Yonsei Medical Journal
Vol. 28, No. 2, 1987

The Effect of Pertussis Vaccine and Cyclosporin on Streptozotocin Induced Diabetic Rats

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The injection of streptozotocin (stz) at a high dose (60 mg/kg) into young male rats produces direct beta cell destruction and leads to insulin dependent diabetes (IDD). In contrast, the injection of multiple smal doses of stz (40 mg/Kg/d for 5 days) produce IDD, which resembles type I diabetes in man. The provocative effects of the pertussis vaccine (PV) and cyclosporin (CA) against the development of IDD induced by stz were studied. When PV in a dose of 3.75×10^{10} microorganism was administered to single or multiple stz treated rats, hyperglycemia still developed and persisted during the experiment. No difference was noted in blood glucose levels, but plasma insulin levels were higher in PV treated rats. When CA (10 mg/Kg) was administered daily to single or multiple stz treated rats, hyperglycemia seemed to be lower, but this was not statistically significant, however, plasma insulin levels were higher in CA treated rats. The results of this experiment suggest that PV and CA provide some protection to the beta cells of the pancreas.

Key Words: Pertussis vaccine, cyclosporin, streptozotocin, insulin dependent diabetes.

Streptozotocin (stz) is a broad spectrum agent possessing antitumor, oncogenic and diabetogenic properties. This last activity is mediated by pancreatic beta cell destruction and is widely used as a method for inducing diabetes in experimental animals (Lide & Rossini 1976). When a single, sublethal, high dose of stz was administered to susceptible animals, rapid destruction of the islet beta cells ensued, followed almost immediately by profound and permanent hyperglycemia (Junod *et al.* 1969).

In contrast, the administration of multiple small does of stz caused pancreatic insulitis associated with the induction of endogenous type C viruse in beta cells, and produced a permanent form of insulin dependent diabetes in experimental animals (Like & Rossini 1967)

The administration of multiple small doses of stz induces the triad of direct beta cell cytotoxicity, virus induction within beta cells and cell mediated autoimmune reaction (Rossini *et al.* 1977).

Thus, this stz-induced diabetes has become an increasingly useful model with which to study the pathogenesis of insulin dependent diabetes because it resembles many of the biochemical, immunologic and histologic changes reported in recent onset insulin dependent diabets in man (Like & Rossini 1976; Huang & Taylor 1981)

It provides an opportunity for the study of intervention at the early stages of diabetes which invariably carry a high frequency of complications in spite of insulin treatment. Several agents such as diazoxide, antilymphocyte antiserum, nicotinamide, adrenergic blockers, hydrocortisone can produce protection from diabetes induced by stz. (Cubert *et al.* 1974; Iwatsuka *et al.* 1974; Sumi and Ui 1975; Katada and Ui 1977).

Pertussis vaccine (PV) also has an immunosuppressive effect and curtails stz induced diabetes (Katada and Ui 1977). Recently cyclosporin (CA) was found to be effective in suppressing the T cell response (Borel 1976; Wiesinger and Borel 1979).

In this study PV and CA were administered to stz treated rats and their protective effects against the development of insulin dependent diabetes were observed.

MATERIALS AND METHODS

A total 140 Sprague-Dawley male rats each weighing 100-150 gm were used. They were divided

Received February 5, 1987
Accepted April 7, 1987
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into groups as follows.

Experiment I : single high dose of stz (60 mg/Kg)

Group 1 : control (n=10)
2 : stz (n=20)
3 : stz+pv (n=20)
4 : stz+cA (n=20)

Experiment II : multiple small doses of stz (40 mg/Kg/day, for 5 days)

Group 1 : control (n=10)
2 : stz (n=20)
3 : stz+pv (n=20)
4 : stz+cA (n=20)

Experiment I : Streptozotocin (Sigma No S-01310, 60 mg/Kg), freshly dissolved in citrated buffer (pH 4.5, 0.1 mol/ℓ) was injected intraperitoneally (IP) into group 2 nonfasting rats. The animals in group 3 were given the pertussis vaccine (DS Co) intraperitoneally (IP) in a dose of 0.25 ml containing approximately 3.75×10^{10} organisms, on the same day. PV was then administered weekly during the experiment. Cyclosporin (Sandimmun®, 10 mg/Kg), diluted in normal saline, was injected intraperitoneally daily into the group 4 rats during the experiment.

Experiment II : Streptozotocin (40 mg/Kg) was injected intraperitoneally daily for 5 days by the same method as in experiment I. In groups 3 and 4, PV and CA were injected respectively as in experiment I. Weekly blood samples were taken from tail vein, and the whole blood glucose level was tested with glucoscot. Urine glucose and ketone were checked with clinistix and ketostix respectively each week. Plasma insulin levels were evaluated by radioimmunoassay at 3 days, 7 days, 4 weeks and 8 weeks of the experimment.

RESULTS

Fig. 1 illustrates the time course of blood glucose levels of rats injected with a single dose of stz (60 mg/Kg) and with pv and cA in experiment I. Hyperglycemia was defined as a blood glucose level of greater than 200 mg% (Huang *et al.* 1984).

Blood glucose levels were higher in the stz treated experimental groups than in the controls ($P<0.05$).

Group 2 which received stz only developed hyperglycemia within 7 days, and it persisted for 8 weeks. Glycosuria was noted 7 days after stz injection. Group 3 which received stz and pv simultaneously developed hyperglycemia 7 days after injection, and it persisted for 8 weeks. The degree of hyperglycemia did not differ from that of group 2.

Group 4 which received stz and cA simultaneously developed hyperglycemia 7 days after injection, and it persisted for 8 weeks. The degree of hyperglycemia seemed to be lower but there was no statistical significance ($P>0.05$). No ketonuria developed during experiment I (Table 1).

The blood glucose and insulin values of experiment I are presented in Table 2. Group 2, stz only, developed hypoinsulinemia (21.1 μU/ml) 4 weeks after

Table 1. Blood glucose levels (mg%) of experiment I.

Time	Mean Glucose Level (mg%) ± SE			
	Group 1	Group 2	Group 3	Group 4
3 days	83.4±2.0	195.0±22.4	175.8±10.9	198.0±27.7
1 week	80.8±2.5	271.8±51.2	232.8±34.3	218.8±29.9
2 week	86.0±7.1	269.0±36.1	242.4±17.0	239.2±24.3
3 week	78.8±1.5	288.6±34.5	247.8±44.4	213.2±22.1
4 week	73.8±5.2	269.4± 7.0	266.8± 6.2	233.4±20.5
5 week	87.4±7.8	298.2±31.6	348.2±37.4	281.4±42.5
6 week	82.4±6.2	301.4±37.2	317.6±32.3	234.8±21.2
7 week	80.4±3.3	385.8±32.8	306.8±16.5	237.8±12.6
8 week	81.8±8.2	391.6±24.9	437.4±45.4	370.8±26.7

Stz: Streptozotocin PV: pertussis Vacine CA: Cyclosporin A

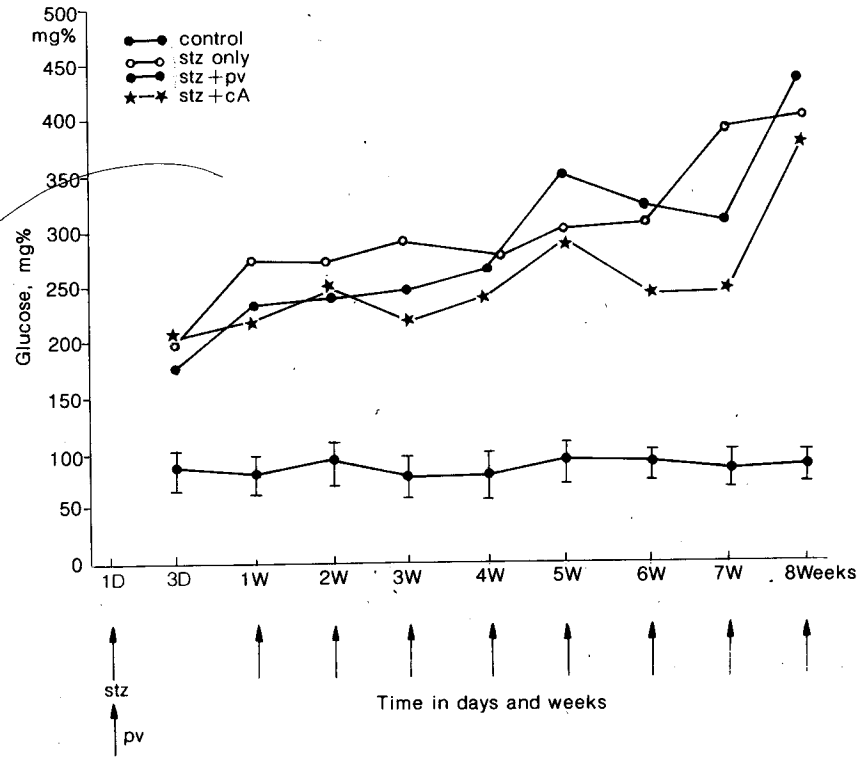

Fig. 1. The sequential changes of blood glucose levels in experiment I.

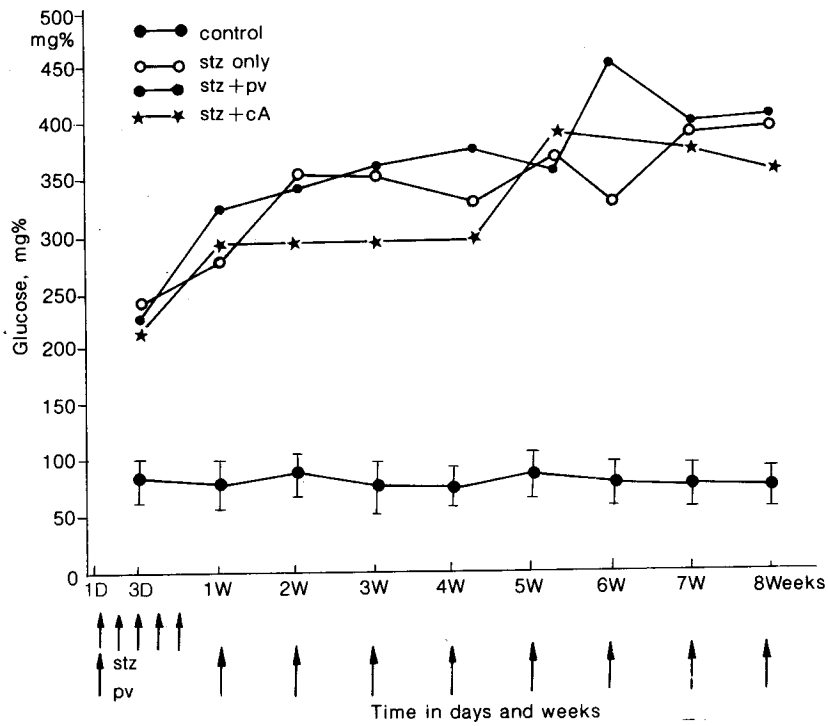

Fig. 2. The sequential changes of blood glucose levels in experiment II.

Table 2. The sequential changes in blood glucose, insulin & urine glucose in experiment I

Mean±SE

Time after stz injection			Group 1	Group 2	Grpup 3	Group 4
3 days	Blood	glucose (mg%)	84.2±2.8	195.0±22.4	175.8±10.9	198.0±27.7
		Insulin (uU/ml)	27.7±1.8	24.9± 1.2	23.8± 1.8	27.9± 1.5*
	Urine	glucose	–	–	–	–
		Ketone	–	–	–	–
7 days	Blood	glucose (mg%)	83.4±3.9	271.8±51.2	232.8±34.3	218.8±29.9
		Insulin (uU/ml)	24.8±1.7	29.7± 1.4	31.4± 1.8	36.2± 1.4*
	Urine	glucose	–	+	–	–
		Ketone	–	–	–	–
4 weeks	Blood	glucose (mg%)	73.8±5.2	269.4± 7.0	266.8± 6.2	233.4±20.5
		Insulin (uU/ml)	28.7±1.6	21.1± 1.8	24.6± 2.5	15.2± 1.8
	Urine	glucose	–	+	–	–
		Ketone	–	–	–	–
8 weeks	Blood	glucose (mg%)	81.8±8.2	391.6±24.9	437.4±45.4	370.8±26.7
		Insulin (uU/ml)	27.9±1.8	13.9± 1.4	*21.8± 1.4	14.4± 1.5
	Urine	glucose	–	++	–	–
		Ketone	–	–	–	–

* $p<0.05$ as compared with Group 2
stz:streptozotocin pv: pertussis vaccine cA: cyclosporin A

Table 3. Blood glucose levels (mg%) in experiment II

Time	Mean Glucose Level (mg%) ± SE			
	Group 1	Group 2	Grpup 3	Group 4
3 days	84.2±2.8	238.8±12.5	226.8±26.1	221.6±18.4
1st week	83.4±3.9	281.6±68.5	328.0±36.4	290.0±28.1
2nd week	86.0±7.1	354.0±46.2	343.8±37.7	290.6±21.8
3rd week	78.8±1.5	353.2±27.0	353.0±32.3	290.4±33.2
4th week	73.8±5.2	334.2±12.4	380.8± 6.8	295.4±43.7
5th week	87.4±7.8	372.8±24.1	360.6±25.3	390.8±25.0
6th week	82.4±6.2	331.2±28.7	462.0±27.6	451.0±25.1
7th week	80.4±3.3	396.8±18.9	406.4±31.0	383.4±23.9
8th week	81.8±8.2	403.0±29.1	412.4±20.9	365.4±14.3

stz : Streptozotocin
pv : pertussis vaccine
cA : cyclosporin A

the single injection of stz.

Group 3, stz and pv, retained relatively higher insulin values at 8 weeks as compared with the stz only group ($P<0.05$).

Group 4, stz and cA retained relatively higher insulin values at 3 days and 7 days as compared with the only treated group ($P<0.05$).

Fig. 2 shows serial blood glucose levels of rats in

Table 4. The sequential changes in blood glucose, insulin & urine glucose in experiment II

Time after stz injection			Group 1	Group 2	Group 3	Group 4
3 days	Blood	glucose (mg%)	84.2±2.8	238.8±12.5	226.8±26.1	198.0±27.7
		Insulin (uU/ml)	27.7±1.8	23.9± 1.2	*39.1± 1.8	23.4± 1.8
	Urine	glucose	–	–	–	–
		Ketone	–	–	–	–
7 days	Blood	glucose (mg%)	83.4±3.9	281.6±68.5	328.0±36.4	290.0±28.1
		Insulin (uU/ml)	24.8±1.7	23.8± 1.4	*36.9± 1.4	18.9± 2.7
	Urine	glucose	–	++	++	++
		Ketone	–	–	–	–
4 weeks	Blood	glucose (mg%)	73.8±5.2	334.2±12.4	380.8± 6.8	295.4±43.7
		Insulin (uU/ml)	28.7±1.6	13.3± 1.5	*16.2± 1.8	15.8± 1.6
	Urine	glucose	–	++	+	–
		Ketone	–	–	–	1
8 weeks	Blood	glucose (mg%)	81.8±8.2	402.0±29.7	412.2±20.9	365.4±14.3
		Insulin (uU/ml)	27.9±1.8	10.1± 1.3	11.4± 2.8	*14.4± 1.8
	Urine	glucose	–	+++	+++	++
		Insulin	–	–	–	–

* $p<0.05$ as compared with Group 2
stz:streptozotocin pv: pertussis vaccine cA: cyclosporin A

experiment II with multiple subdiabetogenic doses of stz (40 mg/Kg/d, 5 days) and with pv and cA.

The blood glucose level in the stz treated experimental group was much higher than that of the control ($P<0.05$), and the degree of hyperglycemia in experiment II appeared to be greater than that in experiment I ($P<0.05$). The blood glucose levels of groups 2, 3 and 4 were increased to the hyperglycemic level by day 3 after stz injection and persisted during the experiment. There were no significant differences among roups 2, 3 and 4 (Table 3). Ketonuria did not develop in any group. Glycosuria was noted in each group one week after the experiment began. The blood glucose and insulin values of experiment II are presented in Table 4.

Group 2 with stz only developed hypoinsulinemia (13.3 μU/ml) 4 weeks after receiving multiple small doses of stz.

Group 3 with stz and pv retained relatively higher insulin levels at 3 days, I week and 4 weeks as compared with group 2 ($P<0.05$).

Group 4 with stz and cA retained relatively higher insulin levels by 8 weeks as compared with group 2 ($P<0.05$).

DISCUSSION

Streptozotocin is an N-nitroso derivative of D-glucosamine, and is a relatively selective beta cytotoxin in certain animal species, causing an initial triphasic glucose response and then permanent diabetes (Junod *et al.* 1969).

The glucose component of stz enhances its uptake into the beta cells where the cytotoxicity of the nitrosourea moiety can be concentrated. Within the beta cells stz is believed to reduce the levle of nicotine adenine dinucleotide (NAD) by both decreasing its synthesis and increasing its breakdown (Gunnarson *et al.* 1974).

The diabetogenic activity of stz depends on the dose (Junod *et al.* 1969; Ganda *et al.* 1976).

Diabetes induced by a small dose of stz is so mild that an overnight fast results in normoglycemia despite an impaired glucose tolerance.

When the dose of stz is further increased, a more severe diabetes developes with a marked hyperglycemia even after prolonged fasting.

Abnormal glucose tolerance and insulin response

are seen when pancreatic immunoreactive insulin (IRI) is depleted by about one-third, while fasting hyperglycemia and gross glycosuria occurs when the depletion has reached two thirds and three-quarters, respectively (Junod *et al.* 1969).

No significant hyperglycemia developed with a dose of 10 to 20 mg/Kg. Diabetogenic activity was induced by doses of 25 to 100 mg/Kg. The ideal dose of stz for experimental diabetes is 60 mg/Kg in a single administration (Ganda *et al.* 1976). With an intravenous injection of 65 mg/Kg, the diabetic state becomes evident after 24 hours, characterized by polyuria, glycosuria and hyperglycemia (Tancrede *et al.* 1983).

Hyperglycemia developed 2 days after a 60 mg/Kg stz of intravenous (IV) injection (Eizirik and Migliorini 1984).

In contrast, hyperglycemia occurred 4-6 weeks after an intraperitoneal (IP) injection of 60 mg/Kg stz (Huang *et al.* 1984).

In this experiment, the blood glucose level increased to 195 mg% at 3 days, and hyperglycemia developed 7 days after an IP injection of stz (60 mg/Kg). The fasting glucose levels were significantly elevated 1 week after a dose of ⩾55 mg/Kg and 16 weeks after a dose of ⩾45 mg/Kg. The basal insulin levels were significantly decreased after 1 week with the ⩾65 mg/Kg doses, after 4 weeks with the ⩾55 mg/Kg and after 16 weeks with the ⩾35 mg/Kg doses (Tancrede *et al.* 1983).

Histopathologically beta cell necrosis and degranulation of the surviving beta cells is usually observed 3 days after a single IP injection of stz (Rerup 1970; Ganda *et al.* 1976).

The administration of five multiple subdiabetogenic doses (40 mg/Kg) to mice IP produces hyperglycemia after the 4th dose of stz and continues to increase the blood glucose level to 350-450 mg/100 ml within 5-6 days after the last injection (Like and Rossini 1976).

On the day after last stz injection, the nonfasting blood glucose is elevated as is the fasting blood glucose 7 days after the last stz injection (Bonnevie-Nielsen *et al.* 1981). In this experiment, nonfasting hyperglycemia occurred 3 days after the administration of stz and continued to rise to progressively higher levels.

The nonfasting blood glucose level correlates beta cell function better than the fasting level (Bonnevie-Nielsen *et al.* 1981).

Morphologically, the pancreatic islets reveal pronounced insulitis with infiltrating lymphocytes and macrophages, architectual distortion and beta cell necrosis. There are numerous type C viruses within the surviving beta cells within 6 days of the last stz injection.

The delayed development of insulitis and the nature of the inflammatory infiltrates suggest a cell mediated immune reaction (Like & Rossini 1976). The insulitis is a consequence of beta cell destruction rather than its cause (Leiter *et al.* 1983).

The multiple injections of stz induce the triad of direct beta cell cytotoxicity, virus induction within beta cells and cell mediated autoimmune reaction (Rossini *et al.* 1977).

Mice, given a single injection of stz, 200 mg/Kg, develops hyperglycemia within 24 hours with complete destruction of beta cells.

In contrast, mice given five daily injections of stz, 40 mg/Kg, develop hyperglycemia only after a latent period of 5 days.

After the latent period, there is a progressive increase in the blood glucose concentration and in the infiltration of the islets by lymphocytes (Like and Rossini 1976).

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) hyperglycemia occurred after a latent period, (2) diabetes cannot be induced by this procedure in athymic nude mice and induced by a thymus graft, (3) diabetes can be transferred to normal mice with spleen cells from mice rendered diabetic by this procedure (Paik *et al.* 1980; Buschard and Rygaard 1977).

T lymphocytes play a crucial 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 the production of autoantibodies specific for beta cells (Kim and Steinberg 1984).

3-0-methyl D-glucose, a nonmetabolizable glucose analoque, given before each stz injection, provides protection against beta cell destruction and hyperglycemia. It is suggested that the sugar may interfere with a possible direct beta cell cytotoxic effect of stz (Rossini *et al.* 1978).

Antilymphocyte sera is effective in preventing this form of diabetes, thus supporting the assertion that immunocytes participate in the development of the disease (Rossini *et al.* 1978).

The blood insulin level is increased by injection of

the Bordetella pertussis vaccine, which stimulates adrenergic beta receptors (Sumi and Ui 1975). It was reported that PV appears to curtail stz-induced diabetes (Katada and Ui 1977). When PV is administered either IP or IV 3 days prior to stz injection, none of the vaccinated group developed diabetes.

The protective effect is evidenced by normoglycemia, normal insulin values and the absence of insulitis in vaccinated rats (Huang *et al.* 1982).

The diabetogenic effect of PV against stz is quite extensive. If the vaccine is given from-10 days to +4 days relative to the day of stz injection, the outcome of the protective effect is identical. If the PV is given 30 days after stz injection, two-thirds of the mice will still revert to normoglycemia after booster injection (Huang *et al.* 1984).

In the multiple stz treated group, the protective effect against the development of hyperglycemia is less complete. After numerous booster injections, the outcome is somewhat improved but is significantly less effective as compared with the results of the single stz treated group (Huang 1984).

The mechanism of the protective effect of PV against diabetes is still unknown. However, at least three important biologic actions may be linked to the antidiabetogenic effect.

Firstly the endotoxin Bordetella pertussis may act as an immunoregulatory agent by altering the immune response in the host. Secondly the lymphocytosis-promoting factor (LPF) of Bordetella pertussis may also participate by suppressing insulitis via its unique effect on lymphocytes.

Thirdly the Islet-activating protein (IAP) of Bordetella pertussus is a powerful adrenergic beta receptor agonist on the cell membrance of beta cells and may serve as on effective deterrent against the damage caused by stz (Dresser and Phillips 1973; Sumi and Ui 1975).

The immunosuppressive effect in the pertussis vaccinated animals is the result of the combined effects of the endotoxin and other biological factors. Thus, PV may sometimes serve as a natural safe guard against insulin dependent diabetes even for a genetically susceptible host and interrupt the diseases in its early stages (Huang and Taylor 1982). PV is more effective when compared with boiled vaccine and is more effective in the single treated group than in the multiple treated group (Huang 1984).

Cyclosporin was purified from fungal extracts in 1973. It influences the early phase of the immune response by blocking the synthesis and/or release of interleukin I from monocytes and interleukin-2 from T-helper cells. Thus, Cyclosporin inhibits the T-cell dependent B-cell activation, expansion of the unprimed T helper and cytotoxic T cell subsets and gamma interferon production.

Recently it has been demonstrated that cyclosporin favors the expansion of antigenspecific suppressor T-cells.

(Wang *et al.* 1981; Tosato *et al.* 1982; Mohagheghpour *et al.* Kupiec-Weglinski 1984).

cyclosporin has been administered to experimental models of autoimmunity such as adjuvant arthritis, experimental allergic encephalomyelitis and experimental allergic neuritis. When cyclosporin is given during the sensitization phase, it prevents the development of autoimmune diseases in experimental animals (Bolton *et al.* 1982; Hinrichs *et al.* 1983). In addition to the prophylactic effect, cyclosporin also has a strong therapeutic effect when administration is initiated at the onset of symptoms (Bolton *et al.*, 1982).

In experimental autoimmune thyroiditis induced in rats by thymectomy and irradiation, cyclosporin mediates a significant improvement in diseased animals, as measured by the fall in antithyroglobulin antibodies and the normalization of the Th: Ts ratio in the course of treatment. Discontinuation of treatment results in a relapse (McGregor *et al.* 1983; Vladutiu 1983).

Preventive treatment with cyclosporin in the BB rats of spontaneous autoimmune diabetes completely suppresses the onset of the disease (Laupacis *et al.* 1983).

It has been suggested that even the simple exposure of the animal to a toxin such as stz had been considered subdiabetogenic, a repeated or successive exposure would greatly shorten the latency of the development of insulin dependent diabetes.

Thus in the case of multiple stz administration, PV protection against the development of hyperglycemia is less complete than in single stz administration (Huang *et al.* 1984).

Judging from the blood glucose levels and plasma insulin values in these experiments, it is conceivable that in the single stz injection, the progression of β cell damage in the islets occurs more slowly than with multiple injections, and PV and CA administration could account for the higher plasma insulin levels, suggesting of protection of the β cells of the pancreas.

But the reason for the persistance of hyperglycemia with relatively high insulin levels is difficult to explain. It may be that relatively high insulin levels can not normalized the nonfasting blood glucose.

Insulin therapy for type 1 diabetes mellitus is not

satisfactory, and a considerable number of patients encounter delayed complications and a reduced life expectancy.

Additionally, data are accumulating in favor of the immune nature of beta cell destruction in type 1 diabetics. There are reports of attempts to halt the disease by employing various immunosuppressants (Elliot *et al.* 1981; Rand *et al.* 1981; Stiller *et al.* 1983). Immune intervention therapy is thought to be too late in the case of overt diabetes. To arrest the process of the disease at an earlier stage, it will be necessary to recognize the prediabetic period.

For this purpose markers including islet cell antibodies and islet cell surface antibodies are needed (Kolb *et al.* 1983). The slow rate of beta cell loss prior to overt diabetes raises the hope that type 1 diabetes may become a preventable illness with early trials of immunosuppressants. Further study in this area should not only aid us in better understanding IDD but also would increase the possibilities of a new strategy for more efficient management of the disease.

REFERENCES

Bolton C, Borel JF, Cuzner ML, Davison AN, Turner AM: Immunosuppression by cyclosporin A of experimental allergic encephalomylitis. *J Neurol Sci 56:147-53, 1982*

Bonnevie-Nielsen V, Steffes MW, Lernmark A: A major loss in islet mass & B-cell function precedes hyperglycemia in mice given multiple low doses of streptozotocin. *Diabetes 30:424-49, 1981*

Borel JF: Comparative study of in vitro and in vivo drug effects on cell mediated cytotoxicty. *Immunology 31:631-41, 1976*

Buschard K & Rygaard J: Passive transfer of streptozotocin induced diabetes mellitus with spleen cells. *Acta Path Microbial Scand (C) 85:469-72, 1977*

Cubert S, Sharp R, Rogers M, Felts P, Burr IM: Diazoxide modification of streptozotocin-induced diabetes in rats. *Diabetes 23:282-6, 1974*

Dresser DW, Phillips JM: the cellular targets for the action of adjuvants: T-adjuvants and B-adjuvants. *Ciba foundation symp 18:3, 1973*

Dizirik DL, Migliorini RH: Reduced diabetogenic effect of streptozotocin in rats previously adaped to high-protein, carbohydrate free diet. *Diabetes: 33:383-88, 1984*

Elliott RB, Crossley JR, Berryman CC, James Ag: Partial preservation of pancreatic β cell function in children with diabetes. *Lancet 2:1-4, 1981*

Ganda OP, Rossini AA, Like AA: Studies on streptozotocin diabetes. *Diabetes 25:595-603, 1976*

Gunnarson R, Berne C, Hellerström C: Cytotoxic effects of streptozotocin and N-nitrosomethylurea on the pancreatic B cell with special regard to the role of nicotinamide-adenine dinucleotide. *Biochem J 140:437-94, 1974*

Hinrichs DJ, Wegmann KW, Peters BA: The influence of cyclosporin A on the development of actively induced and passively transferred experimental allergic encephalomyelitis. *Cell Immunol 77:202-209, 1983*

Huang SW, Taylor GE: Immune insulitis and antibodies to nucleic acid induced with streptozotocin in mice. *Clin Exp Immunol 43:425-29, 1981*

Huang S, Taylor GE: Pertussis vaccine inhibits immune insulitis with streptozotocin. *Clin Exp Immunol 48:375-80, 1982*

Huang s, taylor GE, Basid A: the effect of pertussis vaccine on the insulin dependent diabetes induced by streptozotocin in mice. *Ped Research 18:221-26, 1984*

Iwatsuka H, Shino A, Taketomi S: Streptozotocin resistance of genetically diabetic KK mouse. *Diabetes 23:856-7, 1974*

Junod A, Lambert AE, Stauffacher W, Renold AE: Diabetogenic action of streptozotocin: Relationship of dose to metabolic response. *J Clin Invest 48:2129-39, 1969*

Katada T, Ui M: Spontaneous recovery from streptozotocin induced diabetes in rats pretreated with pertussis vaccine or hydrocortisone. *Diabetologia 13:521-5, 1977*

Kim YT, Steinberg C: Immunologic studies on the induction of diabetes in experimental animals. Cellaular basis for the induction of diabetes by streptozotocin. *Diabetes 33:771-77, 1984*

Kolb H, Schernthaner G, Gries FA: Immunological aspects of type I (insulindependent) diabetes: pathogenesis and therapy. *Diabetologia 24:394-95, 1983*

Kupiec-weglinski JW, Filho MA, Strom TB, Tilney NL: Sparing of suppressor cells, a crucial action of cyclosporin. *Transplantation 38:97-101, 1084*

Laupacis A, Stiller CR, Gardell C, Keown P, Dupre J, wallace AC, Thibert P: Cyclosporin prevents diabetes in BB Wistar rats. *Lancet 1:10-11, 1983*

Leiter EH, Beamer, WG, Schultz LD: the effect of immunosuppression on streptozotocin induced diabetes in C 57 BL/KSJ mice. *Diabetes 32:148-55, 1983*

Like AA, Rossini AA: Streptozotocin-induced pancreatic insulitis, new model of diabetic mellitus. *Science 193:415-17, 1976*

McGregor AM, Rennie DP, Weetman AP, Hasman RA, Foord SM, Dieguez C, Hall R: The influence of cyclosporin A on experimental autoimmune thyroid disease in the rat. *Life Sci 32:97-108, 1983*

Mohagheghpour N, Benike CJ, kansas G, Bieber C, Englerman EG: Activation of antigen-specific suppressor T cells in the presence of cyclosporin A requires interaction between T cells of inducers & suppressor lineage. *J Clin Invest 72:2092-100, 1983*

Paik SG, Fleischer N, Shin S: Insulin dependent diabetes mellitus induced by subdiabetogenic dose of streptozotocin, obligatory role of cell mediated autoimmune process. *Proc Nat Acad Sci 77:6129-33, 1980*

Rand KH, rosenbloom AL, Maclaren NK, Silverstein JH, Riley WJ, Butterworth BE, Yoon JW, Rubenstein AH, Merigao TC: Human leukocyte interferon treatment of two children with insulin dependent diabetes. *Diabetologia 21:116-21, 1981*

Rerup CC: Drugs producing diabetes through damage of the insulin secreting cells. *Pharmac Rev 22:485-518, 1970*

Rossini AA, Like AA, Chick WL, Appel MC, Cahill GF: Studies of streptozotocin-induced insulitis and diabetes. *Proc Nat Acad Sci 74:2485-89, 1977*

Rossini AA, William RM, Appel MC, Like AA: Complete protection from low dose streptozotocin-induced diabetes in mice. *Nature 276:182-84, 1978*

Stiller CR, Laupacis a, Dupre J, Jenner MR, Keown PA, Rodger W, Wolfe BMJ: Cyclosporin for treatment of early type I diabetes. *N Engl J Med 308:1226-27, 1983*

Sumi T, Ui M: Potentiation of the adrenergic beta receptor mediated insulin secretion in pertussis-sensitized rats. *Endocrinology 97:352-58, 1975*

Tancrede G, Rousseau-Migneron R, Nideau A: Long-term changes in the diabetic state induced by different doses of streptozotocin in rats. *Br J Exp Path 64:117-23, 1983*

Tosato G, Pike SE, Koski IR, Blaese RM: Selective inhibition of immunoregulatory cell function by cyclosporin A. *J Immunol 128:1986-91, 1982*

Vladutiu AO: Effect of cyclosporin on experimental autoimmune thyroiditis in mice. *Transplantation 35:518-20, 1983*

Wang BS, Heacock EH, Collins KH, Hutchinson IF, Tilney NL, Mannick JA: Suppressive effects of cyclosporin A on the induction of alloreactivity in vito and in vivo. *J Immunol 127:89-93, 1981*

Wiesinger D, borel JF: Studies on the mechanism of action of cyclosporin A. *Immunobiology 156:454-63, 1979*