

Immunologic Studies on the Induction of Diabetes in Experimental Animals

Cellular Basis for the Induction of Diabetes by Streptozotocin

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SUMMARY

Repeated low doses of streptozotocin (STZ, 40 mg/kg body wt) gradually induce hyperglycemia in mice after a latent period of 5–7 days. The ability of STZ to induce hyperglycemia varies in different mouse strains. Repeated low doses of STZ fail to induce diabetes in T-cell-deficient mice, suggesting a crucial rôle of T-cells in the induction of diabetes by this procedure. Transfer of spleen cells from mice rendered diabetic by repeated low doses of STZ failed to induce hyperglycemia in the recipients. However, pretreating the recipients with a single low dose of STZ permitted efficient transfer of the diabetic state, suggesting that the immunologic reaction resulting in diabetes was actually specific for an STZ-modified beta cell. DIABETES 33:771–777, August 1984.

Several lines of evidence suggest that autoimmune mechanisms may be involved in the induction of type I (juvenile-onset) diabetes. Type I diabetes is usually characterized by destruction of pancreatic beta cells. Although the precise mechanism that leads to this destruction is not known, immune factors have been suggested on the basis of the following observations: (1) the infiltration of the islets by lymphocytes,^{1,2} (2) the occurrence of autoantibodies to islet cells in type I patients,^{3,4} (3) the detection of other autoantibodies (e.g., anti-DNA and anti-thyroid) in sera from diabetic patients,⁵ and (4) the finding that a cell-mediated immune mechanism may contribute to the destruction of pancreatic islets.⁶

Diabetes can be induced by giving streptozotocin (STZ) to experimental animals.⁷ STZ has a highly selective toxicity for the insulin-secreting beta cells and, thereby, induces a diabetic syndrome in mice and other animals that is characterized by severe and permanent hyperglycemia and de-

struction of the pancreatic beta cells. Mice, given a single injection of 200 mg STZ/kg, develop hyperglycemia within 24 h with complete destruction of the beta cells. This is most likely the direct consequence of the toxicity of STZ to beta cells. In contrast, mice given five daily injections of 40 mg STZ/kg develop hyperglycemia only after a latent period of 5 days. After the latent period, there is a progressive increase in blood glucose concentration and an infiltration of the islets by lymphocytes.⁸ It has been suggested by several investigators that the induction of diabetes by multiple injections of low doses of STZ may be due to an immunologic process on the basis of the following observations: (1) during the latent period, lymphocyte infiltration in the pancreatic islets takes place; (2) diabetes cannot be induced by this procedure in athymic nude mice;⁹ and (3) diabetes can be transferred to normal mice with spleen cells from mice rendered diabetic by this procedure.¹⁰

In the present study, we provide further evidence that an immunologic process plays an important role in the induction of diabetes in mice by multiple injection of low doses of STZ. It can be seen that lymphocytes were responsible for the transfer of diabetes from mice rendered diabetic with repeated doses of STZ to recipients that are treated with a subdiabetogenic dose of STZ. These data support the contention of Sandler and Andersson's paper,¹¹ in which intrasplenically implanted islets in mice rendered diabetic by repeated low doses of STZ develop insulinitis only when small doses of STZ are given at the time of implantation.

MATERIALS AND METHODS

Animals and reagents. CD-1 mice were purchased from Charles River Breeding Lab Inc. (Wilmington, Massachusetts), and LAF₁, C57BL/6J, AKR/J, and BALB/c mice were obtained from Jackson Laboratories (Bar Harbor, Maine). BALB/c mice were also obtained from colonies maintained by Dr. Bosma and by Dr. Shin (Albert Einstein Medical School, Bronx, New York). STZ was obtained from Boehringer-Mannheim (Indianapolis, Indiana). Anti-thy 1.2 was obtained from New England Nuclear (Boston, Massachusetts).

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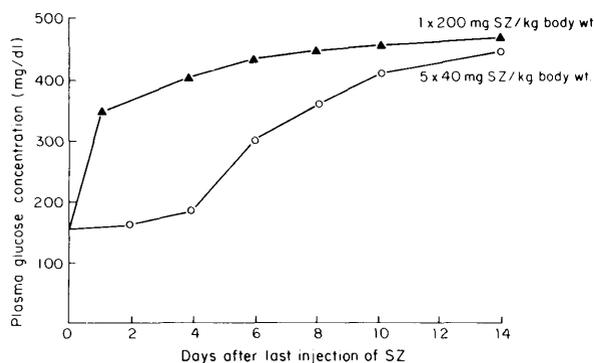


FIGURE 1. Kinetics of induction of hyperglycemia in male C57BL/6J adult mice by STZ treatment. Mice were given either a single injection of 200 mg STZ/kg body wt (▲) or 5 daily injections of 40 mg STZ/kg body wt (○).

Induction of diabetes. Diabetes was induced in mice by the intraperitoneal (i.p.) injection of 40 mg/kg body wt STZ daily for 5 consecutive days.

Glucose measurement. Blood samples were collected in microcapillary tubes from the retro-orbital venous plexus before and 1, 3, 7, 14, 21, and 28 days after the last injection of STZ. Serum glucose concentration was assayed by a glucose-oxidase method using a Beckman glucose analyzer (Beckman Instruments, Fullerton, California).

Preparation of T-cell-depleted mice. T-cell-depleted mice were prepared by surgical thymectomy followed, in 2 wk, by lethal irradiation (770 rad) and reconstitution with 4×10^7 anti-thy 1.2 antibody plus complement-treated syngeneic bone marrow cells. Control mice were not thymectomized and, after lethal irradiation, were reconstituted with 5×10^7 syngeneic spleen cells.

Transfer of cells from mice rendered diabetic by STZ to normal syngeneic mice. Fifty million spleen cells, obtained from C57BL/6J diabetic mice at various times after STZ treatment, were injected into normal syngeneic mice. The serum glucose concentration of the recipients was assayed before and 1, 2, 5, 10, and 15 days after cell transfer.

Immunization of mice. Mice were immunized by the intravenous (i.v.) injection of sheep erythrocytes (SRBC). Their splenic plaque-forming cell (PFC) response was assayed 7 days after antigen injection.

Cell culture system. Spleens were removed aseptically and were teased in Hanks' balanced salt solution (Gibco, Grand Island, New York) containing 0.35 mg/ml Na_2HCO_3 and 0.02 mg/ml sodium heparin sulfate. The cells were filtered through a thin layer of cotton gauze to remove clumps, washed once, and resuspended in an appropriate amount of RPMI 1640 medium (Microbiological Associates, Bethesda, Maryland) supplemented with 10% fetal calf serum (Microbiological Associates), 0.02 M glutamine, 100 IU of penicillin, 100 μg streptomycin/ml, and 1.5×10^{-4} M 2-mercaptoethanol. In 1 ml of medium, 7.0×10^6 cells were cultured in plastic Petri dishes (Falcon no. 1008, Oxnard, California) with 10^7 SRBC, or 1 mg TNP-PAA, for 4 days. Cultures were incubated on a rocking platform (7 cycles/min), at 37°C, in a 5% $\text{CO}_2/95\%$ air environment for 4 days. The cells were then harvested using a rubber policeman, washed once, and assayed for anti-SRBC or anti-DNP PFC.

Assay of plaque-forming cells (PFC). Anti-SRBC PFC and anti-TNP PFC in the cell cultures were assayed by the Dresser and Gréaves¹² slice modification of the method of Jerne et al.¹³ The target cells used for assay of anti-SRBC PFC and anti-TNP PFC were SRBC and TNP-SRBC, respectively. Data are reported as number of PFC/ 10^6 viable cells. Cell viability was assayed, at the termination of culture, by trypan blue exclusion.

RESULTS

Strain differences in the induction of hyperglycemia by STZ. As shown in Figure 1, male C57BL/6J mice given a single injection of 200 mg STZ/kg body wt developed hyperglycemia within 24 h. In contrast, male C57BL/6J mice given 5 daily injections of 40 mg STZ/kg body wt developed hyperglycemia after a latent period of 4–5 days. After this latent period, there was a progressive increase in serum glucose concentration.

Male CD-1, C57BL/6J, AKR/J, LAF₁, and BALB/c mice from three different colonies (Jackson Laboratories, Dr. Bosma, and Dr. Shin) were treated with a single injection of 200 mg STZ/kg body wt or with 5 daily injections of 40 mg STZ/kg body wt. Neither a single nor multiple injections of STZ induced hyperglycemia in AKR/J or Jackson BALB/c mice. LAF₁ mice became hyperglycemic after a single injection of 200 mg STZ/kg body wt, but did not become hyperglycemic

TABLE 1
Strain differences in the induction of hyperglycemia by STZ*

Strain	Glucose concentration (mg/dl) \pm SD	
	1 \times 200 mg STZ/kg body wt	5 \times 40 mg STZ/kg body wt
AKR/J	224 \pm 82 (2/6)	177 \pm 34 (0/5)
C57BL/6J	525 \pm 138 (5/5)	379 \pm 121 (15/15)
LAF ₁	569 \pm 209 (4/5)	176 \pm 17 (0/11)
CD-1	556 \pm 18 (5/5)	397 \pm 200 (5/6)
BALB/c (Jackson Labs.)	212 \pm 124 (2/5)	215 \pm 48 (1/5)
BALB/c AnNlcr (Dr. Bosma)	488 \pm 122 (6/6)	318 \pm 83 (6/6)
BALB/c (Dr. Shin)	510 \pm 62 (14/14)	475 \pm 210 (14/15)

*Male mice of various strains were given either a single injection of 200 mg STZ/kg body wt or five daily injections of 40 mg STZ/kg body wt. Plasma glucose concentrations 10 days after the last injection of STZ are reported. The results are presented as mean \pm SD (number of mice manifesting hyperglycemia/number of mice studied). Glucose concentrations > 200 mg/dl were regarded as hyperglycemic.

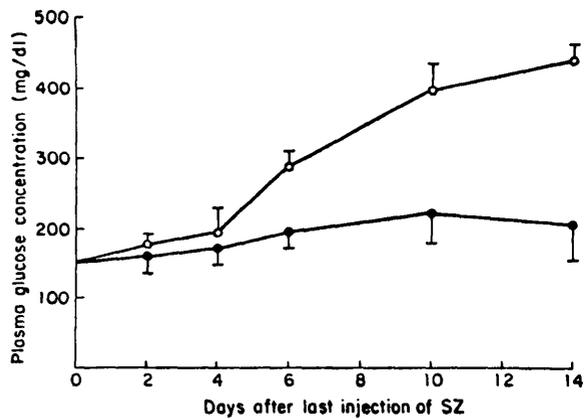


FIGURE 2. Requirements for T-lymphocytes for the induction of diabetes by repeated low doses of STZ. C57BL/6J mice were thymectomized, followed by lethal irradiation and reconstitution with anti-thy 1.2 plus complement-treated bone marrow (●). Controls were not thymectomized and were reconstituted with spleen cells after lethal irradiation (○). All mice were given 5 daily injections of 40 mg STZ/kg body wt starting 4 wk after cell transfer. Average blood glucose levels (\pm SD) for 19 T-cell-depleted and 10 control mice are presented.

after 5 daily doses of 40 mg STZ/kg body wt. C57BL/6J, CD-1, and BALB/c mice from the colonies of Dr. Bosma or Dr. Shin became hyperglycemic after both treatment schedules. The BALB/c mice obtained from different colonies differed markedly in their susceptibility to STZ (Table 1):

Characterization of the lymphocyte subpopulation required for the induction of diabetes by STZ. It has been shown that hyperglycemia cannot be induced in athymic nude mice by either the diabetogenic M-strain encephalomyocarditis virus or multiple injections of a low dose of STZ.^{9,14} These findings suggest that T-lymphocytes may be required for the induction of hyperglycemia by these agents. We decided to test this hypothesis by determining if STZ could induce hyperglycemia in T-cell-deficient mice pre-

pared by thymectomy, lethal irradiation, and reconstitution with anti-thy 1.2 and complement-treated syngeneic bone marrow cells, as described in MATERIALS AND METHODS. Control mice were not thymectomized and, after lethal irradiation, were reconstituted with spleen cells. Four weeks after reconstitution, mice were injected with 40 mg STZ/kg body wt daily for 5 consecutive days. It is clear from the data reported in Figure 2 that T-cell-deficient mice failed to become hyperglycemic, while control mice manifested significant hyperglycemia. Taking serum glucose levels >200 mg/dl as hyperglycemic, only 6 of 19 T-cell-deficient mice (4 experiments) manifested hyperglycemia, while 10 of 10 control mice (3 experiments) showed hyperglycemia. Statistical analysis of the glucose concentrations in these two groups indicated that the difference is significant ($P < 0.01$) as determined by Student's *t* test. In addition, we used the mice thymectomized, irradiated, and reconstituted with normal bone marrow cells as another control for testing our hypothesis that mature T-cells are required for induction of diabetes by multiple low doses of STZ. As shown in Table 2, 6 of 12 mice (3 experiments) that were thymectomized, irradiated, and reconstituted with normal bone marrow cells showed hyperglycemia by the multiple low doses of STZ. These results may be due to the fact that the lack of thymus prevents the maturation of T-cells from bone marrow cells. The incidence of induction of diabetes in the mice reconstituted with normal bone marrow cells was higher (6/12) than in mice reconstituted with anti-thy 1.2 antibody and complement-treated bone marrow cells (6/19). This may be due to the presence of recirculating mature T-cells in the normal bone marrow cells. Statistical analysis of the number of animals that induced hyperglycemia in the recipient mice (reconstituted with bone marrow treated with anti-thy 1.2 and complement in 4 separate experiments) and the number of animals that induced hyperglycemia in the recipient mice (reconstituted with normal bone marrow in 3 separate experiments) was highly significant ($P < 0.05$). However, sta-

TABLE 2
Effect on T-cells of induction of diabetes in mice by multiple low doses of STZ

Recipient reconstituted with	Glucose concentration (after 5 \times 40 mg/kg STZ)	P	Animals showing glucose concentration > 200 mg (%)	P¶
Anti-thy 1.2 plus complement Treated bone marrow*	225 \pm 84 (6/19)§		31.3 \pm 10.3#	
Normal spleen cells†	395 \pm 88 (10/10)	<0.01	100 \pm 0	
Normal bone marrow‡	265 \pm 85 (6/12)	>0.1	50.0 \pm 10.0**	<0.05

*The recipient mice were thymectomized, irradiated, and then received bone marrow cells treated with anti-thy 1.2 antibody and complement. The mice received five injections daily of 40 mg STZ/kg body wt.

†The recipient mice were irradiated and reconstituted with normal spleen cells without prior thymectomy. The mice received five injections daily of 40 mg STZ/kg body wt.

‡The recipient mice were thymectomized, irradiated, and then reconstituted with normal bone marrow cells. The mice received five injections daily of 40 mg STZ/kg body wt.

§The numbers in parentheses indicate mice manifesting hyperglycemia/number of mice studied. Glucose concentrations > 200 mg/dl were regarded as hyperglycemic.

||P-values are based on a comparison, by Student's *t* test, of the results for that group with the results of the recipient mice receiving anti-thy 1.2 and complement-treated bone marrow.

¶P-value is based on a comparison, by Student's *t* test, of the % of animals showing glucose concentrations > 200 mg/dl in serum samples of the total number of animals reconstituted with anti-thy 1.2 and complement-treated bone marrow versus animals reconstituted with normal bone marrow.

#Average % \pm SD from four experiments using anti-thy 1.2 and complement-treated bone marrow (2/5, 2/5, 1/5, and 1/4).

**Average % \pm SD from three experiments using normal bone marrow (2/5, 3/5, and 1/2).

TABLE 3
Correlation between the glucose concentration and the response to SRBC in recipient mice reconstituted with spleen cells or T-cell-depleted bone marrow

Recipient	Glucose concentration 10 days after 5 × 40 mg/kg STZ‡ (mg/dl)	Anti-SRBC PFC/spleen§
Thymectomized, irradiated, and reconstituted with anti- thy 1.2 plus complement- treated bone marrow*	181	270
	185	1200
	198	360
	200	1110
	257	1860
	268	8340
	302	4170
	547	13,080
Irradiated and reconstituted with normal spleen cells†	245	15,180
	325	7800
	416	18,000
	532	27,360
	540	31,900

*Irradiated mice were reconstituted with bone marrow cells treated with anti-thy 1.2 antibody and complement. The mice received five injections daily of 40 mg STZ/kg body wt.

†Irradiated mice were reconstituted with normal spleen cells, and received five injections daily of 40 mg STZ/kg body wt.

‡Glucose concentration was measured in serum samples obtained 10 days after the final injection of STZ.

§All experimental mice were immunized with i.v. SRBC 10 days after the final injection of STZ. Spleen cell suspensions were prepared and anti-SRBC PFC was determined by the method described by Dresser and Greaves.¹²

tistical analysis of the glucose concentration in these two groups (6/12 and 6/19) indicated that the difference was not significant ($P > 0.1$) as determined by Student's *t* test. Statistical analysis of the glucose concentration of the mice reconstituted with normal spleen cells and the mice reconstituted with normal bone marrow indicated that the difference was significant ($P < 0.05$). The results are, thus, consistent with the hypothesis that T-lymphocytes are required for induction of diabetes by low doses of STZ. Since there was considerable variability in the response of T-cell-deficient mice to STZ, we tested the possibility that this might be related to residual T-cell activity. We evaluated the degree of T-cell activity present by immunizing the mice with the T-dependent antigen SRBC. As indicated in Table 3, the magnitude of their anti-SRBC PFC response correlated very well with the degree of hyperglycemia observed in T-cell-depleted mice. That is, those mice that manifested the greater degree of hyperglycemia after STZ treatment gave the higher anti-SRBC PFC response. The correlation coefficient (*r*), obtained from regression analysis, of the anti-SRBC and glucose concentration was 0.91. A correlation coefficient of 0.91 is highly statistically significant ($P < 0.01$) (Figure 3).

Transfer of diabetes by spleen cells from STZ-treated mice to normal mice. Although some workers¹⁰ have reported that spleen cells from mice that have been given multiple low doses of STZ will transfer hyperglycemia to normal mice, several laboratories, including ours, have had difficulty regularly reproducing this finding.^{15,16} This difficulty in transferring hyperglycemia with spleen cells may be due to the fact that cytotoxic T-cells generated in the diabetic mice

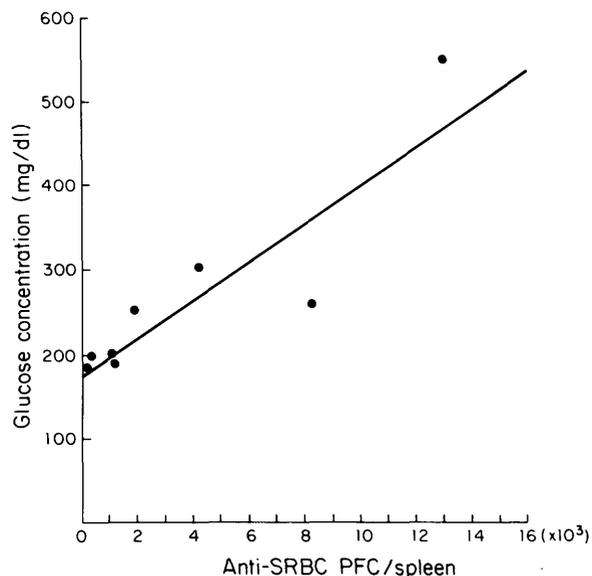


FIGURE 3. Regression analysis between glucose concentrations and anti-SRBC PFC/spleen of the recipient mice reconstituted with bone marrow treated with anti-thy 1.2 and complement. Glucose concentration was determined with serum samples obtained from recipient mice 10 days after the final injection of STZ. Anti-SRBC PFC was determined from spleen cells obtained from the recipient mice immunized with SRBC IV. The correlation coefficient (*r*) of the glucose concentration and anti-SRBC PFC was 0.91. Based on a *t* statistic ($t = \sqrt{vr^2/(1 - r^2)}$; $v = n - 2$, *r* = correlation coefficient, $P < 0.01$).

actually recognize STZ-altered beta cells and do not react (or only partially cross-react) with the normal beta cells of normal mice. To test this hypothesis, we treated normal mice with a single injection of 40 mg STZ/kg body wt before transfer of spleen cells from mice that had been rendered hyperglycemic by repeated low doses of STZ. A single low dose of 40 mg STZ/kg does not, in itself, cause hyperglycemia, but might be sufficient to induce slight modification

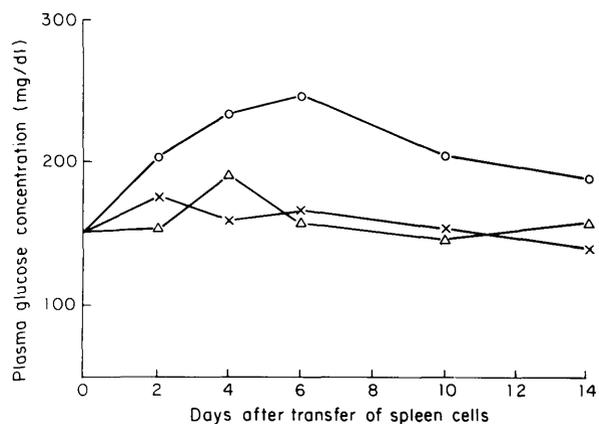


FIGURE 4. Transfer of diabetes from mice treated with 5 daily injections of 40 mg STZ/kg body wt to normal syngeneic mice by spleen cells. C57BL/6J mice were killed 12 days after completing a 5-day course of STZ injections, and their spleen cells were transferred into normal syngeneic mice or into mice that had received 1 injection of 40 mg STZ/kg body wt 5 days before cell transfer. Serum glucose levels were assayed periodically and average values for groups of 8 mice are presented. (x) Normal spleen cell donors and recipients treated with one injection of 40 mg STZ/kg body wt. (Δ) STZ-treated donors (5 doses) and normal recipients. (O) STZ-treated donors (5 doses) and recipients treated with one injection of 40 mg STZ/kg body wt.

TABLE 4
Transfer of hyperglycemia by spleen cells from STZ-treated mice to normal mice*

Recipient	Donor of spleen cells	Glucose concentration (mg/dl)†	
		Expt. 1	Expt. 2
Normal	5 × 40 mg STZ/kg	172 ± 22‡ P < 0.01§ (4)	162 ± 30 P < 0.01 (5)
1 × 40 mg STZ/kg	Normal	168 ± 26 P < 0.01 (5)	164 ± 32 P < 0.01 (5)
1 × 40 mg STZ/kg	5 × 40 mg STZ/kg	252 ± 12 (4)	246 ± 16 (4)

*Spleen cells (5×10^7) from each donor were intravenously transferred to respective recipient mice.

†Glucose concentrations of serum samples from each recipient animal were measured 6 days after transfer of spleen cells.

‡Glucose concentrations are expressed as mean ± SD. The numbers in parentheses indicate the number of animals used for each group.
§P-values are based on a comparison, by Student's *t* test, of the results for that group with the results for recipients (1 × 40 mg STZ/kg) receiving the spleen cells from diabetic mice (5 × 40 mg STZ/kg).

of the beta cells so that cytotoxic T-cells from diabetic mice could recognize them as target cells. As shown in Figure 4, diabetic spleen cell recipients, which were given a single low dose of STZ, showed a moderate but progressive increase in blood glucose concentration. This increase in glucose concentration was seen in all of the eight mice studied in two independent experiments. Recipients of normal spleen cells showed no increase in blood glucose concentration. Thus, pretreatment of recipients with a low dose of STZ was necessary for efficient transfer of hyperglycemia.

As shown in Table 4, glucose concentration of serum samples obtained from the recipient mice (1 × 40 mg STZ/kg) was gradually increased 2 days after the transfer of spleen cells from the diabetic mice (5 × 40 mg STZ/kg). Glucose concentration was highest (252 mg/dl for experiment 1 and 246 mg/dl for experiment 2) 6 days after the transfer, and thereafter glucose concentration gradually decreased. However, the normal recipient mice that received the spleen cells from diabetic mice showed no increment of glucose concentration in their serum samples throughout the experiment (172 mg/dl for experiment 1 and 162 mg/dl for experiment 2). The statistical analysis of glucose concentrations in the recipient mice (1 × 40 mg STZ/kg) receiving the spleen cells from the diabetic mice (5 × 40 mg STZ/kg) and in the re-

ipient mice (1 × 40 mg STZ/kg) receiving the spleen cells from normal mice showed significant difference ($P < 0.01$). Recipient mice (1 × 40 mg STZ/kg) receiving spleen cells from normal mice also showed a normal range of glucose concentration throughout the experiment (168 mg/dl for experiment 1 and 164 mg/dl for experiment 2). The statistical analysis of glucose concentration measured in the recipient mice (1 × 40 mg STZ/kg) receiving the spleen cells from diabetic mice (5 × 40 mg STZ/kg) and in the recipient mice (normal) receiving the spleen cells from diabetic mice (5 × 40 mg STZ/kg) showed significant difference ($P < 0.01$). These observations were confirmed in two independent experiments using 4 or 5 animals in each group.

PFC response in spleen cells in vitro from STZ-diabetic mice. The immune responses to a T-dependent antigen, SRBC, and a T-independent antigen, TNP-PAA, in the spleen cell cultures obtained from diabetic and control mice were investigated in vitro to assess any abnormal function of lymphocytes from diabetic mice.

Spleen cell suspensions were prepared from normal and diabetic mice of the C57BL/6J strain; diabetes was induced both by multiple low doses of STZ and a single large dose of STZ. Spleen cells from normal and both groups of diabetic mice were cultured with SRBC or TNP-PAA for 4 days. Anti-

TABLE 5
Anti-SRBC and anti-TNP PFC responses in spleen cells from STZ-diabetic mice*

C57BL/6J	Anti-SRBC PFC/10 ⁶ cells			Anti-TNP PFC/10 ⁶ cells			
	Expt. 1	Expt. 2	Expt. 3	Expt. 1	Expt. 2	Expt. 3	Expt. 4
Normal spleen cells	1698	2730	1962	1956	2045	1698	1896
Diabetic spleen cells	271	1359	444	1806	2444	1822	1720
by 5 × STZ (40 mg/kg)	(84%)	(50%)	(70%)	(8%)	(-20%)	(-7%)	(9%)
Diabetic spleen cells	—	1092	235	—	—	1579	1820
by 1 × STZ (200 mg/kg)	—	(60%)	(88%)	—	—	(7%)	(4%)
½ Normal spleen cells +	847	2242	1186	1974	1900	1625	2000
½ diabetic spleen cells	(14%)	(-10%)	(1%)	(15%)	(15%)	(8%)	(-11%)
by 5 × STZ	—	—	—	—	—	—	—
½ Normal spleen cells +	—	1758	1483	—	—	1426	2489
½ diabetic spleen cells	—	(8%)	(-35%)	—	—	(13%)	(-34%)

*Spleen cells (7×10^6 /ml) from normal and both groups of diabetic mice were added to 0.1% SRBC (vol/vol) or 1% TNP-PAA (vol/vol) and incubated for 4 days. Anti-SRBC or anti-TNP PFC were assayed using SRBC or TNP-SRBC as target cells. The results are expressed as PFC/10⁶ cells present at the end of the culture period. The numbers in parentheses are percent of depression compared with PFC response from normal spleen cells.

TNP PFC responses to T-independent antigen, TNP-PAA, in normal and diabetic spleen cells obtained both from multiple low doses of STZ and from a single large dose of STZ were similar; the anti-SRBC responses in both groups of diabetic spleen cells, however, were much lower than in those of normal spleen cell cultures (Table 5). It is interesting to note that the spleen cells obtained from diabetic mice, rendered so by a single injection of a large dose of STZ, also showed depressed PFC response to SRBC but not to TNP-PAA, as observed in the culture of spleen cells after multiple low doses of STZ. The mixed culture of normal and both groups of diabetic spleen cells showed no suppression in PFC responses to either SRBC or TNP-PAA. These results suggest that decreased response to anti-SRBC in both diabetic groups is not due to the increased activity of suppressor T-cells but, rather, is due to the impaired activity of T-helper cells.

DISCUSSION

It is generally accepted that induction of diabetes by a large dose of STZ is the consequence of the destruction of beta cells. Morphologic changes in beta cells of STZ-treated mice have been demonstrated.^{17,18} It is possible, however, that damage to beta cells by STZ may, in addition, activate an autoimmune process that results in further damage to beta cells. When mice are injected with a single dose of 200 mg STZ/kg body wt, hyperglycemia appears within a few hours, making it unlikely that immunologic mechanisms play a significant role. However, when mice are injected with 40 mg STZ/kg body wt daily for 5 consecutive days, hyperglycemia slowly develops 5–6 days after the last injection of STZ. A role for immunologic mechanisms in the induction of diabetes by repeated low doses of STZ seems likely. Several lines of evidence support this hypothesis. An infiltration of lymphocytes in the islets has been reported. Shin and Paik⁹ have shown that STZ (in repeated low doses) does not induce diabetes in athymic nude mice. Similarly, the diabetogenic M-strain encephalomyocarditis virus does not induce hyperglycemia in nude mice.¹⁴ A most provocative finding was that lymphocytes from 3 of 6 patients with diabetes induced hyperglycemia in athymic mice.¹⁹ It has been reported that diabetes can be induced in normal mice by the transfer of spleen cells from mice rendered diabetic by repeated injections of low doses of STZ.¹⁰ These results are all consistent with the involvement of an immunologically mediated process in the induction of diabetes in this experimental model.

We have observed that different strains vary in their susceptibility to the induction of diabetes by STZ. Similar observations have been reported by other workers.^{20,21} There is evidence that the major histocompatibility complex influences the susceptibility to a number of experimentally induced autoimmune diseases in animals, including thyroiditis, encephalomyelitis, myasthenia gravis, and, possibly, hemolytic anemia. In addition, there are data suggesting a relationship between diabetes and certain alleles of the major histocompatibility locus in man.²² Thus, genetic factors are undoubtedly important in influencing the induction of diabetes.

Results reported here indicate that T-cell-depleted animals do not develop hyperglycemia after multiple injections of a low dose of STZ, suggesting that T-lymphocytes play a cru-

cial role in the induction of hyperglycemia after repeated low doses of STZ. Two possible roles for T-lymphocytes in the induction of hyperglycemia should be considered. First, T-cells may develop into cytotoxic cells that are specifically reactive with pancreatic beta cells. Second, T-cells may be required as helper cells for production of autoantibodies specific for beta cells. These two roles are, of course, not exclusive and T-cells might act in both ways in the induction of diabetes.

Although some workers have reported the transfer of diabetes from low-dose, STZ-induced diabetic mice to normal animals, several laboratories, including our own, have had difficulty reproducing this transfer in a regular manner.^{16,17} This inefficiency in transfer might be due to the fact that cytotoxic T-cells generated in the STZ-treated mice actually recognize STZ-altered beta cells and do not react (or only partially cross-react) with the normal beta cells of normal mice. This possibility was tested by transferring spleen cells from diabetic mice into mice treated with a single dose of 40 mg STZ/kg body wt. This dose of STZ, in itself, does not induce hyperglycemia. Pretreated mice regularly developed moderate hyperglycemia after transfer of spleen cells from diabetic mice, suggesting that a modification of beta cells by STZ is important in the specificity of the induction of diabetes by STZ. It was interesting to note that the moderate increment of glucose concentrations in the recipient mice that received the spleen cells from diabetic mice was correlated with an appearance of insulinitis in pancreatic islets. This should be confirmed by further histologic evidence. Since the induction of hyperglycemia by multiple low doses of STZ mediates immunologic processes, the functional aspects of lymphocytes in production of antibodies to specific antigens *in vitro* were studied with spleen cells obtained from mice with diabetes induced by both multiple low doses of STZ and a single large dose of STZ.

Both spleen cell preparations obtained from the mice with diabetes induced by multiple low doses of STZ or a single large dose of STZ showed normal response to the T-independent antigen TNP-PAA. However, responses to the T-dependent antigen SRBC in the spleen cell preparation of both groups of diabetic mice were about 60% lower than those of spleen cell preparations from normal mice. These results suggest that T-helper cell functions may be impaired in both groups of diabetic mice. However, the relationship between induction of diabetes in mice by multiple low doses of STZ and the impairment of T-helper function is not known. Further investigation will be required on the functional aspects of lymphocytes treated with STZ.

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