14. Leiter, E. H., J. W. Fewell, and E. L. Kuff. 1986. Glucose induces intracisternal type A retroviral gene transcription and translation in pancreatic beta cells. *J. Exp. Med.* 163:87.
15. Vlassara, H., M. Brownlee, and A. Cerami. 1985. High affinity-receptor mediated uptake and degradation of glucose-modified proteins: a potential mechanism for the removal of senescent macromolecules. *Proc. Natl. Acad. Sci. USA.* 82:5588.
16. Leiter, E. H. 1985. Type C retrovirus production by pancreatic beta cells. Association with accelerated pathogenesis in C3H-db/db ("diabetes") mice. *Am. J. Pathol.* 119:22.
17. Cornell, R. P., and C. C. McClellan. 1982. Modulation of hepatic reticuloendothelial system phagocytosis by pancreatic hormones. *J. Reticuloendothel. Soc.* 32:397-407.