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# Effect of Antilymphocyte Serum and Neonatal Thymectomy upon Onset of Diabetes in the Chinese Hamster (42148)

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*Abstract.* Prediabetic Chinese hamsters were treated with antilymphocyte serum (ALS), or thymectomized in order to test the hypothesis that β-cell loss leading to diabetes in this animal model was related to cell-mediated autoimmunity. In addition, passive transfer of diabetes from the Chinese hamster to the nude mouse was attempted by transplantation of lymphocytes. Treatment of prediabetic Chinese hamsters with ALS or thymectomy did not alter development or severity of diabetes in this animal model. Lymphocytes from newly diagnosed diabetic Chinese hamsters did not cause hyperglycemia in nude mice. These three lines of evidence suggest that cell-mediated autoimmunity does not contribute to the etiology of diabetes in the Chinese hamster. The Chinese hamster remains a good model for the study of those forms of diabetes not related to cell-mediated autoimmune phenomena. 

Lymphocytic infiltration of the islets of Langerhans in human patients with recent onset of Type I IDDM has been described by Lecompte (1) and Gepts (2). This is suggestive of a cellular autoimmune component in the onset of diabetes. Further support for this concept are recent clinical studies which suggest that immunosuppression may ameliorate development of diabetes (3, 4). Some animal models also exhibit lymphocytic infiltration. Insulitis along with glycosuria and hyperglycemia has been described in the NOD mouse (5, 6) and BB rat (7). These observations suggest that lymphocytes may play a role in the destruction of β cells in the islets of Langerhans in these animal models. This concept is supported by observations that treatment with antilymphocyte serum or neonatal thymectomy prevented onset of diabetes in the BB rat (8, 9). Buschard *et al.* (10) have reported hyperglycemia in nude mice by transferring lymphocytes from newly diagnosed IDDM patients. Although unconfirmed, it is an interesting observation and lends further support to the possibility that cellular autoimmunity may be involved in the onset of some types of diabetes.

The Chinese hamster is another animal model with spontaneous diabetes mellitus. Chinese hamsters have been bred for and against diabetes so that inbred lines exist that are essentially either 100% diabetic or nondiabetic. Hamsters are nonobese and display hyperglycemia, decreased pancreatic insulin levels, and relative or absolute plasma insulin deficiency. Diabetes in the Chinese hamster appears to be due to loss of β cells (11). In order to test the hypothesis that loss of β cells is due to cellular autoimmunity, three lines of investigation were pursued. Prediabetics were treated with antilymphocyte serum, neonatal thymectomies were performed, and lymphocytes from newly diagnosed diabetics were transferred to athymic nude mice.

**Materials and Methods.** *Hamsters for ALS study.* Ten-day-old prediabetic and nondiabetic Chinese hamsters were obtained from The Upjohn Company's stock. They were housed in plastic cages fitted with stainless-steel lids and provided with Purina 5015 mouse breeder chow (Purina, St. Louis, Mo.) and water *ad libitum.* Urine was tested for glucose by expressing fresh samples directly onto TesTape (Eli Lilly and Co., Indianapolis, Ind.) every third day. Time of onset of overt diabetes was defined as the age of the animal when marked glycosuria was first detected by a 3+ TesTape value.

*Blood glucose.* Blood samples were obtained from the retro-orbital sinus with a hematocrit tube (12). Glucose was determined by autoanalyzer by the method of Hoffman (13).

*Goat normal serum.* Prior to immunization for production of ALS, a young male goat



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was bled repeatedly over the course of several weeks to provide normal goat serum as control for ALS.

*Preparation of ALS.* Lymphocytes obtained from the spleen, lymph nodes, and thymus of Chinese hamsters were administered subcutaneously at multiple sites in the flank and shoulders of a young male goat. Injections consisting of a total of $2–3 \times 10^7$ lymphocytes were given once a week for 6 weeks. Serum was obtained 1 week after the final immunization.

*Potency of ALS.* Dose finding studies indicated that 0.2 ml of ALS was the most effective on lymphocyte and total white count suppression. When a single injection of ALS (0.2 ml) was given ip to a group of 18 Chinese hamsters, their total white count was depressed to 60% of normal within 3 days. In addition, the percentage of lymphocytes in the differential shifted from 80 to 50%. Further, the effect of ALS was similar in the experimental animals (Table I). Although blood glucose data for the ALS and control serum-treated animals are shown in the manuscript for only 45 days (Fig. 1, Table II), the animals were actually treated for 90 days, and the percentage lymphocytes was monitored by differential. Approximately 30 days after cessation of ALS treatment the percentage of lymphocytes returned to normal levels as indicated at age 129 days. Total white counts were also monitored during the study and ALS-treated animals were 60% of normal serum-treated animals but returned to control levels by age 129 days. To further test the potency of the ALS, reciprocal skin grafts were exchanged between 10 ALS-pretreated Chinese hamsters of incompatible sublines. Ten normal serum-treated animals served as controls. The normal serum-treated control animals rejected their grafts by 10 days of age. Grafts on animals pretreated with ALS did not show signs of rejection until 28 days. Thus, the skin grafts were tolerated about three times as long in ALS-treated animals suggesting an effective ALS preparation.

*Effect of ALS administration on prediabetic Chinese hamsters.* Prediabetic and nondiabetic Chinese hamsters were divided into treatment and control groups when they were 10 days of age. The treatment group received ALS at 0.1 ml/10 grams body wt via the intraperitoneal route every third day for a total of 90 days. The control group received goat normal serum on the same schedule. Animals in both treatment and control groups were TesTaped every third day. Nonfasted glucose values were determined once a week. All hamsters were negative for glycosuria and exhibited a glucose value in the normal range on the day of initiation of the study.

*Thymectomy of Chinese hamsters.* Newborn Chinese hamsters less than 24 hr of age were placed in ice up to the neck for 1–3 min until no movement was detected. Thymectomy was performed with a suction catheter through a midline anterior incision in the lower neck–upper thorax region. Controls received a sham operation. After completion of the operation, the newborns were warmed under a lamp until movement and even breathing were restored, and promptly returned to their mothers. Surviving hamsters were placed at weaning into sterile cages in a Laminar Flow Hood and provided with sterile water and mouse breeder chow 5015 (Purina).

Animals were monitored for glycosuria. They were killed 10 days after onset (3+ TesTape) and subjected to autopsy and the

TABLE 1. PERCENTAGE LYMPHOCYTES OF CHINESE HAMSTERS DURING TREATMENT WITH GOAT ALS OR GOAT NORMAL SERUM

| Treatment | Genotype | Age (days) | | | |
|---|---|---|---|---|---|
| | | 17 | 36 | 60 | 129 |
| Goat normal serum | Nondiabetic | 89.0 ± 2.4 | 91.3 ± 3.1 | 86.7 ± 6.4 | ND |
| Goat ALS | Nondiabetic | 74.0 ± 9.4 | 52.3 ± 1.5 | 56.0 ± 6.9 | 81.8 ± 4.2 |
| Goat normal serum | Diabetic | 87.6 ± 4.1 | 89.0 ± 1.4 | 87.7 ± 2.5 | ND |
| Goat ALS | Diabetic | 59.5 ± 5.5 | 52.3 ± 1.5 | 53.3 ± 3.1 | 83.6 ± 4.6 |

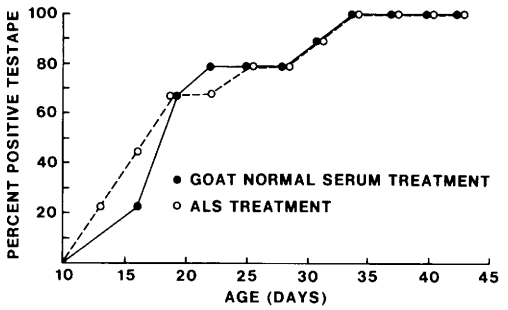


FIG. 1. Cumulative percentage of genetically diabetic Chinese hamsters ($N = 9$) exhibiting a positive (3+) TesTape after treatment with ALS serum versus hamsters ($N = 9$) treated with goat normal serum.

thoracic region was fixed for histologic examination.

*Histologic examinations.* The entire thoracic region was fixed in Bouins solution, paraffin embedded, and serially sectioned. All serial sections were stained with hematoxylin and eosin and examined for thymic remnants.

*Transfer of Chinese hamster lymphocytes into nude mice.* Prediabetic Chinese hamsters (21 days) were monitored daily for glycosuria. Immediately after the first strongly positive TesTape (3+), they were killed and lymph nodes, spleen and thymus tissues taken aseptically to prepare single cell suspensions of lymphocytes for injection. Age and sex matched nondiabetics served as control lymphocyte donors. Nude mice (*nu/nu*) of Swiss background were obtained at 4–5 weeks of age from (Sprague–Dawley, Inc., Indianapolis, Ind.), and injected via the intraperitoneal route with $1 \times 10^8$ lymphocytes from either newly diagnosed or nondiabetic Chinese hamsters. Throughout the course of the experiment the nude mice were housed in sterile cages in a Laminar Flow Hood equipped with HEPA filters and supplied with sterile food and water *ad libitum.* The nude mice were monitored for glycosuria and blood glucose every 3 days for a total of 18 days.

**Results.** *Effect of antilymphocyte serum on the onset and severity of diabetes.* Administration of ALS to diabetic hamsters had no effect on the onset of glycosuria (Fig. 1). The graph shows the percentage of animals testing at least 3+. There is no apparent difference between animals given ALS versus those given goat normal serum. Although not shown, all nondiabetic hamsters were negative for glycosuria whether they received ALS or normal serum. Nonfasted blood glucose values for both nondiabetic and diabetic hamsters are shown in Table II. Comparing the values of normal animals given ALS with those receiving normal serum shows that ALS had no effect on glucose levels of normal animals with the exception of the 17-day values (1 week after initiation of treatment). There is no significant difference between diabetic hamsters (prediabetic at initiation) given ALS versus those given normal serum.

*Effect of thymectomy on the onset and severity of diabetes.* Thymectomized hamsters did not differ significantly from their sham-operated litter mates as to onset of glycosuria (Fig. 2) or body weight, blood glucose, and plasma insulin (Table III). At autopsy, all thymectomized animals were checked for completeness of thymectomy by gross examination and microscopic evaluation of

TABLE II. NONFASTED BLOOD GLUCOSE VALUES (mg/dl) OF DIABETIC AND NONDIABETIC CHINESE HAMSTERS AFTER REPEATED INTRAPERITONEAL INJECTIONS WITH GOAT NORMAL SERUM OR GOAT ALS

| Treatment | *N* | 17 Days | 24 Days | 31 Days | 38 Days | 45 Days |
|---|---|---|---|---|---|---|
| | | | Nondiabetic | | | |
| Goat normal serum | 8 | 110 ± 2.1 | 110 ± 3.3 | 101 ± 2.8 | 106 ± 2.6 | 107 ± 3.3 |
| Goat ALS | 9 | 124 ± 3.7* | 106 ± 3.7 | 108 ± 4.6 | 111 ± 2.2 | 104 ± 3.8 |
| | | | Diabetic | | | |
| Goat normal serum | 9 | 82 ± 6.9 | 193 ± 14.3 | 213 ± 22.5 | 259 ± 21.4 | 259 ± 29.6 |
| Goat ALS | 9 | 103 ± 8.4 | 195 ± 14.3 | 178 ± 12.0 | 255 ± 23.0 | 254 ± 25.6 |

*Note.* $N$ = number of animals per group.
* $P < .01$.

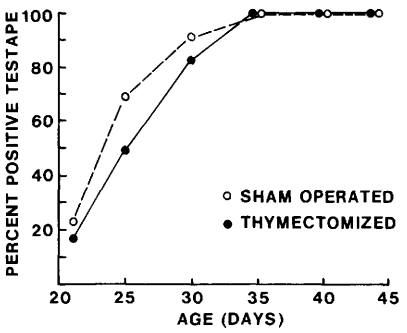


FIG. 2. Cumulative percentage of thymectomized and sham-operated genetically diabetic Chinese hamsters exhibiting a positive (3+) TesTape.

sections from the thoracic region. Gross examination did not reveal thymic tissue but microscopic examination revealed thymic remnants in all cases indicating that animals were partially thymectomized.

*Effect of transplantation of Chinese hamster lymphocytes into nude mice.* Both experimental and control nude mice were negative for glycosuria throughout the course of the experiment. Blood glucose values appear in Table IV. There was no statistical difference between controls and those mice receiving diabetic lymphocytes at any timepoint through Day 18.

**Discussion.** Lymphocytic infiltration of the islets of Langerhans of human patients with recent onset IDDM has been reported by Lecompte (1) and Gepts (2). This is suggestive of a cellular autoimmune component in the etiology of some types of diabetes. Insulitis has also been reported in the diabetic BB rat (7) and the NOD mouse (5, 6). When pancreatic tissue was examined well after onset of diabetes in Chinese hamsters, insulitis was not observed (14–18); however, a steady loss of $\beta$ cells has been reported (11). Lymphocytic infiltration has been reported in the islets of diabetic Chinese hamsters soon after onset by Boquist (19).

In order to determine if the loss of $\beta$ cells might be due to a cellular autoimmunity, we have investigated the effect of lymphocyte suppression by both ALS treatment and neonatal thymectomy. In addition, we have attempted to passively transfer diabetes from the Chinese hamster to the nude mouse via lymphocytes. Within the constraints of these experimental protocols, no differences in body weights, food consumption, or physiologic state of experimental versus control animals were observed.

The ALS serum used in this study lowered the percentage of circulating lymphocytes as determined by differential. Diabetic and nondiabetic animals treated with ALS had lymphocytes ranging from 52 to 59% during the course of the experiment compared with a range of 87 to 91% for animals treated with normal goat serum. Percentage of lymphocytes in the ALS-treated groups returned to normal by 30 days after conclusion of ALS treatment. Although the level of ALS used significantly depressed lymphocytes, the time of onset of glycosuria and hyperglycemia was not affected. Although not shown, plasma insulin values were also not statistically different. This is in contrast to the results of lymphocyte suppression in the BB rat model in which ALS decreased the incidence of clinical disease (8).

Neonatal thymectomy has also been shown to prevent onset of insulitis and diabetes in the BB rat (9). Partial thymectomy also provided significant protection in this animal model (9). It would have been expected that if cellular autoimmunity were a factor in onset of diabetes in the Chinese hamster,

TABLE III. EFFECT OF THYMECTOMY ON WEIGHT, GLUCOSE, AND INSULIN VALUES OF GENETICALLY DIABETIC CHINESE HAMSTERS

| Treatment | *N* | 21-Day weight | 45-Day weight | 45-Day glucose (mg/dl) | 45-Day insulin ($\mu$U/ml) |
|---|---|---|---|---|---|
| Thymectomy | 12 | 13.6 ± 0.2 | 21.1 ± 0.8 | 238 ± 18 | 144 ± 18 |
| Sham thymectomy | 13 | 13.8 ± 0.3 | 21.5 ± 0.5 | 285 ± 18 | 119 ± 14 |

*Note.* N = Number of animal per group.

TABLE IV. AVERAGE NONFASTED BLOOD GLUCOSE OF ATHYMIC NUDE MICE INJECTED WITH LYMPHOCYTES FROM EITHER DIABETIC OR NONDIABETIC CHINESE HAMSTERS (mg/dl ± SEM)

| Days after lymphocyte transfer | N | Genotype of donor chinese hamster | |
|---|---|---|---|
| | | Diabetic | Nondiabetic |
| 0 | 25 | 163 ± 2.0 | 163 ± 2.0 |
| 1 | 8 | 139 ± 4.0 | 138 ± 5.0 |
| 3 | 10 | 150 ± 3.0 | 146 ± 4.0 |
| 5 | 11 | 183 ± 10.0 | 171 ± 10.0 |
| 7 | 8 | 168 ± 12.0 | 189 ± 8.0 |
| 10 | 4 | 143 ± 3.0 | 139 ± 10.0 |
| 14 | 4 | 143 ± 4.0 | 135 ± 5.0 |
| 15 | 7 | 137 ± 10.0 | 136 ± 6.0 |
| 18 | 15 | 140 ± 5.0 | 140 ± 4.0 |

*Note.* $N$ = Number of animals per group.

that partial thymectomy would have afforded some protection against onset of clinical disease but partially thymectomized animals exhibited no alteration in the onset of diabetes.

Passive transfer of chemically induced diabetes from mouse to mouse (20) by transplant of lymphocytes has been demonstrated. In those experiments the results indicate that glucose values are elevated by 3 to 5 days after lymphocyte transfer. It was also noted in the man to mouse transfer (10) that lymphocytes obtained at the onset of severe clinical disease were best able to transfer diabetes to the nude mouse. Therefore, diabetic Chinese hamsters were used as lymphocyte donors in our transfer experiments just at the time of onset of glycosuria, since this appeared to be the time when the lymphocytes are most likely capable of a diabetogenic effect. Under the conditions of the experiment, diabetes was not passively transferred from the Chinese hamster to the nude mouse, since no elevation of glucose occurred through the 18 days of the experiment.

It has been reported that histoincompatibility may be a problem in transfer studies between allogenic mouse strains and nude mice (21, 22). Lack of histocompatibility could explain the lack of effect of Chinese hamster lymphocytes in nude mice. Nakhooda (23) reported mild insulitis but not hyperglycemia in nude mice after injection of BB rat lymphocytes. This could have occurred in the Chinese hamster studies. Unfortunately, pancreata from the nude mice injected with Chinese hamster lymphocytes were not evaluated for insulitis due to technical difficulties. Therefore, this question remains unanswered.

Koevary *et al.* (24) reported insulitis and hyperglycemia in nondiabetic BB rats after injection of congenic concanavalin A-treated spleen cells from BB rats with acute diabetes. This interesting observation suggests that transfer of diabetic Chinese hamster lymphocytes to congenic prediabetics could provide definitive data on cell-mediated autoimmunity in the diabetic Chinese hamster. Since antibody production has been reported to be abnormal in the diabetic Chinese hamster (25, 26), it would have been appropriate and interesting to complement this series of experiments by looking at subsets of lymphocytes. This is impractical, however, since no specific probes are available. We have found in this laboratory that Chinese hamster lymphocytes do not cross-react with antiserum to mouse T-cell subsets.

In this paper we report the failure of ALS treatment or neonatal thymectomy to alter the course of diabetes in the spontaneously diabetic Chinese hamster. Gishizky *et al.* (27) have found that suppressing the lymphocyte population 50% with cyclophosphamide did not alter the onset of glycosuria or hyperglycemia in the Chinese hamster. These observations coupled with failure to observe insulitis in most morphological studies appear to exclude cellular autoimmunity as an etiologic factor in the development of diabetes in the Chinese hamster. In order to resolve this question more completely, a lymphocyte transfer study should be done between the newly diagnosed diabetic hamster and prediabetics of the same genotype.

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