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Insulin-dependent diabetes mellitus induced by subdiabetogenic doses of streptozotocin: Obligatory role of cell-mediated autoimmune processes

(juvenile-type diabetes mellitus/autoimmune mechanism/*nude* mice/thymus graft)

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ABSTRACT **The role of thymic functions in the development of insulin-dependent diabetes was investigated in athymic *nude* (*nu/nu*) mice and euthymic heterozygous (+/*nu*) littermates of BALB/c origin treated with streptozotocin. The injection of a single high dose of streptozotocin (200 mg/kg body weight) induced rapid and permanent hyperglycemia in both *nu/nu* and +/*nu* mice. In contrast, the injection of the same total dose divided into multiple "subdiabetogenic" doses (40 mg/kg per day for 5 consecutive days) caused the development of delayed but progressive hyperglycemia only in the thymus-competent +/*nu* mice. Female mice of either genotype were significantly less susceptible to streptozotocin at both doses. Restoration of thymic immunity in *nu/nu* mice by thymus grafts also restored the susceptibility to the hyperglycemic effects of multiple low doses of streptozotocin. Moreover, splenic lymphocytes from +/*nu* mice previously made diabetic with the multiple low-dose injections of streptozotocin induced transient glucose intolerance in *nu/nu* mice. The ability of the diabetic spleen cells to transfer the diabetic state was abolished when the splenic lymphocytes were depleted of the T cells but not when they were depleted of B cells. These results provide direct proof that thymus-dependent functions play an obligatory etiologic role in the development of diabetes in mice treated with repeated subdiabetogenic doses of streptozotocin. These observations also add to the growing evidence that autoimmune amplification mechanisms may be critically involved in the etiology of juvenile-onset diabetes mellitus in humans.**

There is increasing evidence that the development of the juvenile-type insulin-dependent diabetes mellitus is mediated by autoimmune processes. Pancreatic islets of children with newly diagnosed insulin-dependent diabetes frequently contain infiltrating mononuclear lymphocytes (1), and a large fraction of juvenile-onset diabetes patients have both circulating antibodies against the islet cells (2) and lymphocytes with cytolytic activity against human insulinoma cells in culture (3). In addition, epidemiologic studies in several countries have independently established that a number of alleles of the *HLA* gene complex, especially of the *HLA-D* locus, are associated with a significantly increased risk of this form of diabetes (reviewed in ref. 4).

Evidence implicating autoimmune processes in the etiology of insulin-dependent diabetes comes also from experimental model systems utilizing laboratory rodents. In a series of experiments, Like and Rossini and their coworkers have shown that the induction of diabetes in mice and rats by the beta-cell toxin streptozotocin typically proceeds in two quite different pathways. When a single, sublethal, high dose of streptozotocin was administered to susceptible animals, rapid destruction of the islet beta cells ensued, followed almost immediately by a profound and permanent hyperglycemia (5). In contrast, the administration of the same total dose of streptozotocin, divided into multiple small "subdiabetogenic" doses, caused insulitis associated with the induction of endogenous type C viruses in the beta cells; after several days, delayed but progressive hyperglycemia followed (5). Significantly, both the insulitis and the subsequent development of hyperglycemia were prevented by rabbit antisera directed against mouse lymphocytes (6). Similarly, rabbit antisera against rat lymphocytes could prevent the development, and sometimes even reverse the progression, of spontaneous diabetes that occurs with a high frequency in the nonobese BB/W rats (7). On the basis of these observations, Rossini and his colleagues proposed that the induction of insulin-dependent diabetes by multiple injections of subdiabetogenic doses of streptozotocin involved "the triad of direct beta cell cytotoxicity, virus induction within beta cells, and cell-mediated autoimmune reaction" (6).

The involvement of host immunological mechanisms in the development of diabetes is supported also by the work of Buschard and Rygaard (8, 9), who reported that streptozotocin could induce hyperglycemia in euthymic heterozygous (+/*nu*) mice but not in the athymic (*nu/nu*) mice. Certain viruses known to induce the diabetic syndrome in susceptible mice, such as the M strain of encephalomyelocarditis virus (10), could induce hyperglycemia in +/*nu* BALB/c mice but not in *nu/nu* BALB/c mice (11). Moreover, *nude* mice injected with spleen cells from streptozotocin-induced diabetic mice developed clinical evidence of diabetes (12). Most provocative in this regard, however, was the report by Buschard *et al.* (13) that peripheral blood lymphocytes from some newly diagnosed juvenile-onset diabetes patients were also able to induce hyperglycemia in *nude* mice. These observations, although preliminary, appeared to imply that the induction of experimental hyperglycemia in mice is critically mediated by autoreactive immune lymphocytes that recognize, and perhaps destroy, the pancreatic beta cells in an organ-specific species-nonspecific manner. Subsequent efforts in several laboratories to confirm the reports of Buschard and coworkers have been, unfortunately, largely unsuccessful (14–18).

In view of the very significant implications of the observations reported by Buschard and his colleagues, we investigated the experimental parameters needed for induction of insulin-dependent diabetes mellitus in normal and congenitally athymic BALB/c mice treated with streptozotocin. Specifically, we sought to reexamine critically the possible involvement of the thymus-dependent immunity in the induction process.

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Abbreviation: SRBC, sheep erythrocytes.

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MATERIALS AND METHODS

Mice. The colony of *nude* (*nu/nu*) mice used in this study was established in this laboratory in 1973, with a breeding stock derived from the fourth backcross generation in BALB/c mice obtained from the Gl. Bomholtgard, Ry, Denmark. The colony was bred by mating homozygous *nude* (*nu/nu*) males with heterozygous (+/*nu*) females, in a semi-aseptic environment using presterilized cages, cage tops, and bedding inside sterile-air-flow cabinets ("Stay Clean Racks," Lab Products, Garfield, NJ). Mice were given boiled tap water and autoclaved feed (Autoclavable Mouse Chow no. 5021, Ralston Purina, St. Louis, MO), which were supplied ad lib. Nursing females and preweanling young were also fed with autoclaved whole-wheat bread soaked in condensed milk, and liquid vitamin complexes (ABCDE Pet Drops, Hart-Delta, Baton Rouge, LA). The entire mouse colony was housed in a limited-access clean animal facility, maintained under a positive pressure with particle-free air, strictly isolated from nonresearch personnel and other laboratory animals. Periodic checks on random animals removed from the colony during the course of this study showed that the colony was free of pathogenic microorganisms, including mouse hepatitis virus. The life span of untreated *nu/nu* mice in the colony was greater than 12 months. Mice used in this study were 4–8 weeks old at the start of each experiment.

Streptozotocin. Streptozotocin was purchased from Sigma (S0132, mixed anomers; lots 28C-0385 and 88C-0032). It was dissolved in sterile 0.1 M sodium citrate buffer, pH 4.5, and injected into mice intraperitoneally with sterile syringes within 5 min after preparation.

Determination of Plasma Glucose and Insulin Levels. Blood samples (about 80 μl for each assay) were collected between 9 a.m. and 11 a.m. from the paraorbital venous plexus of nonfasting animals, using heparinized hematocrit capillary tubes (DADE Capilets, American Hospital Supply, Miami, FL). The plasma was quickly separated by centrifugation and was assayed immediately or stored at −20°C. Plasma glucose was measured with the Beckman Glucose Analyzer 2 by the glucose-oxidase method. Plasma immunoreactive insulin was determined by radioimmunoassay (19).

Thymus Implantation in *Nude* Mice. The entire thymuses of 3- to 5-day-old heterozygous (+/*nu*) mice were removed under sterile conditions immediately after decapitation and implanted subcutaneously into 4- to 6-week-old male *nu/nu* mice through a small dorsal incision. Each recipient mouse received the whole thymus of one donor animal. During the operation, the mice were kept under light anesthesia with Epontol (Bayer, Leverkusen, West Germany). The incision was closed with a sterile surgical glue (Histoacryl Blue, B. Braun, Melsungen, West Germany).

Test of T Cell Reconstitution in Thymus-Grafted *Nude* Mice. Four weeks after the thymus implantation, the recipient *nude* mice and control +/*nu* mice were immunized with 0.1 ml of 10% (vol/vol) fresh sheep erythrocytes (SRBC) suspended in neutral phosphate-buffered saline, injected intraperitoneally. Each mouse was boosted 7 days later with an identical SRBC

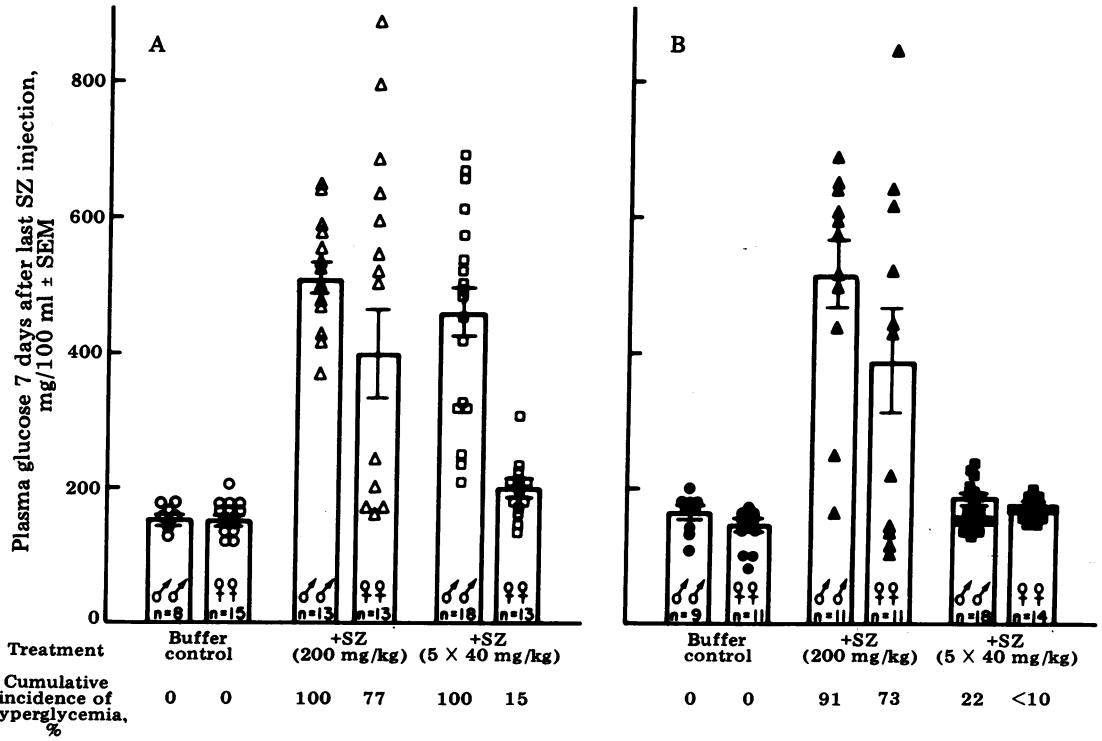

FIG. 1. Induction of hyperglycemia with streptozotocin (SZ): Effects of the genotype, sex, and dose schedule on hyperglycemic incidence. Male and female mice of +/*nu* (*A*) or *nu/nu* (*B*) genotype were injected intraperitoneally with streptozotocin, either as a single high dose of 200 mg/kg body weight or as multiple low doses of 40 mg/kg per day for 5 consecutive days. The plasma glucose values obtained on the seventh day after the end of the injection schedules are shown here, together with the number (*n*) of total surviving mice on that day for each experimental group. The cumulative incidence of persistent hyperglycemia as of 4 weeks after the final injection is also given for each group. The mean plasma glucose values in pretreatment mice allowed free access to food and water were, in units of mg/100 ml ± SD: +/*nu* males, 170 ± 18 (n = 39); +/*nu* females, 154 ± 23 (n = 41); *nu/nu* males, 173 ± 24 (n = 38); and *nu/nu* females, 167 ± 24 (n = 36). Plasma glucose levels higher than 200 mg/100 ml were rarely observed in untreated mice; therefore, for the purpose of this study, glucose values exceeding 220 mg/100 ml (normal mean + 2 SD) were considered to be hyperglycemic.

injection, and serum was collected 7 days after the second injection. The titer for hemagglutinating antibodies against SRBC was determined in microtiter wells, using 1:2 serial dilutions (20).

RESULTS

Induction of Hyperglycemia with Streptozotocin. The development of hyperglycemia in euthymic +/*nu* mice and their athymic *nu/nu* littermates was examined by monitoring the changes in the plasma glucose level after the injection of streptozotocin, given either as a single high dose of 200 mg/kg body weight or as multiple low doses of 40 mg/kg per day for 5 consecutive days. Control mice received only the solvent. The results are shown in Fig. 1. The single high dose of streptozotocin induced severe hyperglycemia in both +/*nu* and *nu/nu* mice, which was apparent within 2 days after the injection and persisted for the duration of the experiment (up to 60 days) (data not shown). The mortality of mice by 2 weeks after the injection was 15% (7 of 48 mice); all of the mice that died had severe hyperglycemia. The induction of hyperglycemia was much more effective in males than in females; 23% of +/*nu* females and 27% of *nu/nu* females in this group did not develop hyperglycemia at all.

In mice injected with multiple low doses of streptozotocin, however, there was a very striking difference between +/*nu* and *nu/nu* animals. The majority of +/*nu* males developed pronounced hyperglycemia by the seventh day after the final injection; eventually, by the third week, 100% of these mice became hyperglycemic. In contrast, only a few of the +/*nu* females and *nu/nu* mice of either sex became hyperglycemic, ultimately reaching a cumulative hyperglycemic incidence of 15%, 22%, and <10% for each of these groups of mice (see Fig. 1). Furthermore, even in those mice that did develop detectable hyperglycemia, the plasma glucose levels were only minimally higher than the pretreatment level; almost none reached the degree of hyperglycemic levels seen in the +/*nu* males treated identically. The combined mortality for all of the mice given the multiple low-dose injections was less than 1% during the 60 days after the treatment. Subsequent studies confirmed repeatedly that female mice of both genotypes are in general less susceptible to streptozotocin-induced hyperglycemia, irrespective of the dosage schedules employed. In later experiments, therefore, only male mice were utilized.

In Fig. 2 are illustrated the time course and magnitude of the development of hyperglycemia in male mice treated with streptozotocin, either with a single high dose or with multiple low doses. In +/*nu* mice (Fig. 2*A*), both dosage schedules induced severe and persistent hyperglycemia in all of the animals. However, the development of hyperglycemia in the mice given the multiple low doses was not apparent until several days after the final injection, and the mean glucose levels in this group of mice were significantly lower compared to +/*nu* mice given the single high dose. Again, consistent with earlier observations, *nu/nu* males injected with the high dose of streptozotocin developed severe hyperglycemia as rapidly as +/*nu* males treated similarly (Fig. 2*B*). In contrast, *nu/nu* males injected with the multiple low doses failed to develop hyperglycemia at all. A small number of these mice (about 20%) showed mild and sporadic elevations in the plasma glucose level, but the remainder showed no evidence of impairment in glucose metabolism, either in the nonfasting plasma glucose levels or in glucose tolerance tests (data not shown).

The severe hyperglycemia induced in these mice was in every case associated with a pronounced reduction in the plasma insulin level (data not shown). The insulin dependence of the streptozotocin-induced hyperglycemia was demonstrated also by implanting moribund hyperglycemic *nude* mice with a hamster islet cell tumor, previously shown to maintain its insulin-producing capacity through serial transplantation in *nude* mice (21). As the hamster tumor grew in size, the plasma glucose levels of the tumor-bearing mice decreased gradually and finally returned to the normal range. Eventually, these once *hyper*glycemic mice became *hypo*glycemic.

Induction of Hyperglycemia in Thymus-Reconstituted *Nude* Mice. We next examined whether the inability to induce hyperglycemia in the athymic mice with multiple low-dose injections of streptozotocin was indeed due to the absence of thymus-dependent immunity in these mice. Young adult male *nu/nu* mice were grafted with whole thymuses removed from newborn +/*nu* mice, and, 4 weeks later, the degree of T-cell reconstitution was determined by the titer of hemagglutinating antibodies produced after immunization with SRBC, which are known to be thymus-dependent antigens (20). Typical response in eight adult euthymic (+/*nu*) mice similarly challenged with SRBC ranged in hemagglutinating titers of from 2^{10} to 2^{12} (see

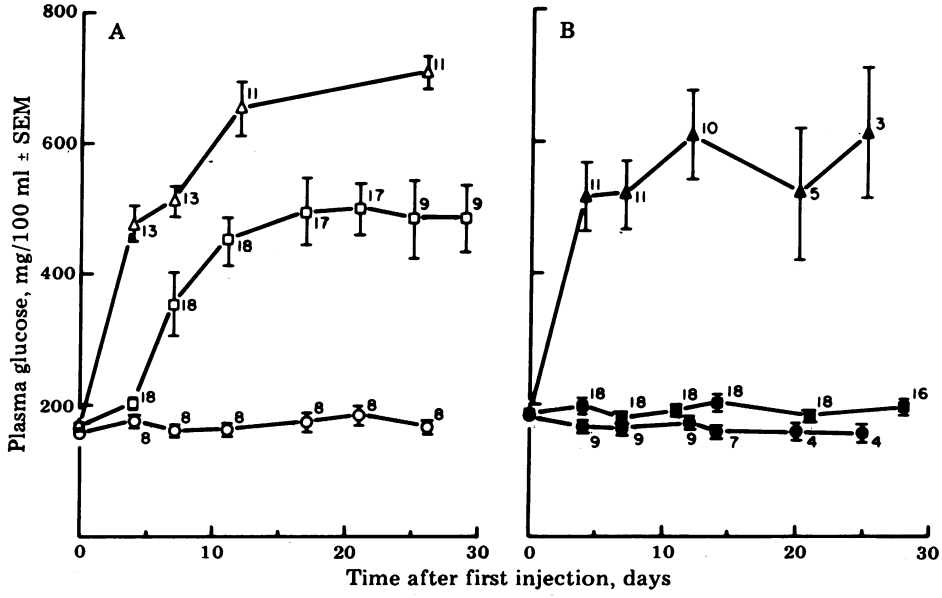

FIG. 2. Induction of hyperglycemia with streptozotocin: Time course and magnitude of hyperglycemia in male mice. Male +/*nu* (*A*) and *nu/nu* (*B*) mice were injected with streptozotocin, either as a single high dose or as multiple low doses, and changes in nonfasting glucose levels were determined periodically for 4 weeks. Numbers at each data point refer to the total number of mice of that group used for the glucose determination; the vertical bar represents the SEM. The doses of streptozotocin given were: △ and ▲, 200 mg/kg body weight on day 0; □ and ■, 40 mg/kg per day for 5 days on days 0 to 4; ○ and ●, citrate buffer alone on day 0.

Fig. 3). Among the nine *nu/nu* mice grafted with thymus, five showed no evidence of functional restoration: the anti-SRBC titers in these mice were not detectably higher than in sham-operated *nu/nu* mice ($<2^3$). However, in the remaining four *nu/nu* mice, the graft was at least partially successful in restoring T-cell immunity: the anti-SRBC antibody titers in these mice ranged from 2^6 to 2^9.

All of these mice were injected with multiple low doses of streptozotocin. Progressive and permanent hyperglycemia was induced in all of the four *nude* mice in which the thymus graft had resulted in successful functional reconstitution, but in none of the five *nude* mice in which the thymus graft failed to restore T-cell functions (Fig. 3). The severity of the hyperglycemia induced in the partially T-cell-reconstituted *nude* mice was considerably less than in the fully thymus-competent +/*nu* mice, showing that there is an approximate correlation between the degree of hyperglycemia induced by multiple low doses of streptozotocin and the degree of T-cell-dependent immune functions present in the animal.

Passive Transfer of Glucose Intolerance by Splenic Lymphocytes of Diabetic Mice. Now we asked whether the +/*nu* mice that develop severe hyperglycemia as a consequence of multiple low-dose injections of streptozotocin have activated lymphocytes capable of adversely affecting beta-cell functions in mice not previously treated with streptozotocin. Spleen cells recovered from hyperglycemic +/*nu* males, treated 17 days earlier with multiple low doses of streptozotocin, were injected into a group of male *nude* mice. A second group of *nude* males was injected with spleen cells from control (buffer-treated) +/*nu* mice. The nonfasting plasma glucose levels in both groups of recipients remained within the normal range. However, the *nude* mice given the spleen cells from hyperglycemic mice showed significantly abnormal glucose tolerance tests beginning 4 days after the transfer (data from one such experiment are summarized in Table 1). The fraction of mice showing impaired glucose tolerance reached a peak (about 80%) between 10 and 20 days after the spleen cell transfer, and decreased afterwards so that by the fourth week most of the spleen cell recipients again responded normally to glucose tolerance tests (data not shown).

Table 1. Induction of glucose intolerance in *nude* mice by spleen cells from diabetic heterozygous mice

Recipient *nude* mouse no.	Spleen cell donor	Response to glucose tolerance test, plasma glucose level, mg/100 ml					
		Day 4		Day 11		Day 19	
		0 min	60 min	0 min	60 min	0 min	60 min
187	Normal	71	150	118	199	113	171
188	Normal	110	196	152	200	140	156
182	Diabetic	67	140	100	95	97	398
183	Diabetic	86	206	83	375	99	173
184	Diabetic	81	137	90	494	91	147
185	Diabetic	87	148	95	448	96	297
186	Diabetic	106	272	86	331	100	161

Spleen cells were removed from severely diabetic male +/*nu* mice treated with multiple low doses of streptozotocin 17 days earlier, and 4×10^7 cells were injected intraperitoneally into each male *nude* mouse, using one donor spleen for each recipient. Control *nu/nu* mice received spleen cells from normal (buffer-treated) +/*nu* mice. On days 4, 11, and 19 after the spleen cell transfer, each mouse was tested for glucose tolerance after an overnight fast, by intraperitoneally injecting glucose at 2 mg/g body weight, in a 20% aqueous solution.

In order to identify the cell type responsible for the passive transfer of glucose intolerance, the spleen cells removed from the diabetic mice were first depleted of the T cells or the B cells and then injected into *nude* mice. The results of this experiment (Fig. 4) showed that the capacity to induce glucose intolerance in recipient mice was abolished only when the T lymphocytes were selectively removed from the spleen cell preparations.

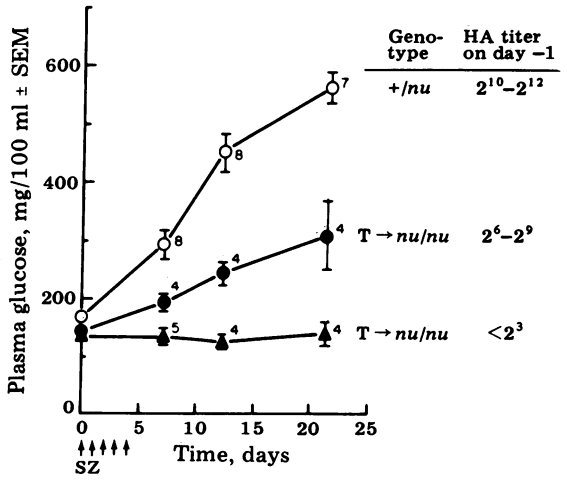

FIG. 3. Induction of hyperglycemia in thymus-reconstituted *nu/nu* mice with multiple low doses of streptozotocin. Male *nu/nu* mice were grafted with whole thymus glands from +/*nu* mice and, 4 weeks later, tested for production of hemagglutinating (HA) antibodies to SRBC. The thymus-grafted *nu/nu* mice (▲ and ●) and euthymic control male +/*nu* mice (○) were injected with streptozotocin (SZ) at 40 mg/kg per day for 5 days beginning on day 0, and the development of hyperglycemia was measured for 3 weeks. The total number of surviving mice in each experimental group and the SEM (vertical bar) are indicated for each data point.

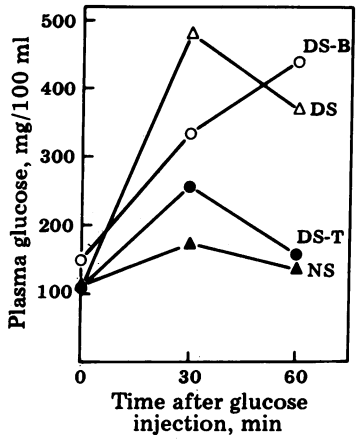

FIG. 4. Induction of glucose intolerance in *nude* mice by splenic lymphocytes from diabetic +/*nu* mice: Effect of depletion of T cells. Spleen cells from two severely diabetic +/*nu* males (glucose levels of 640 and 672 mg/100 ml, respectively), which had been treated with multiple low doses of streptozotocin 24 days earlier, were pooled, and one fraction was treated with anti-Thy 1.2 antiserum and complement to deplete T cells, and another fraction was passed through a column packed with nylon wool to deplete the B cells. For control, spleen cells from two buffer-treated nondiabetic +/*nu* males (glucose levels of 173 and 161 mg/100 ml, respectively), were used without further fractionation. Male *nu/nu* mice were injected intravenously via the tail vein with 10^7 spleen cells of each of the 4 different preparations and, 20 days later, tested for glucose tolerance as described in the legend to Table 1. DS (△), diabetic spleen cells, unfractionated; DS-B (○), diabetic spleen cells after passage through nylon wool column; DS-T (●), diabetic spleen cells depleted of T cells with anti-Thy 1.2 and complement; NS (▲), normal (nondiabetic) spleen cells.

DISCUSSION

These results provide direct proof that, for the induction of delayed insulin-dependent diabetes mellitus in mice treated with multiple subdiabetogenic doses of streptozotocin, the host thymic functions play an obligatory role. The results of the spleen cell transfer experiments, in particular, lend strong support to the view that the development of juvenile-type diabetes is mediated by autoimmune T lymphocytes, presumably directed against beta-cell-specific self-antigens that are generated, in this case, by the beta-cell toxin, which by itself is not diabetogenic at the doses used.

Our observations are in general consistent with the previous reports by Like and Rossini and their colleagues (5–7). Rossini *et al.* (6) proposed, however, that the generation of autoimmune lymphocytes directed against the beta cells might involve the participation of beta-cell-tropic type C viruses induced by streptozotocin. This proposal would explain the development of diabetes in mice infected with various viruses (10, 22), as well as the clinical observations that suggest a possible link between viral infections in children and subsequent development of rapid-onset diabetes (23–25). Early results from our own studies have so far failed to support this notion. Electron microscopic examination of thin sections of the islet tissue from +/*nu* and *nu*/*nu* mice, before and after the induction of the diabetic state by either the single high-dose or the multiple low-dose procedure, revealed no evidence that type C viruses were present in these cells (unpublished results). The fact that T-cell-depleted splenic lymphocytes can no longer induce transient glucose intolerance in *nude* mice also tends to argue against the obligatory role of a newly induced endogenous virus in the streptozotocin-treated mice, because it is improbable that such a virus would have been selectively eliminated only in the T-cell-depleted spleen cell populations.

One may speculate, therefore, that the diabetogenic viruses and subdiabetogenic doses of streptozotocin are alternative triggering mechanisms, each of which can act independently by inducing the appropriate beta-cell surface modifications that are then recognized as neoantigens by the host T cells. The strain dependence of the susceptibility to diabetogenic viruses (10, 26) or to streptozotocin (27) in mice, and the association between an increased incidence of juvenile-onset diabetes and certain specific alleles of the *HLA* complex in man (28), may then be seen as reflecting in part the degree of ease with which the relevant autoantigens are generated on the beta-cell surface. The formation of such autoantigens would depend on the existence of the proper surface molecular substrates on the beta cell (perhaps provided, on human cells, by the appropriate *HLA-A* and *HLA-B* determinants), and their recognition as potential immunogens would in turn require the proper T-cell clonal repertoire (and thus the appropriate *HLA-D* haplotypes in human cells). On the other hand, the rapid development of profound and permanent hyperglycemia in mice given the high dose of streptozotocin would not require the amplification by autoimmune host T cells, because the hyperglycemia in this case would be a direct consequence of the overwhelming physical destruction of the beta cells by the toxin, as suggested by Like and Rossini (5). Similarly, an islet-tropic virus may also cause a rapid insulin-dependent diabetes by destroying the majority of beta cells in an animal. The isolation, from the pancreas of a child with diabetic ketoacidosis, of a strain of Coxsackievirus B4 capable of inducing hyperglycemia in mice (25) may have been an example of this, even though viremia is a very rare event even in fulminant juvenile-type diabetes.

Our results differ from the observations of Buschard and Rygaard (8) in that we never observed the induction of severe hyperglycemia in *nude* mice treated with streptozotocin according to the multiple low-dose procedure. In addition, we were unable to confirm their report (9) that the implantation of spleen cells from diabetic +/*nu* mice caused frank hyperglycemia in *nu*/*nu* recipients.

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1. Gepts, W. (1965) *Diabetes* **14**, 619–633.
2. Lendrum, R., Walker, G. & Gamble, D. R. (1975) *Lancet* **i**, 880–883.
3. Huang, S.-W. & Maclaren, N. K. (1976) *Science* **192**, 64–66.
4. Craighead, J. E. (1978) *N. Engl. J. Med.* **299**, 1439–1445.
5. Like, A. A. & Rossini, A. A. (1976) *Science* **193**, 415–417.
6. Rossini, A. A., Like, A. A., Chick, W. L., Appel, M. C. & Cahill, G. F., Jr. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 2485–2489.
7. Like, A. A., Rossini, A. A., Guberski, D. L., Appel, M. C. & Williams, R. M. (1979) *Science* **206**, 1421–1423.
8. Buschard, K. & Rygaard, J. (1977) *Acta Pathol. Microbiol. Scand. Sect. C* **85**, 469–472.
9. Buschard, K. & Rygaard, J. (1978) *Acta Pathol. Microbiol. Scand. Sect. C* **86**, 23–27.
10. Notkins, A. L. (1977) *Arch. Virol.* **54**, 1–17.
11. Buschard, K., Rygaard, J. & Lund, E. (1976) *Acta Pathol. Microbiol. Scand. Sect. C* **84**, 299–303.
12. Buschard, K. & Rygaard, J. (1978) *Acta Pathol. Microbiol. Scand. Sect. C*, **86**, 277–282.
13. Buschard, K., Madsbad, S. & Rygaard, J. (1978) *Lancet* **i**, 908–910.
14. Lipsick, J., Beattie, G., Osler, A. G. & Kaplan, N. O. (1979) *Lancet* **i**, 1290–1291.
15. Thurneyssen, O., Jansen, F. K., Vialettes, B., Vague, P., Selam, J. L. & Mirouze, J. (1979) *Lancet* **i**, 1291–1292.
16. Serra, A. S., Farndon, J. R., Shenton, B. K. & Johnston, I. D. A. (1979) *Lancet* **i**, 1292.
17. Beattie, G., Lannom, R., Lipsick, J., Kaplan, N. O. & Osler, A. G. (1980) *Diabetes* **29**, 146–150.
18. Buschard, K., Rygaard, J. & Madsbad, S. (1979) *Lancet* **ii**, 303.
19. Morgan, C. R. & Lazarow, A. (1963) *Diabetes* **12**, 115–126.
20. Kindred, B. & Loor, F. (1974) *J. Exp. Med.* **139**, 1215–1227.
21. Shin, S., Baum, S. G., Fleischer, N. & Rosen, O. M. (1975) *J. Cell Sci.* **18**, 199–206.
22. Onodera, T., Jenson, A. B., Yoon, J.-W. & Notkins, A. L. (1978) *Science* **201**, 529–531.
23. Gamble, D. R., Taylor, K. W. & Cumming, H. (1973) *Br. Med. J.* **4**, 260–262.
24. Craighead, J. E. (1975) *Prog. Med. Virol.* **19**, 161–214.
25. Yoon, J.-W., Austin, M., Onodera, T. & Notkins, A. L. (1979) *N. Engl. J. Med.* **300**, 1173–1179.
26. Yoon, J.-W., Lesniak, M. A., Fussganger, R. & Notkins, A. L. (1976) *Nature (London)* **264**, 178–180.
27. Rossini, A. A., Appel, M. C., Williams, R. M. & Like, A. A. (1977) *Diabetes* **26**, 916–920.
28. Rubinstein, P., Suciu-Foca, N. & Nicholson, J. F. (1977) *N. Engl. J. Med.* **297**, 1036–1040.