transferred, PLP, as a gene transfer system, offers greater efficiency than the widely used calcium phosphate coprecipitation method. In addition, the PLP system provides a novel capability for application to gene transfer studies in vitro and, potentially, in vivo.

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Passive Transfer of Diabetes in the BB/W Rat

Abstract. *Severe diabetes with insulitis was produced in young diabetes-prone BB/W rats by passive transfer of concanavalin A–treated spleen cells from BB/W animals with acute diabetes. Spleen cells alone or in combination with lymph node cells were active in transferring disease.*

Autoimmunity may play an important role in the pathogenesis of insulin-dependent diabetes (*1*). The spontaneously diabetic Bio Breeding (BB) rat constitutes an important model for the study of autoimmune diabetes. These nonobese animals generally first manifest hyperglycemia between 60 and 120 days of age, with a mean age of onset close to 90 days. Approximately 30 to 50 percent become affected (*2*). Once diabetic, the animals require daily injections of insulin for survival.

A salient feature of the disease is severe insulitis in animals with acute diabetes (*3*). Other abnormalities of the immune system include lymphocytic thyroiditis and the presence of antibodies to the islet cell surface as well as to lymphocytes and other tissues (*4–6*). Administration of an antiserum to lymphocytes prior to or at the time of onset of hyperglycemia is useful in preventing the development of permanent diabetes, as is neonatal thymectomy (*7, 8*) and bone marrow transplantation (*9*).

These studies in the BB rat and other experimental systems suggest a role for cell-mediated immunity in the pathogenesis of diabetes. Strong support for this hypothesis would be provided by the demonstration that diabetes or insulitis can be produced by the passive transfer of cells from affected animals. This has proved difficult to do (*10–12*). A recent study described the passive transfer of mild insulitis from BB rats with acute diabetes to nude mice by means of spleen and peripheral blood cells (*13*): another described the transfer of insulitis to x-irradiated BB recipients (*9*). Other attempts to transfer insulitis from diabetic BB/W rats to nude rats or mice were unsuccessful (*14*).

It seemed possible that the efficiency of transfer might be enhanced by culturing donor cells with the T cell mitogen concanavalin A (Con A) (*15*), as is the case for another autoimmune disease in rodents, experimental allergic encephalomyelitis (*16*). We therefore attempted to transfer diabetes in the BB/W rat using spleen and lymph node cells cultured with Con A (*17*). We first injected cells from BB/W donors with acute diabetes into young, 30- to 40-day-old diabetes-prone BB/W recipients (*18*). We reasoned that passive transfer would produce early appearance of diabetes, and that a much higher than expected percentage would become diabetic.

The results (Table 1) supported these assumptions. Spleen cells alone or in combination with smaller numbers of lymph node cells produced severe hyperglycemia in 19 of 21 recipients (*19*). In some recipients, hyperglycemia appeared within 7 days after transfer, with a mean interval of 12 ± 1 days (± standard error of the mean). The age of onset was 50 ± 1 days in the 19 recipients which became diabetic. This is significant since spontaneous diabetes rarely appears before 60 days of age. Within the range of cell numbers used in these experiments (50×10^6 to 120×10^6) there

Table 1. Passive transfer of diabetes by means of cells cultured with Con A. The recipients received Con A–treated spleen cells (50×10^6 to 100×10^6) alone or in combination with lymph node cells (5×10^6 to 20×10^6). Plasma glucose concentrations in rats were determined from blood samples from a tail vein before the donors were killed and at least three times weekly in recipients by means of a Beckman glucose analyzer. The values, in milligrams per deciliter, were (mean ± standard error of the mean): donors with acute diabetes, 399 ± 24; Wistar Furth, 124 ± 4; diabetes-prone rats prior to transfer of cells or before being injected with medium alone, 126 ± 4; low-incidence rats, 125 ± 3; and diabetes-prone rats after transfer of cells from rats with acute diabetes, 520 ± 40 (when killed). Glycosuria in nude mice (BALB/c and SWR, *nu/nu*) was determined with Testape.

Donor	Recipient	Number of diabetic rats at 60 days of age	Number tested
BB/W with acute diabetes	Diabetes-prone BB/W	19	21
BB/W with acute diabetes	Low-incidence BB/W	0	14
BB/W with acute diabetes	Wistar Furth	0	8
BB/W with acute diabetes	Nude mice	0	8
Diabetes-prone BB/W	Diabetes-prone BB/W	0	6
Low-incidence BB/W	Diabetes-prone BB/W	0	5
Wistar Furth	Diabetes-prone BB/W	0	4
None (RPMI-1640 medium alone)	Diabetes-prone BB/W	0	30

was no apparent correlation between numbers of cells injected and speed of onset of hyperglycemia. Of the 32 control rats that received an injection of medium alone, two were killed within 2 weeks for evaluation of pancreatic morphology, which appeared normal. Of the remaining 30 rats, ten eventually became diabetic, with a mean age of onset of 86 ± 5 days. The other 20 animals were followed for at least 118 days and remained normoglycemic.

Most of the animals were killed within 1 week of onset of hyperglycemia in order to assess the presence of insulitis (*20*). This was present in each of the 12 animals examined. The insulitis appeared similar to that in spontaneously diabetic animals.

Of the two diabetes-prone recipients that failed to develop hyperglycemia when injected with cells from donors with acute diabetes one appeared unhealthy and died 31 days after transfer. Autopsy was not performed because of autolysis. The second animal was killed at 120 days of age, and the pancreatic morphology appeared normal.

We also investigated a number of other donor-recipient combinations. We were unable to transfer diabetes to partially inbred BB/W rats in which there was a low incidence of the disease (*21*). Transfer to immunologically deficient athymic nude mice was also unsuccessful. Furthermore, to date we have been unable to transfer diabetes using diabetes-prone donors prior to the onset of hyperglycemia.

Further experiments must be conducted to determine the precise role of Con A in activating cells so that they transfer insulitis and diabetes, and to identify the specific cell type or types responsible for transfer. The question of whether transferred cells become localized in the pancreatic islets and whether there is recruitment of host cells in the resultant insulitis and β cell necrosis must also be examined. The model described in this report may furnish a means for delineating the β cell antigen against which presumptive pancreatic autoreactive T cells are directed in the spontaneously diabetic BB rat.

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16. Experimental allergic encephalomyelitis is an inflammation of the central nervous system produced in susceptible strains of mice or rats by injection of myelin basic protein. Passive transfer of the clinical disease is possible with the use of 10^8 freshly isolated lymph node cells but not with freshly isolated spleen cells. As few as 2×10^7 spleen cells that have been cultured with Con A for 3 days are capable of transferring the disease.
17. Spleen and pancreatic plus cervical node cells were prepared by teasing tissue apart. The cells were cultured in RPMI-1640 medium containing Con A (5 μg/ml), 10 percent heat-inactivated fetal calf serum, 5 m*M* L-glutamine, 5×10^{-5} *M* β-mercaptoethanol, and sodium penicillin (400 U/ml). Spleen and node cells from individual animals were cultured separately for 3 days in flasks containing 150 ml and 100 ml of medium, respectively. Cells were collected, washed, resuspended in 1 to 2 ml of serum-free RPMI-1640 medium, and injected into the tail vein. Recipients received either spleen cells (50×10^6 to 100×10^6), node cells (5×10^6 to 20×10^6), or a combination of the two (55×10^6 to 120×10^6).
18. BB/W designates Bio Breeding/Worcester rat. Donors with acute diabetes and diabetes-prone recipients were produced by random matings between diabetic males and nondiabetic females. Male and female rats with acute diabetes, 56 to 115 days old, were used within 4 days of the onset of hyperglycemia. The term "diabetes-prone" refers to rats that may eventually become hyperglycemic.
19. Although our first experiments suggested that a combination of spleen and node cells produced more rapid onset of disease than spleen cells alone, this was not borne out in subsequent experiments. Lymph node cells alone from donors with acute diabetes failed to produce hyperglycemia in the young diabetes-prone recipients ($N = 7$). However, the number of node cells harvested from each of the animals with acute diabetes was only 10 to 20 percent of the number of spleen cells.
20. Tissue was fixed in Bouin's solution and embedded in paraffin, and sections were stained with hematoxylin and eosin.
21. This low-incidence line, designated VB, was produced by ten generations of inbreeding of nondiabetic BB/W rats. There have been no diabetic VB animals for the past three generations.
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Monocyte Chemotaxis: Stimulation by Specific Exosite Region in Thrombin

Abstract. *Human α-thrombin is a potent chemoattractant for human monocytes, with optimum activity occurring at about 10 nanomoles per liter. A variety of thrombins that were chemically modified to alter procoagulant or esterolytic functions showed a similar optimum activity, but complexes of prothrombin or α-thrombin with either antithrombin III or hirudin did not. These findings indicate that the regions in thrombin responsible for monocyte chemotaxis are proximate to those involved in certain protein recognition interactions of α-thrombin (for example, hirudin binding) but are distinct from the catalytic site and from certain exosites required for clotting.*

Thrombin (E.C. 3.4.21.5) is generated from its circulating zymogen, prothrombin, during blood coagulation and, once activated, plays multiple roles in hemostasis (*1*). In addition to clotting fibrinogen and enzymatically activating other parts of the plasma-clotting system (for example, factors V, VIII, and XIII), thrombin stimulates the aggregation of release of platelets (*2*), various endothelial cell functions (*3*), smooth muscle contraction (*4*), and mitogenesis (*5*). We reported that thrombin, at concentrations consistent with those generated in vivo (*6*), also elicits a chemotactic response from human peripheral blood monocytes and that this activity does not require a catalytically functional enzyme (*7*).

A variety of thrombins with modifications of the catalytic site and exosite were tested in an effort to identify the domain or domains in thrombin that are responsible for stimulating monocyte chemotaxis. Chemotaxis was assessed in Boyden-type chambers by the double filter method (*7, 8*). In brief, mononuclear cells were isolated from peripheral blood (*9*) drawn from ten healthy donors and transferred in equal portions into the upper compartment of the chemotaxis chambers. Native or modified thrombins were added to the upper compartment, lower compartment, or both compartments. After 2 hours of incubation at 37°C, the pairs of filters were removed, stained, mounted on slides, and scored microscopically for the number of mono-