Synergistic Effects of Adjuvants, Endotoxin, and Fasting on Induction of Diabetes With Multiple Low Doses of Streptozocin in Rats

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Three weekly intraperitoneal injections of complete Freund's adjuvant (CFA) and, 1 day later, low-dose streptozocin (STZ; 25 mg/kg i.p.) have been reported to cause immune destruction of β-cells and a gradual onset of diabetes mellitus. In this study, male Lewis rats were injected intraperitoneally with CFA and 1 day later with low-dose STZ; these were repeated at weekly intervals for 3 wk. The incidence of diabetes mellitus (nonfasted plasma glucose >200 mg/dl) in wk 1, 2, 3, and 4 was 50, 80, 93, and 100%, respectively. Rats receiving either CFA or STZ only did not develop diabetes. Injections of either the components of CFA (incomplete Freund's adjuvant and *Mycobacterium butyricum*), another granuloma-inducing organism (*Listeria monocytogenes*), or endotoxin before STZ induced diabetes, but the onset was slower and the diabetes was less severe than with CFA and STZ. Because intraperitoneal CFA injections caused peritoneal irritation, acute weight loss, and hypoglycemia on the day after injection, we examined whether fasting alone potentiated low-dose STZ. Fasting for 24 h before and 24 h after low-dose STZ caused diabetes that was similar in rapidity of onset and severity to that induced with CFA and STZ. Administration of CFA subcutaneously before STZ did not cause hypoglycemia or weight loss but did cause diabetes. Thus, the fasting effect of intraperitoneal CFA was not responsible for the induction of diabetes with CFA and STZ. These data indicate that immunologic adjuvants, endotoxin, and fasting all potentiate the diabetogenic action of low-dose STZ. The 50% incidence of diabetes in rats within 48 h of a single injection of CFA and low-dose STZ suggests that the immune system does not mediate diabetes in this model. Furthermore, transplants of islet isografts were not rejected by eight rats made diabetic with CFA and low-dose STZ. *Diabetes* 37:112–18, 1988

Streptozocin (STZ), a methylnitrosourea with a 2-substituted glucose, is a pancreatic β-cell toxin that has been widely used to induce experimental diabetes mellitus (1,2). Its mechanism of action has not been fully elucidated. In single high doses, STZ is believed to act as an alkylating agent and damage the DNA of β-cells (3–5). Studies examining the effects of multiple injections of low (i.e., subdiabetogenic) doses of STZ in certain strains of mice suggest that the thymus-dependent, cellular immune systems may be involved in at least part of STZ's diabetogenic effect (6–9).

Ziegler et al. (10) recently reported the induction of diabetes in rats by the combined use of multiple injections of low doses of STZ and complete Freund's adjuvant (CFA). In their study, Wistar rats were injected intraperitoneally with 0.5 ml of CFA emulsified with saline followed 1 day later by an injection of 25 mg/kg STZ. This regimen was repeated twice at weekly intervals. They observed a gradual onset of severe diabetes mellitus, marked depletion of pancreatic insulin content, and the presence of cytotoxic antibodies to islet cells. The authors speculated that *1*) STZ damaged the pancreatic β-cells sufficiently to be immunogenic but not enough to cause β-cell necrosis; *2*) CFA, a powerful immunological adjuvant, amplified the humoral response to these β-cell neoantigens; and *3*) the cytotoxic antibodies destroyed the STZ-modified β-cells, precipitating the onset of diabetes.

In this study we examine this new model and offer evidence that autoimmunity is not involved in the pathogenesis of diabetes mellitus in this model.

MATERIALS AND METHODS

ANIMALS

Male Lewis rats (Harlan-Sprague-Dawley, Indianapolis, IN) weighing 200–300 g were individually caged and given

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water and rat chow (Purina, Richmond, IN) ad libitum except where indicated otherwise. Rats were divided into groups to receive the treatment regimens.

STUDIES

Intraperitoneal adjuvants and STZ. Group 1: 0.5 ml of CFA (Cappel, West Chester, PA) was emulsified in 0.5 ml of saline. Emulsions were injected intraperitoneally on days 1, 8, 15, and 22; STZ (lot #2408A, Upjohn, Kalamazoo, MI) was dissolved in citrate buffer and immediately injected (25 mg/kg i.p.) on days 2, 9, 16, and 23. Group 2: same as in group 1 except that no CFA was given. Group 3: same as in group 1 except that no STZ was given. Group 4: same as in group 1 except that incomplete Freund's adjuvant (ICFA) was substituted for CFA. Group 5: same as in group 1 except that *Mycobacterium butyricum* (1 mg in 1 ml of saline; Difco, Detroit, MI) was substituted for CFA. Group 6: same as in group 1 except that heat-killed *Listeria monocytogenes* (10^7 organisms in 1 ml saline) was substituted for CFA. Group 7: same as in group 6 except that 10^8 *L. monocytogenes* organisms were used.

Endotoxin and STZ. Group 8: endotoxin (LPS from *Salmonella typhi,* 1 μg in 1 ml saline i.p.; RIBI, Immunochem, Hamilton, MT) was injected 1 h before STZ (25 mg/kg i.p.) on days 2, 9, 16, and 23. Group 9: same as group 8 except that 10 μg LPS was used. Group 10: LPS only (1 μg in 1 ml saline) was given on days 2, 9, 16, and 23.

Fasting and STZ. Group 11: rats were fasted on days 1, 2, 8, 9, 15, 16, 22, and 23; STZ (25 mg/kg i.p.) was injected on days 2, 9, 16, and 23. Group 12: rats were fasted as in group 11 and administered CFA as in group 1; no STZ was given.

Subcutaneous CFA and STZ. Group 13: CFA emulsions, prepared as in group 1, were divided into two parts and injected subcutaneously (0.5 ml/flank) on days 1, 8, 15, and 22. Injection site was varied slightly each week. The STZ (25 mg/kg i.p.), prepared as in group 1, was injected on days 2, 9, 16, and 23.

PLASMA GLUCOSE DETERMINATION

Plasma glucose and animal weights were monitored at least 2–3 times/wk. Blood samples were collected from the tail veins of nonfasted rats with heparinized capillary tubes. On days that injections were given, blood was drawn before the injections. Plasma glucose concentrations were analyzed by the glucose-oxidase method in a Beckman glucose analyzer (Fullerton, CA). Rats were considered to be diabetic when nonfasting plasma glucose concentrations were >200 mg/dl.

HISTOLOGICAL STUDIES

After 12 wk, the rats were killed, and pancreatic tissue was fixed in Bouin's solution, processed for light microscopy, and stained with either hematoxylin and eosin or aldehyde fuchsin. Pancreatic tissue was also obtained from rats dying during the study.

Time-course studies to evaluate histologically the progression of β-cell necrosis were performed as follows. Individual rats, treated as in groups 1–3, were killed on days 8, 15, 22, and 29. Two group 1 rats per day were killed on days 3, 4, and 5.

ISLET ISOLATION AND TRANSPLANT

Islets were isolated from male Lewis rats by the collagenase technique (11,12) and separated on a discontinuous Ficoll gradient (13). Islets were removed from the gradient, washed, resuspended in tissue culture medium, and picked with a Pasteur pipette and dissecting microscope. Portal vein isografts (1200–1500 islets/rat) were performed immediately as previously described on eight rats from group 1 to determine whether the antibodies against β-cell antigens or some other immune mechanism would initiate isograft rejection (14).

RESULTS

Effects of intraperitoneal CFA and low-dose STZ. As shown in Table 1, rats receiving both STZ and CFA (group 1) rapidly developed diabetes mellitus. Seven of 13 rats in group 1 were diabetic on day 4 of the treatment protocol (i.e., within 48 h of the 1st injection of STZ); 80 and 93% were diabetic by the end of the 2nd and 3rd wk, respectively. Maximum plasma glucose levels, a measure of the severity of diabetes, during the 12th wk are shown in Fig. 1. The range for group 1 rats was 393–587 mg/dl.

Rats receiving STZ alone (group 2) or CFA alone (group 3) did not develop diabetes (Table 1), except for one rat in group 2 that developed mild hyperglycemia (239–259 mg/dl) immediately after the 4th STZ injection but reverted to normoglycemia during the 5th wk.

Histological studies of the pancreases of group 1 rats killed after 12 wk revealed small islets containing a few partially degranulated, often hypertrophied, β-cells surrounded by a mantle of α-cells or a complete absence of β-cells (Fig. 2). No inflammatory infiltrates involving islets (i.e., insulitis) were

TABLE 1
Induction of diabetes with adjuvants and 25 mg/kg STZ

Group no.	Treatment protocol*		No. rats with nonfasting plasma glucose >200 mg/dl					
	Adjuvant	STZ	wk 1	wk 2	wk 3	wk 4	wk 5	wk12
1	Complete Freund's adjuvant	+	8/16	12/15	13/14	13/13	12/12	10/10
2		+	0/9	0/8	0/7	1/6	1/5	0/5
3	Complete Freund's adjuvant		0/6	0/5	0/4	0/3	0/2	0/2
4	Incomplete Freund's adjuvant	+	1/12	4/11	8/10	9/9	8/8	8/8
5	*Myobacterium butyricum* (1 mg)	+	2/6	4/6	5/6	6/6	6/6	6/6
6	*Listeria monocytogenes* (10^7 organisms)	+	1/3	1/3	3/3	3/3	2/3	1/3
7	*Listeria monocytogenes* (10^8 organisms)	+	0/3	1/3	3/3	3/3	3/3	3/3

Injections made at beginning of wk 1, 2, 3, and 4. Numbers for groups 1–4 include rats used in time-course study. A randomly selected rat from each group was killed at end of wk 1, 2, 3, and 4.

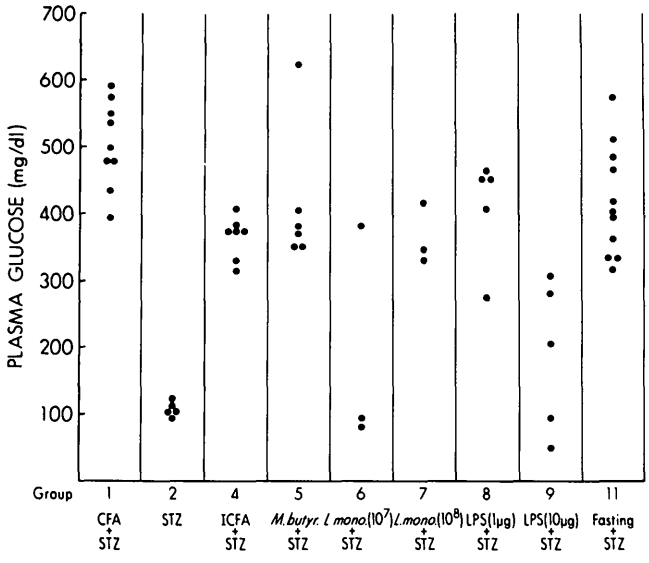

FIG. 1. Maximum plasma glucose levels during wk 12 for rats in experimental groups. Groups 3, 10, 13, 14, and 15 excluded because none of these rats showed transient or permanent hyperglycemia. CFA, complete Freund's adjuvant; ICFA, incomplete Freund's adjuvant.

seen. Islets from rats receiving either STZ only (group 2) or CFA only (group 3) appeared normal.

Histological studies of the pancreases of rats receiving CFA and STZ (group 1), STZ alone (group 2), and CFA alone (group 3) were accomplished at 3, 4, 5, 8, 15, and 22 days. During the 1st wk (i.e., after a single injection of CFA and STZ), β-cell degranulation was the most prominent finding. Widespread β-cell necrosis was observed in only one rat in the group receiving CFA and STZ. This rat was killed on day 3 (Fig. 3). Neutrophilic or lymphocytic infiltrates were not present within islets; macrophages were found in the central portions of some islets with necrotic centers.

Islets from group 1 rats killed on days 8, 15, 22, and 29 showed either degeneration (Fig. 4) or absence of β-cells and were similar to those seen after 12 wk. Islets from rats receiving STZ alone (group 2) were essentially unremarkable before wk 4 but showed a moderate degree of β-cell degranulation and hypertrophy during wk 4 and 5 (Fig. 5). Islets from rats treated with CFA alone (group 3) were normal.

In the animals receiving CFA (groups 1 and 3), there was progressive replacement of the peripheral lobules of the pancreas by fat necrosis and granuloma. During the first few weeks, inflammatory cells accumulated over the surface of the pancreas, between lobules or acini, and in the peripancreatic adipose. Some of the peripheral pancreatic lobules were totally destroyed after the 3rd wk; however, islets and ducts were preferentially spared where replacement was partial. In all rats, large areas of the pancreas remained normal.

Effects of components of CFA and low-dose STZ. Because of the surprising finding that 50% of the rats receiving CFA and STZ became diabetic within 48 h of the first STZ injection, we examined the effect of the components of CFA in conjunction with STZ. As shown in Table 1, either of the components of CFA [ICFA (group 4) or heat-killed *M. butyricum* (group 5)] and STZ also produced diabetes. However, the onset of hyperglycemia was slower than in group 1. The incidence of diabetes in group 4 rats after 1, 2, and 3 wk was 8, 36, and 80%, respectively, whereas in group 5 it was 33, 67, and 83%.

The magnitude of the hyperglycemia was less severe than that induced in group 1. Plasma glucose ranges during the 12th wk were 315–409 mg/dl for group 4 and 345–637 mg/dl (5 of 6 rats at 345–407) for group 5 compared to 393–587 mg/dl for group 1 (Fig. 1).

Histologically, the islets of rats receiving either ICFA plus STZ or *M. butyricum* plus STZ were indistinguishable from those of rats that had received CFA and STZ. The pancreases of rats receiving ICFA showed extensive interstitial infiltrates of foamy macrophages, granulation tissue around oil vesicles, and preservation of the acinar tissue. The pancreases of rats receiving *M. butyricum* showed a moderate degree of replacement of peripheral acinar tissue by granulomatous inflammation.

Effects of *L. monocytogenes* and low-dose STZ. Having found that both *M. butyricum* and low-dose STZ caused

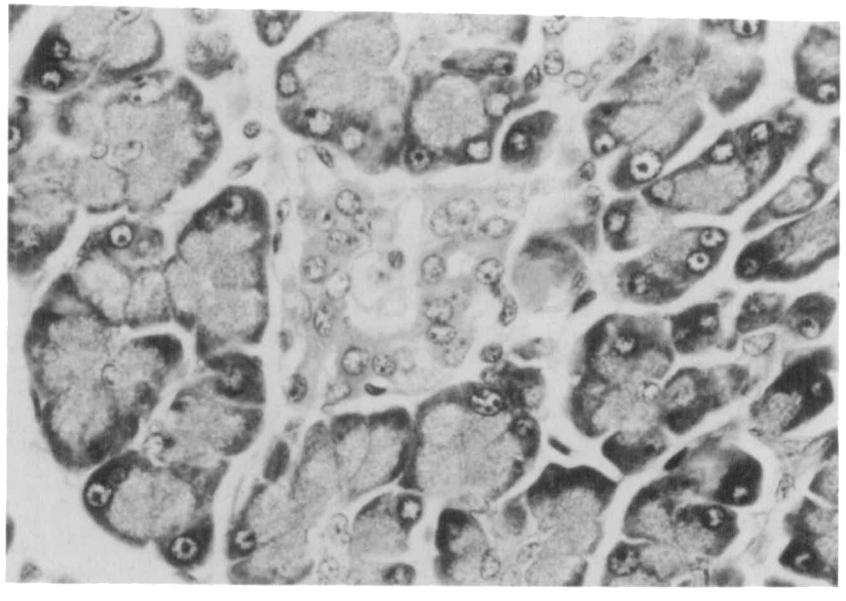

FIG. 2. Section of pancreas from rat treated with complete Freund's adjuvant and low-dose STZ showing small islet consisting of thin rim of α-cells surrounding cellular debris. Stained with hematoxylin and eosin. ×500.

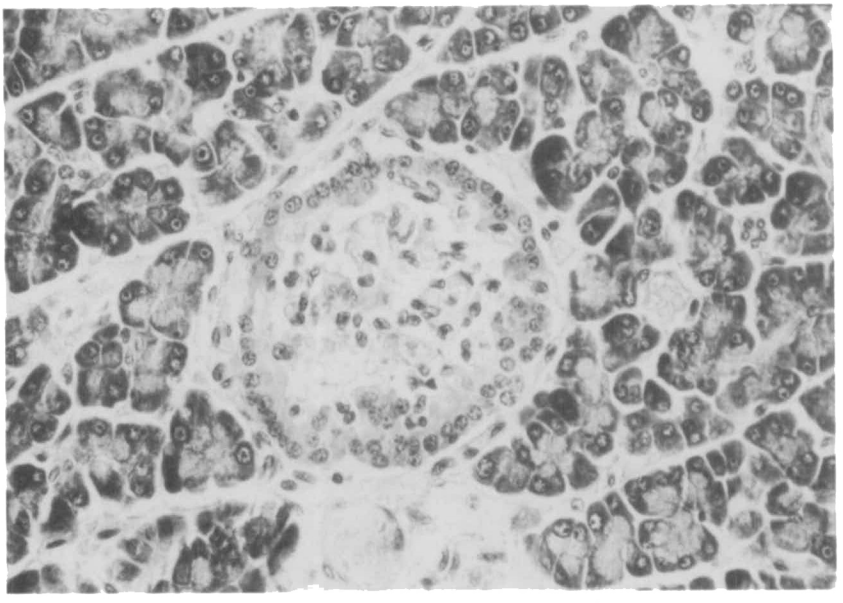

FIG. 3. Section from group 1 rat showing active β-cell necrosis and infiltration by macrophages in center of islet. Peripheral mantle of α-cells is unaffected. Rat was killed on day 3 of time-course study. Stained with hematoxylin and eosin. ×400.

diabetes, we examined whether another granuloma-inducing organism, *L. monocytogenes*, would also enhance the diabetogenicity of low-dose STZ. Table 1 (groups 6 and 7) shows that two different doses of heat-killed *L. monocytogenes* (10^7 and 10^8 organisms) and low-dose STZ also produced diabetes, but neither was as diabetogenic as CFA plus STZ. The incidence of diabetes at the end of 1, 2, and 3 wk for rats receiving 10^7 organisms (group 6) was 33, 33, and 100%, and for rats receiving 10^8 organisms (group 7) it was 0, 33, and 100%. Although both groups developed a 100% incidence of diabetes, incidence was transient in two of three rats receiving 10^7 organisms.

The severity of diabetes, as judged by level of glycemia during the 12th wk, was not as great as that induced by CFA and STZ (group 1). The range of plasma glucose levels for rats receiving *L. monocytogenes* and low-dose STZ was 337–422 mg/dl (Fig. 1).

Histologically, islets from rats that developed permanent hyperglycemia were similar to those made diabetic with CFA and STZ. Rats with transient diabetes had small islets but more abundant β-granules. Neither dose of *L. monocytogenes* caused significant acinar loss.

Effects of endotoxin and low-dose STZ. Because all of the above adjuvants activate peritoneal macrophages, we examined whether intraperitoneal administration of endotoxin (LPS), a potent stimulator for the release of the monokine interleukin 1, would potentiate the diabetogenic effect of low-dose STZ. LPS, given 1 h before STZ (25 mg/kg) at doses of 1 or 10 μg i.p., also induced diabetes (Table 2). The lower dose (group 8) was more diabetogenic than the higher dose (group 9). The incidence of diabetes at the end of 1, 2, and 3 wk was 0, 20, and 100%, respectively, in rats receiving 1 μg LPS plus STZ and 0, 40, and 60% in rats receiving 10 μg LPS plus STZ. All rats in both groups were diabetic by the end of the 4th wk, but the hyperglycemia was transient in two of five rats receiving the higher dose of LPS. Onset

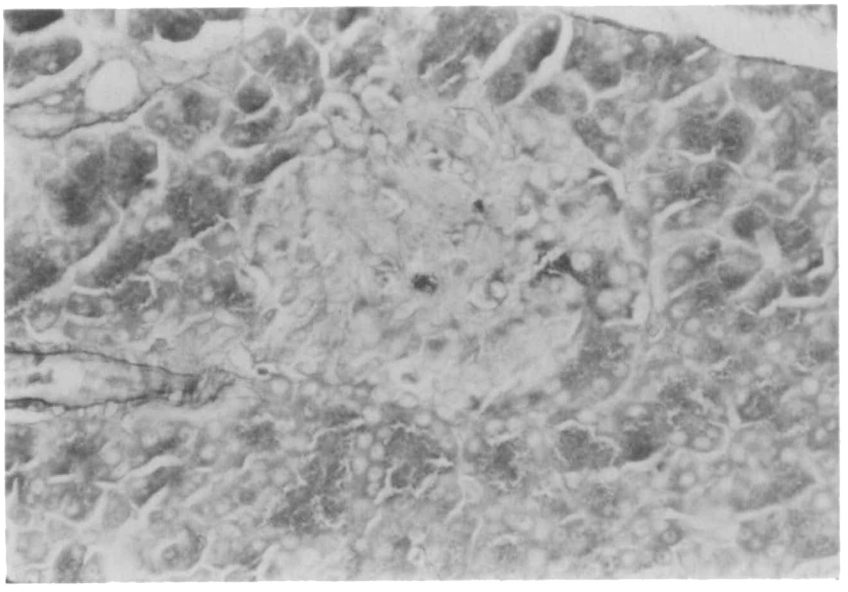

FIG. 4. Section from group 1 rat showing marked β-cell degranulation. Many β-cells are hyperplastic and without evidence of nuclear necrosis. Several well-granulated β-cells are present. Rat was killed during 2nd wk of time-course study. Stained with hematoxylin and eosin. ×400.

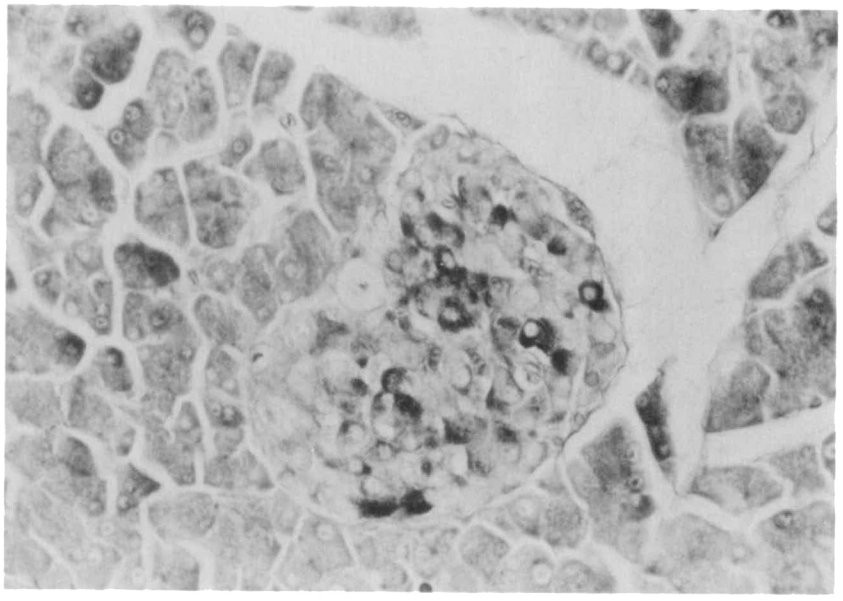

FIG. 5. Section of pancreas from rat treated with low-dose STZ only. Note that many β-cells are partially degranulated. Islet is otherwise normal. Stained with hematoxylin and eosin. ×400.

of diabetes in rats receiving LPS and STZ was slower than in rats receiving CFA and STZ (group 1). LPS (1 μg) alone did not cause diabetes (group 10).

The severity of diabetes, based on maximum level of plasma glycemia during wk 12 (Fig. 1), was greater in rats receiving 1 μg LPS (271–472 mg/dl) than in rats receiving 10 μg LPS (47–311 mg/dl). Hyperglycemia induced with LPS and STZ was not as severe as that induced with CFA and STZ (group 1).

Islet histology was similar to that of rats receiving CFA and STZ. LPS did not cause acinar destruction.

Effect of fasting and low-dose STZ. Because of the marked decrease in plasma glucose on the day after each injection of CFA in the rats receiving both CFA and STZ (Fig. 6), we were concerned that the peritoneal irritation caused by intraperitoneal injections of CFA might be causing the rats to fast. Therefore, we examined whether fasting alone might effect the diabetogenicity of low-dose STZ. Rats were fasted for 24 h, injected with 25 mg/kg i.p. STZ, then fasted for an additional 24 h. Table 3 shows that fasting for 2 days markedly enhances the diabetogenicity of low-dose STZ in Lewis rats. Nonfasted rats did not develop diabetes after four weekly STZ injections (group 2, Table 1), but fasted rats rapidly developed diabetes after STZ injections (group 11). The incidence of diabetes after 1, 2, and 3 wk was 73, 82, and 100%. Fasting and CFA were not diabetogenic when combined in the absence of STZ (group 12).

The diabetes induced with fasting and 25 mg/kg STZ was severe (Fig. 1). Hyperglycemia range was 318–575 mg/dl.

The histologic appearance of islets from rats with diabetes induced by fasting and low-dose STZ was similar to that seen in rats given CFA plus STZ. Fasting did not alter the acinar tissue histologically.

Because of the surprising finding that the fasting and low-dose STZ regimen was as diabetogenic as the CFA and low-dose STZ regimen, we compared acute weight loss to determine whether this fasting effect could account for the development of diabetes in rats receiving CFA and STZ. Fasted rats lost an average of 13.2% of their body weight during the initial 2-day fasts, whereas rats receiving CFA lost an average of 6.8% of their body weight. Clearly, the rats experienced significant weight loss after intraperitoneal administration of CFA, but this was not as severe as in fasted rats. That LPS administered 1 h before STZ injections potentiated the diabetogenic effect of low-dose STZ without causing hypoglycemia or weight loss during the 1-h interval suggests that some mechanism in addition to fasting was involved.

Effects of subcutaneous CFA and low-dose STZ. To better evaluate whether the potentiation of low-dose STZ by intraperitoneal CFA was the result of fasting secondary to peritoneal irritation, we repeated our first experiment with subcutaneous CFA and low-dose STZ. This regimen was also highly diabetogenic. Five (71.4%) of seven rats became diabetic during the 1st wk; 100% were diabetic by the end of the 2nd wk. Figure 6 shows that the severity of hyperglycemia

TABLE 2
Induction of diabetes with LPS and 25 mg/kg STZ

Group no.	Treatment protocol		No. rats with nonfasting plasma glucose >200 mg/dl					
	LPS (μg)	STZ	wk 1	wk 2	wk 3	wk 4	wk 5	wk 12
8	1	+	0/6	1/5*	5/5	5/5	5/5	5/5
9	10	+	0/6	2/5*	3/5	5/5	4/5	3/5
10	1		0/3	0/3	0/3	0/3	0/3	0/3

Injections made at beginning of wk 1, 2, 3, and 4.
*One rat died during 2nd wk of experiment.

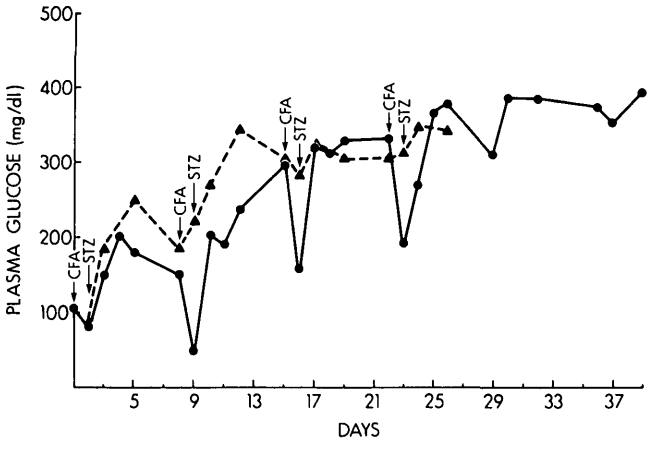

FIG. 6. Mean daily nonfasting plasma glucose levels as function of time for rats receiving weekly injections of intraperitoneal (*solid line*) or subcutaneous (*broken line*) complete Freund's adjuvant (CFA; days 1, 8, 15, and 22) and low-dose STZ (days 2, 9, 16, and 23). Note precipitous decrease in glucose levels immediately after intraperitoneal CFA injections; no hypoglycemia occurred when injections were given subcutaneously. Standard errors of the mean on days 2, 9, 16, and 23 were 5.8, 11.2, 26.6, and 44.0 for rats receiving intraperitoneal CFA and 3.8, 31.9, 21.8, and 14.4 for rats receiving subcutaneous CFA. *n* = 5–13.

was similar to that of the rats in group 1 and that subcutaneous administration did not result in transient hypoglycemia after each CFA injection. The magnitude of weight loss during the first weekly treatment regimen was 3.3%, less than half of that in group 1 rats. Together, these findings clearly demonstrate that the fasting effect, although a potentiating factor, cannot account for the enhanced diabetogenicity of STZ in the rats treated with intraperitoneal CFA and low-dose STZ and that granulomatous destruction of a portion of the pancreas is not a necessary factor in this model. These findings suggest that some systemic mediator, released during granulomatous inflammation, may be ultimately responsible for the potentiation of the diabetogenic effect of low-dose STZ in rats.

Transplantation studies. Finally, we performed transplantation studies to pursue further whether an immune mechanism is responsible for the onset of diabetes in rats treated with CFA and STZ. Lewis rat islets (1200–1500) were isolated and injected into the portal veins of rats made diabetic with CFA and low-dose STZ (group 1). None of the recipient rats rejected the islet isografts. Six of eight diabetic rats transplanted with Lewis rat islets 2–18 wk after the last STZ injection remained normoglycemic for >60 days after transplantation. Two rats died of pneumonia 33 and 53 days after transplantation, but both were normoglycemic at the time of their deaths. Histological evaluation of the livers revealed well-granulated islets without lymphocytic infiltration.

DISCUSSION

These experiments underline the complexity of the diabetic state induced in rats by low-dose STZ. Three distinct types of stimuli promoted the development of diabetes: *1*) those that produce severe inflammation with activation of T-lymphocytes and macrophages (CFA, *M. butyricum,* and *L. monocytogenes*; 15), *2*) those that primarily activate macrophages and B-lymphocytes (ICFA and LPS; 15), and *3*) those that are nonimmunologic (fasting).

Several lines of evidence oppose an autoimmune mechanism, as proposed by Ziegler et al. (10), for diabetes induced with CFA and low-dose STZ. First, the onset of diabetes in our study was too rapid to have been mediated by a specific immune response. Although Ziegler et al. were able to demonstrate the presence of islet cell antibodies in the serum of rats on experimental day 26, most of the rats in our study were diabetic within 48 h of the first injection of CFA and STZ. Islet cell antibodies were probably produced as a result of islet destruction rather than having initiated islet destruction. Second, our transplantation studies suggest these autoantibodies must be very weak. Islet portal vein isografts performed on rats made diabetic with CFA and low-dose STZ did not reject within 60 days of transplantation. Third, the absence of inflammatory infiltrates in the pancreatic islets or the islet isografts also suggests that autoimmunity is not involved.

This study also demonstrates that diabetes induced with CFA and low-dose STZ is not strain specific. Ziegler et al. (10) used Wistar rats in their investigation; we used Lewis rats. Because induction of diabetes was not strain dependent, it seems unlikely that the mechanism is strain specific, but this possibility cannot be excluded.

CFA, ICFA, *M. butyricum,* and *L. monocytogenes* activate peritoneal macrophages and stimulate them to produce interleukin 1, a monokine that has recently been shown to be, at least temporarily, toxic to β-cells (16,17). LPS activates rat macrophages to secrete interleukin 1 within 1 h of intraperitoneal injection (18). We examined whether intraperitoneal administration of LPS had a synergistic effect with low-dose STZ. LPS injected intraperitoneally 1 h before each of four weekly STZ injections also caused severe diabetes, suggesting that interleukin 1 may act synergistically with STZ.

Because intraperitoneal CFA caused peritoneal irritation and hypoglycemia on the day after injection, we examined whether fasting potentiated the diabetogenic action of STZ. Fasting before and after administration (total 48 h) of 25 mg/kg STZ resulted in rapid, severe diabetes. Although fasting

TABLE 3
Effect of fasting on induction of diabetes with low-dose STZ

Group no.	Treatment protocol			No. rats with nonfasting plasma glucose >200 mg/dl					
	48-h fast	STZ	Complete Freund's adjuvant	wk 1	wk 2	wk 3	wk 4	wk 5	wk 12
11	+	+		8/11	9/11	11/11	11/11	11/11	11/11
12	+		+	0/3	0/3	0/3	0/3	0/3	0/3

Injections made at beginning of wk 1, 2, 3, and 4.

for ≥24 h is known to decrease glucose-induced insulin secretion and fasting for >48 h reduces the total extractable insulin content of the pancreas in rats (19,20), we are not familar with any prior reports that fasting potentiates the action of high- or low-dose STZ. Our data suggest that adjuvant-enhanced diabetogenicity of low-dose STZ is not due to a fasting effect because subcutaneous CFA and STZ caused diabetes without transient hypoglycemia or significant weight loss.

Finally, many of the adjuvant treatments used in this series of experiments cause peritoneal irritation and hyperemia. Although these factors may improve the access of intraperitoneal STZ to the bloodstream, they cannot increase the effectiveness of the drug beyond its intravenous effect. Single intravenous doses of 25 mg/kg are not diabetogenic in rats (data not shown; 21), and many of the rats treated once with an adjuvant and STZ were severely diabetic. Furthermore, rats treated with subcutaneous CFA and STZ also developed severe diabetes. Therefore, enhanced absorption of STZ cannot account for increased susceptibility to low-dose STZ.

In essence, the autoimmune pathogenesis of diabetes induced with CFA and low-dose STZ may require reinterpretation. How inflammatory stimuli potentiate the action of low-dose STZ is not known, but it could be the result of a combination of interleukin 1, fasting, and perhaps other unknown mediators. The mechanism by which fasting enhances the diabetogenicity of low-dose STZ is unknown.

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