Our results demonstrate the potential utility of polymercurimethanes as labels in conjunction with STEM microscopy for the study of biological structure at high resolution. The clustered TAMM · L_3 units are readily visualized and can be used to identify selectively stained components of macromolecular assemblies, such as the modified fd coat protein in our study.

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Spontaneous Diabetes Mellitus: Reversal and Prevention in the BB/W Rat with Antiserum to Rat Lymphocytes

Abstract. *Injections of rabbit antiserum to rat lymphocytes reversed hyperglycemia in 36 percent of spontaneously diabetic rats (Bio Breeding/Worcester) and prevented diabetes in susceptible nondiabetic controls. These findings strengthen the hypothesis that cell-mediated autoimmunity plays a role in the pathogenesis of diabetes in this animal model that mimics many morphologic and physiologic characteristics of human insulin-dependent diabetes mellitus.*

Diabetes mellitus occurs spontaneously in approximately 30 percent of a nonobese, outbred colony of Bio Breeding/Worcester (BB/W) (*1*) rats. The rapidly progressive syndrome is characterized by abrupt early onset (60 to 120 days), pronounced hyperglycemia, reduced concentrations of pancreatic and circulating insulin, hyperglucagonemia, and ketoacidosis. Without insulin replacement therapy, most animals succumb within 1 to 2 weeks of the detection of glycosuria (*2*). A unique feature of this model is the presence of profound insulitis prior to and early in the syndrome, with lymphocytes, macrophages, and occasionally eosinophils infiltrating the pancreatic islets. Late in the disease the islets are small and marked by the absence of insulin-synthesizing B (β) cells (*2*). The physiologic and morphologic characteristics of these animals closely resemble those of insulin-dependent humans with juvenile-onset diabetes. The demonstration that selective inbreeding of diabetic animals increases the frequency of diabetes, and the lymphocyte and macrophage nature of the insular infiltrate, suggests a cell-mediated autoimmune pathogenesis of the syndrome.

We present evidence here that the administration of rabbit antiserum to rat lymphocytes (ALS) normalized the concentration of plasma glucose (PG) in 36 percent of acutely diabetic rats and prevented the occurrence of diabetes in susceptible nondiabetic littermates. In contrast, hyperglycemia persisted in rats that were untreated or were injected with normal rabbit serum (NRS). Furthermore, untreated and NRS-injected euglycemic littermates became diabetic with the expected frequency. The effectiveness of ALS in the treatment and prevention of diabetes supports an autoimmune pathogenesis of the syndrome.

The BB/W rats in the susceptible age range (60 to 120 days) were tested for glycosuria three times weekly. Rats were defined as diabetic if their urine glucose indicated 2+ with Testape (*3*) and if they had PG concentrations greater than 180 mg/dl. The disease was detected at a mean age of 101 days. The PG concentration in most animals exceeded 300 mg/dl. Diabetics (N = 93) and nondiabetic

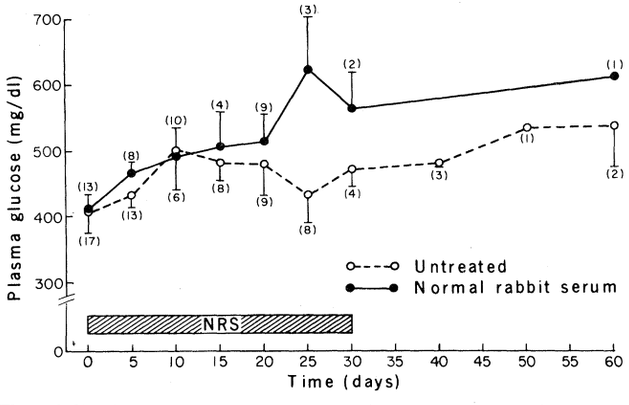

Fig. 1 (left). Plasma glucose concentrations in untreated and NRS-injected BB/W rats studied from the first detection of glycosuria (day 0). The concentrations for both groups of animals remained elevated for the duration of the experiment. Four rats survived beyond 30 days. The others died or were killed at earlier times when moribund. In one untreated rat the concentration spontaneously returned to normal (data not shown). Vertical bars indicate standard errors of the means; numbers of animals are shown in parentheses.

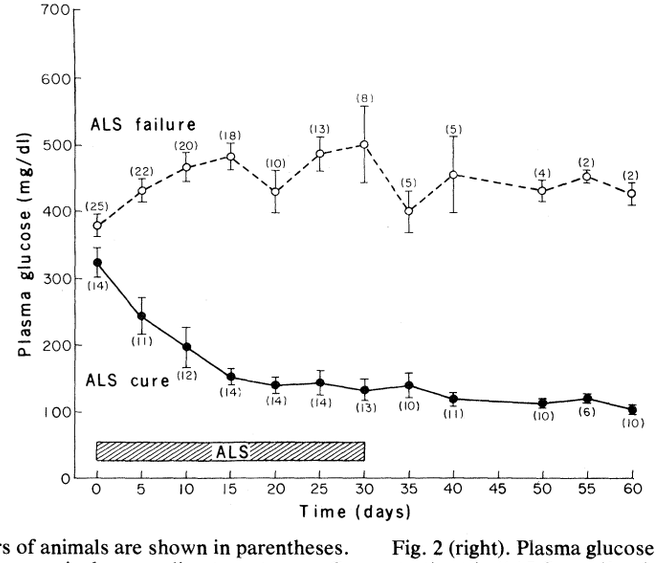

Fig. 2 (right). Plasma glucose concentrations (means ± S.E.) of ALS-injected BB/W rats, plotted retrospectively according to outcome. Concentrations in "ALS cure" animals returned to normal 10 to 15 days after the first injection and remained normal for the duration of the experiment. Concentrations in "ALS failure" rats resemble those of NRS-injected and untreated animals (Fig. 1). In ALS cure rats at day 0 the PG concentrations were significantly lower than in the ALS failure rats ($P < .05$).

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littermates (or age-matched euglycemic animals) (N = 86) were randomly assigned to four treatment groups. The rats in each group received ALS (*4*), NRS (*5*), or whole body x-irradiation (500 rads) on the day diabetes was detected, or remained untreated. The animals were tested weekly for 60 days for urine glucose (Testape) and PG concentrations. Surviving nondiabetic rats were subjected to glucose tolerance tests at 30 and 60 days and killed at 60 days. Diabetics were killed at 60 days, or earlier, when moribund. Samples of tail blood for PG were collected in heparin-treated pipettes and assayed as described (*6*). The unpaired t-test (*7*) was used in statistical analyses. Pancreatic tissues obtained at death were fixed for light microscopy (*8*).

Figure 1 illustrates the pattern of unremitting hyperglycemia among 30 untreated and NRS-injected rats. Only a small number of these animals survived to 60 days. Most died or were killed early in the study when death appeared imminent. One of the 18 untreated rats (not included in Fig. 1) showed normalization of PG, bringing to six the total number of spontaneous cures in more than 1000 diabetic animals studied (data not illustrated). In contrast, glucose concentrations returned to normal within 10 to 15 days in 14 out of 39 (36 percent) ALS-treated diabetics and remained in the normoglycemic range for the duration of the experiment (Fig. 2). All 14 "cured" animals were glucose-intolerant when tested at 60 days (*9*). In 3 out of 23 (13 percent) irradiated diabetics, the PG concentration also returned to normal. Radiation sickness and mortality were frequent, making it difficult to interpret these results.

Of great interest was the response of susceptible nondiabetic animals to the four treatment regimens. None of the 27 ALS-injected or 18 irradiated animals became diabetic. In contrast, 8 out of 24 (33 percent) and 3 out of 17 (18 percent) untreated and NRS-injected rats, respectively, became diabetic, with patterns of PG indistinguishable from those illustrated in Fig. 1. Glucose tolerance tests at 60 days were abnormal in 48 percent of ALS-injected euglycemic littermates.

Morphologically, the pancreatic islets of diabetic rats that did not respond favorably to ALS or irradiation, and the islets of untreated and NRS-injected euglycemic animals that became diabetic, were almost all identical (Fig. 3, A and B). Most were small and revealed no evidence of surviving β cells. These "end-stage" islets were composed predominantly of A (α) cells and a small number of D (δ) and pancreatic polypeptide (PP) cells (not illustrated). The pancreases of cured diabetics were variable: A mixture of enlarged or normal, focally scarred and inflamed, and end-stage islets were almost always present (Fig. 3, C and D). The islet histology of ALS-injected or irradiated euglycemic littermates was usually but not always normal. Focal insulitis, scarring, and architectural distortion were observed, most frequently in animals with abnormal glucose tolerance tests.

Although numerous spontaneously diabetic laboratory animals have been studied in the past 25 to 30 years, very few have provided true examples of nonobese, insulin-dependent, ketosis-prone diabetes. Furthermore, none of the animals developed the pancreatic insulitis that is so characteristic of the the early stages of juvenile-onset diabetes in the human (*10*). Insulitis has been reported in experimental virus-induced diabetes (*11*), after unsuccessful attempts to produce an immune-type diabetes (*12*), and in mice injected with subdiabetogenic doses of streptozotocin (*13*). The BB/W rat provides the only reported example of spontaneous insulin-dependent diabetes in the rat and is the only species that also develops destructive insulitis resembling the lesions described in the human. The successful normalization of PG in 36 percent of diabetic BB/W rats and the prevention of hyperglycemia in susceptible littermates by injections of ALS support the idea that autoimmunity has a role in the pathogenesis of this syndrome.

The fact that PG concentrations did not normalize in all ALS-treated diabetic rats suggests that only those animals with sufficient numbers of viable β cells at the time glycosuria was detected would respond favorably to immune intervention therapy. The mean PG concentration on day zero of ALS-treated diabetic rats that eventually developed normal PG was signficantly lower ($P < .05$) than the PG of rats that did not respond to ALS. The frequent presence of healed insulitis and end-stage islets in ALS-cured rats is further evidence that significant islet injury occurred prior to the institution of ALS therapy.

Since none of the ALS-treated or irradiated euglycemic littermates became hyperglycemic, it is assumed that properly timed immunosuppression, of the magnitude used, can prevent BB/W diabetes. The abnormal glucose tolerance

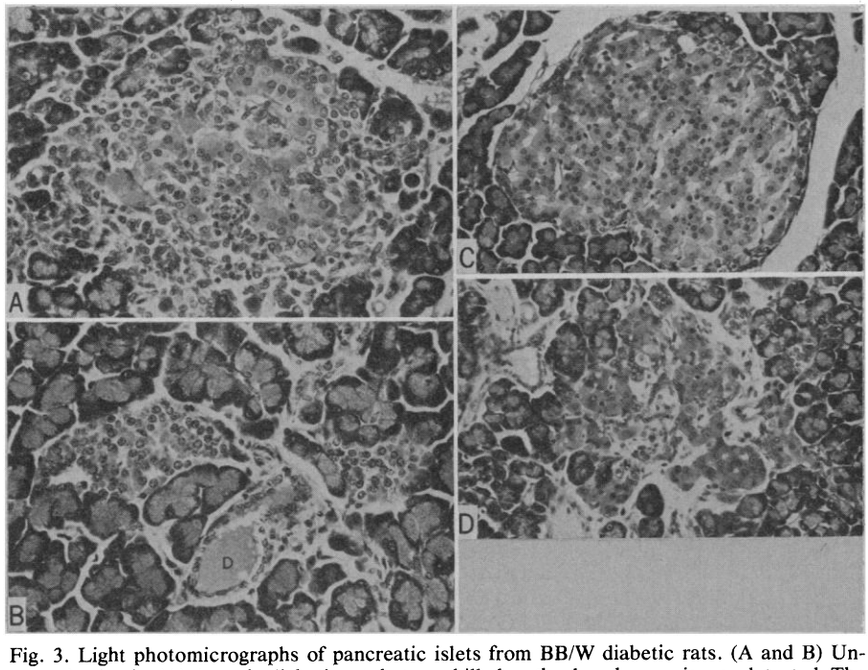

Fig. 3. Light photomicrographs of pancreatic islets from BB/W diabetic rats. (A and B) Untreated animals. (A) Acutely diabetic rat that was killed on the day glycosuria was detected. The islet is infiltrated with lymphocytes and macrophages that distort the islet architecture and obscure the exocrine-islet interface. (B) Chronically diabetic rat that was killed several months after the onset of diabetes. Small end-stage islets are adjacent to a pancreatic duct (*D*). Insulitis is absent. Immunocytochemistry (not illustrated) revealed the virtual absence of β cells, relatively increased numbers of α and infrequent D and PP cells (hematoxylin and eosin; ×350). (C and D) ALS-cured rats. (C) This enlarged islet is virtually normal. Insulitis is absent, the exocrine-islet interface is distinct, and all component endocrine cells are present. (D) Islet distorted by intra- and peri-islet scarring reveals an irregular contour and the peripheral intermingling of islet and exocrine cells. ALS-cured rats also showed small end-stage islets indistinguishable from those in (B) (hematoxylin and eosin; ×224).

tests and the focal islet lesions among many ALS-injected and irradiated euglycemic littermates are interpreted as evidence that ALS did not completely protect against the hypothesized cell-mediated autoimmune process, even when therapy preceded the detection of glycosuria. It is not known whether insulitis and β cell injury may have preceded ALS or irradiation therapy, since these animals were not tested for glucose tolerance prior to the experiments (*14*). It is conceivable that complete or more lasting protection of the pancreatic β cell mass might result from different modes or schedules of immunosuppression. The data presented, however, do establish that empiric immunosuppression may ameliorate or prevent spontaneous diabetes in the BB/W rat, in a manner analogous to the immunological prevention of virus-induced (*15*) or chemically induced (*16*) diabetes in mice.

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Clonal Characteristics of Experimentally Induced "Atherosclerotic" Lesions in the Hybrid Hare

Abstract. *The female hybrid hare (*Lepus timidus × Lepus europaeus*) is heterozygous for eletrophoretically separable, X-linked isoenzymes of glucose-6-phosphate dehydrogenase. The isoenzymes of this animal have been used as cellular markers in the study of the clonal origins of experimentally induced atherosclerotic lesions. Aortic lesions produced in the hybrid hare by feeding cholesterol and injuring the aortic wall with a catheter have been shown to have polyclonal characteristics and in this way are fundamentally different from atherosclerotic fibrous plaques in man.*

The X-linked enzyme, glucose-6-phosphate dehydrogenase (G6PD) (E.C. 1.1.1.49), has been used as a cellular marker to investigate the clonal origins of a variety of human lesions. Studies carried out on tissue from black American females heterozygous for G6PD isoenzymes showed that a number of types of tumors, both benign and malignant, originate from a single clone of cells (*1*). Other studies have been performed on atherosclerotic lesions, leading to the observation that the majority of atherosclerotic plaques have monoclonal characteristics (*2*).

Progress in our understanding of the biology of human atherosclerosis has been hindered by the lack of appropriate animal models. The rabbit has been used extensively, and "atherosclerotic" lesions have been produced by feeding the animal cholesterol (*3*) or by injury to the intimal surface of an artery from a catheter (*4*). However, these lesions differ

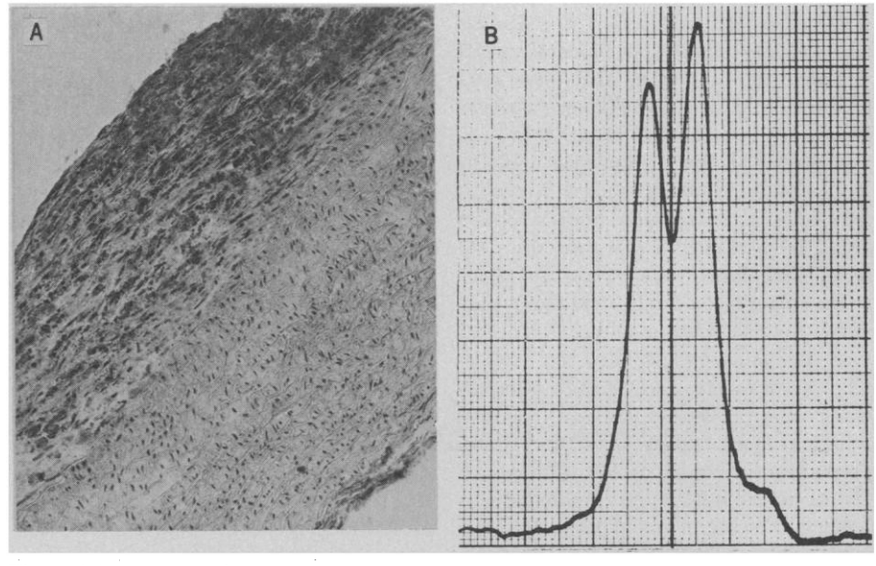

Fig. 1. (A) Frozen section of "atherosclerotic" lesion showing abundant lipid and elongated cells. Oil Red O stain; ×110. (B) Densitometric scan of G6PD isoenzyme bands from an "atherosclerotic" lesion of animal 1. Dissection and cellulose acetate electrophoresis were carried out with the use of standard techniques (*2*). A tris-EDTA-glycine buffer (*p*H 9.2; Gelman) was used. Eight samples were applied to each electrophoresis plate, and electrophoresis was carried out at 360 V for 23 minutes in a Helena electrophoresis chamber. Following specific enzyme staining, the amount of enzyme activity in each isoenzyme band was quantitated with the use of a Helena Quick-Scan densitometer. This densitometric scan of G6PD bands shows a very adequate separation of isoenzyme bands. The results are expressed as the percentage contribution of the slower (*L. timidus*) isoenzyme band (right side) to the total G6PD activity.

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