Clin. exp. Immunol. (1992) **89**, 174–178

Rapamycin prevents the onset of insulin-dependent diabetes mellitus (IDDM) in NOD mice

W. L. BAEDER, J. SREDY*, S. N. SEHGAL, J. Y. CHANG & L. M. ADAMS *Inflammation/Bone Metabolism Division and *Cardiovascular/Metabolic Disorders Division, Department of Experimental Therapeutics, Wyeth-Ayerst Research, Princeton, NJ, USA*

(Accepted for publication 30 April 1992)

SUMMARY

The effect of the immunosuppressive agent rapamycin (RAPA) was assessed in the non-obese diabetic (NOD) mouse which is an autoimmune model of IDDM. RAPA was prepared in a vehicle of 8% cremophor EL/2% ethanol and investigated in two studies. NOD/MrK female mice (six per group, study no. 1; 10 per group, study no. 2) were dosed three times per week p.o. by gavage from 56 to 170 days of age (study no. 1) or from 64 to 176 days of age (study no. 2). Mice treated with RAPA at 0·6 mg/kg, 6 mg/kg, or 12 mg/kg maintained normal plasma glucose through 170 or 176 days of age with 10%, 0%, and 0% incidence of diabetes respectively. In contrast, naive, vehicle-treated, or RAPA 0·06 mg/kg-treated mice exhibited elevated plasma glucose and disease incidence typical for female NOD mice. Mice which became diabetic had elevated levels of β-hydroxybutyrate, triglycerides and cholesterol. These plasma lipid concentrations were positively correlated with the duration of hyperglycaemia ($r = 0{\cdot}85$, 0·87 and 0·84 respectively). Outside of its ability to prevent diabetes, RAPA itself did not affect the lipid profile of the mice. Intervention therapy with RAPA was ineffective at reversing the course of disease after IDDM onset under these experimental conditions. Finally, we report here that prophylactic treatment with RAPA was able to protect against IDDM development in some RAPA-treated mice 41 weeks after cessation of treatment. These data show that orally administered RAPA is effective in preventing onset of disease in the NOD mouse, a relevant model of autoimmune type I diabetes in man.

Keywords autoimmunity insulin-dependent diabetes mellitus NOD mice rapamycin

INTRODUCTION

The non-obese diabetic mouse (NOD), an autoimmune model of type I IDDM, exhibits approximately 70% incidence of diabetes at 160 days of age [1,2]. Genetic susceptibility involving class II MHC genes plays a major role in this autoimmune disease for both humans and NOD mice [3–8]. The pancreatic islets become infiltrated with lymphocytes (insulitis) and insulin-producing beta cells are destroyed. Adoptive transfer studies in NOD mice [9] have shown that T cell-mediated events occur initially in IDDM while humoral abnormalities (cytoplasmic islet cell, insulin, and 64-kD protein autoantibodies) contribute later during the disease progression (see review [10]).

Rapamycin (RAPA) is a novel macrolide which appears to have a different mechanism of action *in vitro* from the immunosuppressants cyclosporin A (CsA) and the macrolide FK506 [11] (see review [12–14]). Rapamycin appears to affect both calcium-dependent and independent pathways of T cell proliferation by working later in the cell cycle (G_1 phase) as compared with FK506 or CsA which primarily act on calcium-dependent pathways in the earlier G_0 phase. Rapamycin, unlike FK506 or CsA, has little effect on mRNA levels for IL-2, IL-2 production, or IL-2R expression, but does have the ability to inhibit proliferation stimulated by exogenous addition of this cytokine as well as IL-4.

Rapamycin is efficacious in animal models of transplantation in suppressing rejection and prolonging survival time of both skin [15] and organ allografts (see review [13]). Rapamycin also demonstrates an immunosuppressive effect in murine T cell-mediated autoimmune models including collagen-induced arthritis (CIA) [16] and the MRL lupus model [17]. Since CsA and FK506 have been reported to prevent onset of IDDM in both the NOD mouse [1,2,18] and BB rat models [19,20], and CsA has partial clinical efficacy in patients with recent IDDM onset [21], we studied the effects of rapamycin in the NOD model and analysed incidence of diabetes by measuring plasma glucose, water consumption, and body weight at regular

J.Y.C. present address: Osteoarthritis Sciences, Inc., One Kendall Square, Bldg. 200, Cambridge, MA 02139, USA.

L.M.A. present address: Spinx Pharmaceuticals Corporation, Two University Place, Durham, NC 27717, USA.

Correspondence: W. L. Baeder, Inflammation/Bone Metabolism Division, Department of Experimental Therapeutics, Wyeth-Ayerst Research, CN 8000, Princeton, NJ 08543, USA.

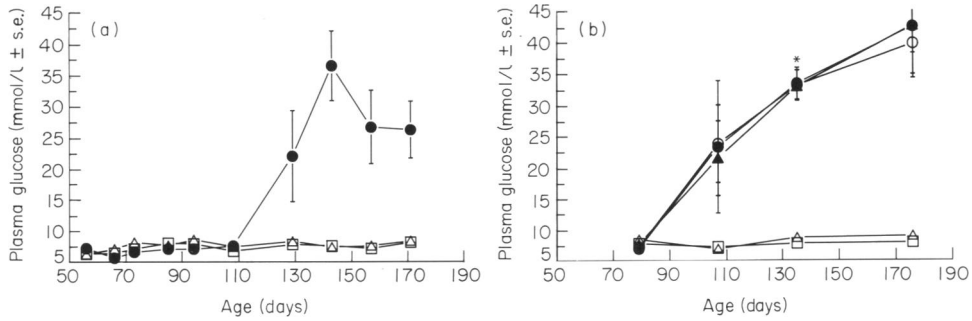

Fig. 1. The effect of rapamycin (RAPA) on plasma glucose in non-obese diabetic (NOD) mice. (a) Study no. 1. ●, Naive; △, RAPA 6 mg/kg; □, RAPA 12 mg/kg. (b) Study no. 2. ●, Naive; ▲, vehicle; ○, RAPA 0·06 mg/kg; □, RAPA 0·6 mg/kg; △, RAPA 6 mg/kg. *Elevated plasma glucose observed in one of 10 mice from the RAPA 0·6 mg/kg group.

intervals. In addition, a cross-sectional evaluation of plasma lipids including triglyceride, cholesterol, and β-hydroxybutyrate was conducted.

MATERIALS AND METHODS

Two studies (nos 1 and 2) were used to determine the effects of rapamycin in the NOD model.

Treatment protocol

Female NOD/MrKTACfBR mice (Taconic), 8 weeks of age (study no. 1) or 9 weeks of age (study no. 2), were housed 3–4 mice per cage in a barrier facility and given food and water *ad libitum*. Weight and water consumption were measured on a weekly basis. In the initial study, six mice per group were randomly distributed into three groups including naive control, RAPA 6 mg/kg, and RAPA 12 mg/kg. Rapamycin was dissolved in a vehicle containing 8% cremophor EL/2% ethanol and administered in a volume of 0·1 ml/10 g body weight. In the second study, 10 mice per group were randomly distributed into six groups (A–F) as described below. The effects of vehicle (A) as compared to naive control (B) were tested. A dose response to rapamycin (0·06, 0·6 and 6 mg/kg (C–E)) was determined and the ability to reverse diabetes with RAPA 6 mg/kg immediately after onset (F) was assessed. Administration of drug began at 56 days (study no. 1) and 64 days (study no. 2) and continued three times per week p.o. by gavage until mice were killed at 170 days of age (study no. 1 only) and through 176 days (study no. 2). Non-diabetic rapamycin-treated mice from study no. 2 were then followed for an additional 41-week period after cessation of rapamycin and analysed for incidence of IDDM.

Biochemical analysis

At regular intervals blood was collected from the tail vein into tubes containing sodium fluoride and plasma removed for analysis of glucose using the Abbott Biochromatic Analyzer. A plasma glucose level consistently $>11{\cdot}1$ mmol/*l* was the criterion used to determine overt onset of diabetes. On day 176 of study no. 2, blood was collected from the retroorbital sinus and plasma levels of triglyceride and cholesterol were investigated using the above method. Plasma levels of β-hydroxybutyrate were analysed enzymatically using the method of Williamson *et al.* [22].

Water consumption determination

Mice were housed 3–4 in cages, and thus water consumption (millilitres of water consumed/mouse per day) was calculated by measuring total consumption (ml) for a 7-day period in a cage and dividing by the total number of mice housed. This was done for each of the 2–3 cages per treatment group. A final weighted mean per group was determined by using consumption data per mouse in a cage as a value for each of the mice housed in that cage and then averaging these values for each of the mice in the group. Although this method does lead to considerable variation by accounting for water consumption of both diabetic and non-diabetic mice, and does not allow for calculation of statistical differences between groups, it did serve as a valuable non-invasive indicator of new onset and as an indicator of osmotic diuresis due to glycouria.

Statistical analysis

The data were expressed as mean ± s.e. Fisher's exact test was used for comparison of per cent incidence of diabetes between the groups. Student's *t*-test was used for comparison of weight between groups and two-way analysis of variance was used to determine significance between treatment groups for the plasma lipid levels. All significance levels were set at $P<0{\cdot}05$. Finally, Pearson's correlation, set at the 95% confidence interval, was used to compare lipid levels and the number of days of overt diabetes.

RESULTS

Incidence of diabetes

In the initial study, rapamycin-treated mice at 170 days of age had no incidence of diabetes (0/10) which was significantly lower from control incidence (4/6, 67%) at $P=0{\cdot}008$ by Fisher's exact test. In the second study, six out of ten (60%) vehicle-treated mice became diabetic by 176 days of age which was not significantly different from naive control mice (6/10, 60%). Rapamycin treatment again protected against diabetes at 6 mg/kg (0/10) and also at 0·6 mg/kg (1/10, 10%) which was significantly lower than incidence for naive or vehicle control at $P=0{\cdot}005$ and $P=0{\cdot}029$, respectively. A no effect concentration of rapamycin was determined at 0·06 mg/kg (60% incidence).

Plasma glucose levels

Plasma glucose levels for all groups were below 7·2 mmol/*l* (study no. 1) and below 8·3 mmol/*l* (study no. 2) at the beginning of the studies as shown in Figs 1a and b, respectively. Elevated plasma glucose levels for diabetic mice were first observed by 129 days of age in study no. 1 for naive control and by 107 days for naive control, vehicle, and RAPA 0·06 mg/kg in study no. 2.

Table 1. Plasma levels of β-hydroxybutyrate, triglyceride and cholesterol in non-obese diabetic (NOD) mice at 176 days of age

	β-hydroxybutyrate (mmol/l)		Triglyceride (mmol/l)		Cholesterol (mmol/l)	
Treatment	Non-diabetic	Diabetic	Non-diabetic	Diabetic	Non-diabetic	Diabetic
Naive	0·61±0·38 (4)	15·2±5·6 (4)*	1·24±0·22	5·06±1·14*	2·55±0·25	5·85±1·30*
Vehicle†	0·66±0·13 (4)	12·8±5·4 (4)*	0·92±0·06	4·06±1·00*	2·60±0·10	4·75±0·85*
RAPA 0·06 mg/kg†	0·45±0·03 (4)	7·6±5·4 (2)*	0·84±0·08	5·04±2·76*	2·65±0·05	4·85±0·50*
RAPA 0·6 mg/kg†	0·58±0·12 (9)		0·74±0·04		2·50±0·05	
RAPA 6 mg/kg†	0·52±0·08 (10)		1·30±0·06		2·90±0·10	

* $P < 0·05$ when compared to vehicle non-diabetic.
† Administered at 64 days of age and continued three times per week p.o.
Values are mean ± s.e.m.
Numbers in parentheses are *n* values.

Table 2. Profile of rapamycin-protected mice after cessation of treatment

Weeks after cessation	RAPA 6 mg/kg, glucose (mmol/l)					RAPA 0·6 mg/kg, glucose (mmol/l)				
	Incidence (%)	Non-diabetic	Diabetic	Water	Status	Incidence (%)	Non-diabetic	Diabetic	Water	Status
1	0/10 (0)	—	—	5·2±0		0/9 (0)	—	—	4·7±0·2	
3	0/10 (0)	7·8±0·3 (10)	—	4·8±0		0/9 (0)	6·8±0·3 (9)	—	4·4±0·2	
6	0/10 (0)	6·8±0·3 (10)	—	5·1±0·1		1/9 (11)	6·8±0·2 (8)	13·9±0 (1)	4·7±0·2	
9	0/10 (0)	7·4±0·2 (10)	—	—		1/9 (11)	7·2±0·4 (8)	29·4±0 (1)	—	
14		—	—	4·0±0·1	†		—	—	9·4±2·6	
15	0/10 (0)	6·8±0·4 (9)	—	4·3±0·1	†	1/9 (11)	7·4±0·3 (8)	41·1±0 (1)	8·9±2·3	
20		—	—	5·1±0·2	†		—	—	5·3±0·4	‡
21	0/10 (0)	7·6±0·4 (7)	—	5·0±0·2		2/9 (22)	7·3±0·5 (7)	17·3±0 (1)	4·7±0·2	
24		—	—	5·4±0	†		—	—	5·5±0·3	
26	0/10 (0)*	10·1±1·6 (7)*	—	5·3±0·4		2/9 (22)	9·9±0·8 (7)	36·6±0 (1)	7·9±1·5	
29		—	—	5·5±0·3			—	—	10·8±2·5	‡
31		—	—	5·3±0·4			—	—	5·3±0·6	
33	0/10 (0)*	—	—	5·7±0·5		3/9 (33)	—	—	8·0±1·8	
35		—	—	4·6±0·3	†		—	—	4·3±0·2	‡
37	0/10 (0)*	8·0±1·3 (5)*	—	4·7±0·3		3/9 (33)	8·5±0·5 (6)	—	4·2±0·2	
39		—	—	4·5±0·6	†		—	—	4·3±0·2	
41	0/10 (0)*	9·7±1·8 (4)*	—	4·6±0·7		3/9 (33)	8·6±0·5 (6)	—	4·2±0·2	

* One mouse had a slightly elevated plasma glucose level from 361 to 466 days of age with no other clinical signs of IDDM including loss of weight or increased water consumption.
† Death not due to hyperglycaemia.
‡ Death as a result of diabetes.
Values are mean ± s.e.m.
Numbers in parentheses are *n* values of surviving mice.
Water consumption values are millilitres of water consumed/mouse per day.

These initial hyperglycaemic levels increased further with duration of disease for diabetic mice in the above groups at 170 and 176 days of age, respectively. In addition, one mouse in the RAPA 0·6 mg/kg group (study no. 2) became diabetic by 135 days and expired at 166 days of age (Fig. 1b). Mice treated with either 6 mg/kg or 12 mg/kg RAPA (study no. 1), 0·6 mg/kg or 6 mg/kg RAPA (study no. 2), maintained normal plasma glucose throughout the experiment.

Water consumption

Water consumption in study nos 1 and 2 increased from baseline (4·3 ml to 4·8 ml water consumed/mouse per day) to peak levels concomitantly with elevated glucose (Figs 1a, b) for naive control (×4·6 increase), vehicle control (×5·2), and RAPA 0·06 mg/kg (×4·6) treated mice. A slightly elevated consumption level reaching 8·8±2·2 ml consumed/mouse per day was observed for the RAPA 0·6 mg/kg group concomitant with the 10% incidence of diabetes. This decreased to baseline levels by 166 days after this mouse expired. In contrast, rapamycin (6 mg/kg or 12 mg/kg, study no. 1; 0·6 mg/kg or 6 mg/kg, study no. 2) treated mice exhibited only a slight increase in water consumption at the end of the studies (×1·4, ×1·7, ×1·2, and ×1·0, respectively) over baseline levels, concomitant with normal weight gain in the mice.

Body weight

Body weights at 8 weeks of age for naive control (20·8 ± 0·5 g), RAPA 6 mg/kg (20·6 ± 0·3 g), RAPA 12 mg/kg (21·1 ± 0·7 g), and at 9 weeks for naive control (22·2 ± 0·4 g), vehicle control (22·2 ± 0·4 g), RAPA 6 mg/kg (22·1 ± 0·4 g), RAPA 0·6 mg/kg (22·0 ± 0·4 g), and RAPA 0·06 mg/kg (22·2 ± 0·5 g) were similar. The non-diabetic mice in all groups gained weight normally and by the end of the study there were no significant differences in per cent change of body weight between rapamycin-treated mice and control mice (study no. 1, 170 days) or between rapamycin-treated mice and vehicle control mice (study no. 2, 176 days). All diabetic mice in the naive, vehicle and RAPA 0·06 mg/kg groups, as well as the single mouse in the RAPA 0·6 mg/kg group, lost weight which varied with the duration of hyperglycaemia (data not shown).

Lipid analysis

In study no. 2, plasma levels of triglyceride, cholesterol, and β-hydroxybutyrate were analysed from the mice at 176 days of age. Although all mice were the same age, their treatment and duration of overt diabetes varied. As is evident from the group means in Table 1, all mice that developed diabetes had elevated levels of plasma triglyceride, cholesterol, and β-hydroxybutyrate. Rapamycin treatment at 0·6 mg/kg and 6 mg/kg in addition to preventing hyperglycaemia (Figs 1a, b) also prevented the associated hyperlipidaemia (Table 1). Furthermore, administration of rapamycin from 0·06 mg/kg to 6 mg/kg did not significantly affect the lipid levels of either the non-diabetic or diabetic mice as compared with the non-diabetic vehicle and diabetic vehicle group, respectively. An examination of individual mice revealed that the plasma levels of β-hydroxybutyrate, triglyceride, and cholesterol were positively correlated ($P < 0·05$) with the duration of hyperglycaemia ($r = 0·85$, 0·87 and 0·84 respectively).

Intervention therapy with rapamycin

Rapamycin administered 6 mg/kg, three times per week p.o., therapeutically to eight NOD mice immediately after IDDM onset at 130–144 days of age was unable to reverse the course of the disease through 176 days of age (data not shown).

Incidence of diabetes after cessation of rapamycin treatment

The 19 non-diabetic mice remaining after 176 days of age from the RAPA 6 mg/kg and RAPA 0·6 mg/kg protected groups were followed for an additional 41-week period after cessation of rapamycin treatment. As shown in Table 2, three of nine mice (33% incidence) from the RAPA 0·6 mg/kg group exhibited delayed onset of IDDM by week 41. As described earlier, there was also a corresponding increase in the mean water consumption for this treatment group which normalized after the three diabetic mice expired. The RAPA 6 mg/kg group continued to have no incidence of diabetes (0/10) with no dipsesis displayed. However, one mouse which had no other clinical signs of IDDM did have a slightly elevated plasma glucose level from 361 days of age through the end of the study at 466 days (Table 2). During this 41-week period, six of the 10 mice previously treated with RAPA (6 mg/kg) expired. Visible tail infections were observed at this time in some mice requiring significant amputation of the remaining tissue. Four of these six deaths may be due to bacterial or viral infection as a result of overimmunosuppression. Necropsy in these mice showed ear infections or white growth present in the chest cavity which contained 'pus' and infiltrated the trachea and lungs. The remaining mice which expired at weeks 35 (425 days of age) and 39 (449 days of age) after cessation of treatment had no visually apparent signs of disease and probably died of natural causes.

DISCUSSION

The present studies show that intermittent oral dosing (three times per week) of rapamycin at concentrations ranging from 0·6 to 12 mg/kg, p.o., prevents the onset of diabetes in NOD mice. Rapamycin-treated mice maintained normal glucose levels throughout the duration of the study and the drug significantly inhibited the progressive water consumption observed in this model. Since water consumption reflects water excreted, the observed normal water consumption of the RAPA-treated mice indicates no osmotic diuresis due to glucosuria. Thus the normal plasma glucose levels observed with RAPA treatment are due to the prevention of diabetes and not due to the elimination of glucose through the urine as is the case for a compound such as phlorizin which lowers plasma glucose by inducing glucosuria [23].

No significant differences in per cent change of body weight were observed in non-diabetic treated mice. In the NOD mice that did become hyperglycaemic, the progression of diabetes followed a typical course of a severe IDDM, i.e. weight loss, and a rise in plasma ketone bodies, triglycerides, and cholesterol [24,25]. The degree of hyperlipidaemia and ketosis was correlated with the duration of uncontrolled hyperglycaemia. Rapamycin-treated mice at both 0·6 mg/kg and 6 mg/kg maintained normal non-diabetic levels of the three plasma lipids. Outside of this ability to prevent diabetes, rapamycin itself did not affect the plasma lipid profile of the mice.

Intervention therapy with 6 mg/kg rapamycin, three times per week p.o., immediately after IDDM was detected, was ineffective at reversing the course of the disease under these experimental conditions. Changes in experimental design are planned to investigate further this parameter as the combination of oral dosing and an intermittent dosing schedule may not have raised rapamycin blood levels to a therapeutic range soon enough to abrogate the attack by T lymphocytes on the pancreatic beta cells. Insulin cotherapy at this stage may also help to 'rest' the remaining beta cells [10,26].

We are encouraged by the level of protection against IDDM demonstrated for both the RAPA 0·6 mg/kg and 6 mg/kg groups in study no. 2, 41 weeks after the cessation of treatment. Our observation of 33% incidence for the RAPA 0·6 mg/kg mice was at reduced levels from expected incidence. Consistent with the findings of Formby *et al.* [2] who reported 25% new incidence of IDDM in NOD mice 5 months (20 weeks) after cessation of CsA treatment, continuous treatment of rapamycin may be needed to prevent IDDM. In this situation, the effectiveness of rapamycin at lower doses using an intermittent dosing schedule may be advantageous in preventing possible complications due to overimmunosuppression. In addition, the administration of insulin prophylactically has been reported to reduce the incidence of development of IDDM in NOD mice [27] and cotherapy with rapamycin may reduce the levels of rapamycin required for prevention. A higher level of rapamycin at 6 mg/kg was able to abrogate the development of IDDM 41 weeks after cessation of treatment. However, since four of 10

mice in this group may have died from complications as a result of overimmunosuppression, we are uncertain whether IDDM would have eventually developed. Our study was not designed to demonstrate the immunological effects of this protection. However, Calne *et al.* [28] and Collier *et al.* [29] have reported that rapamycin induced a state of 'operational tolerance' more than 6 months after treatment ended in three of nine pigs receiving major histoincompatible renal allografts. Morris *et al.* [30] also recently demonstrated that they could induce a state of specific immune unresponsiveness in highly histoincompatible rat heart allografts after cessation of rapamycin treatment. They suggested, among several prospects, the possibility of clonal deletion in their rat model due to the observation of involuted thymi during the rapamycin treatment period. Studies are planned to address these questions.

In conclusion, since the onset of IDDM is becoming more predictable [31], rapamycin may be useful for the prophylactic treatment of autoimmune type I diabetes in man.

ACKNOWLEDGMENTS

The authors thank Terri Cummons for expert assistance with animal care, Lisa Lenskold and Brenda Flam for determining plasma glucose and lipid concentrations, and Hsu-Lin Su for assistance with statistical analysis of data.

REFERENCES

1 Mori Y, Suko M, Okudaira H *et al.* Preventive effects of cyclosporin on diabetes in NOD mice. Diabetologia 1986; **29**:244–7.
2 Formy B, Miller N, Garret R, Peterson CM. Effects of low-dose cyclosporine prophylaxis in nonobese diabetic mice. J Pharm Exp Ther 1987; **241**:1106–11.
3 Kim SJ, Holbeck SL, Nisperos B, Hansen JA, Maeda H, Nepom GT. Identification of a polymorphic variant associated with HLA-DQw3 and characterized by specific restriction sites within the DQ B-chain gene. Proc Natl Acad Sci USA 1985; **82**:8139–43.
4 Hattori M, Buse JB, Jackson RA *et al.* The NOD mouse: recessive diabetogenic gene in the major histocompatibility complex. Science 1986; **231**:733–5.
5 Nishimoto H, Kikutani H, Yamamura K, Kishimoto T. Prevention of autoimmune insulitis by expression of I-E molecules in NOD mice. Nature 1987; **328**:432–4.
6 Todd JA, Bell JI, McDevitt HD. HLA-DQ beta gene contributes to susceptibility and resistance to insulin-dependent diabetes mellitus. Nature 1987; **329**:599–604.
7 Acha-Orbea H, McDevitt HD. The first external domain of the nonobese diabetic mouse class II I-A beta chain is unique. Proc Natl Acad Sci USA 1987; **84**:2435–9.
8 Morel PA, Dorman JS, Todd JA, McDevitt HD, Trucco M. Aspartic acid at position 57 of the HLA-DQ beta chain protects type I diabetes: a family study. Proc Natl Acad Sci USA 1988; **85**:8111–5.
9 Serreze DV, Leiter EH, Worthen SM, Shultz LD. NOD marrow stem cells adoptively transfer diabetes to resistent (NOD × NON) f1 mice. Diabetes 1988; **37**:252–5.
10 Atkinson MA, Maclaren NK. What causes diabetes? Scientific American July 1990; **263**:62–71.
11 Dumont FJ, Staruch MJ, Koprak SL, Melino MR, Sigal NH. Distinct mechanisms of suppression of murine T cell activation by the related macrolides fk-506 and rapamycin. J Immunol 1990; **144**:251–8.
12 Kahan BD, Chang JY, Sehgal SN. Preclinical evaluation of a new potent immunosuppressive agent, rapamycin. Transplantation 1991; **52**:185–91.
13 Morris RE. Rapamycin: fk506's fraternal twin or distant cousin? Immunol Today 1991; **12**:137–40.
14 Morris RE. Rapamycins: antifungal, antitumor, antiproliferative, and immunosuppressive macrolides. Transplant Rev 1992; **6**:39–87.
15 Eng CP, Gullo-Brown J, Chang JY, Sehgal SN. Inhibition of skin graft rejection in mice by rapamycin: a novel immunosuppressive macrolide. Transpl Proc 1991; **23**:868–9.
16 Adams LM, Caccese R, Cummons T, Sehgal SN, Chang JY. Effect of the immunosuppressants rapamycin (RAP) and cyclosporin A (CsA) on collagen-induced arthritis (CIA) in mice. FASEB J 1990; **3**:537 (abstract).
17 Warner LM, Cummons TA, Chang JY, Sehgal SN, Adams LM. Comparison of rapamycin and cyclosporin A effects on murine systemic lupus erythematosus. FASEB J 1990; **3**:538 (abstract).
18 Kurasawa K, Koike T, Matsumura R, Takabayaski K, Tomioka H, Ito I, Yoshida S. The immunosupressant fk-506 prevents progression of diabetes in nonobese diabetic mice. Clin Immunol Immunopathol 1990; **57**:274–9.
19 Laupacis A, Stiller CR, Gardell C, Keown P, Dupre J, Wallace AC. Cyclosporin prevents diabetes in BB wistar rats. Lancet 1983; **1**:10–12.
20 Murase N, Lieberman I, Nalesnik MA, Mintz DH, Todo S, Drash AL, Starzl TE. Effect of fk-506 on spontaneous diabetes in BB rats. Diabetes 1990; **39**:1584–6.
21 Stiller CR, Dupre' J, Gent M *et al.* Effects of cyclosporine immunosuppression in insulin-dependent diabetes mellitus of recent onset. Science 1984; **223**:1362–7.
22 Williamson DH, Mellanby J, Krebbs HA. Enzymatic determinations of D(-)-β-hydroxybutyric acid and acetoacetic acid in blood. Biochem J 1962; **82**:90.
23 Rossetti L, Smith D, Shulman GI, Papachristou D, DeFromzo RA. Correction of hyperglycemia with phlorizin normalizes tissue sensitivity to insulin in diabetic rats. J Clin Invest 1987; **79**:1510–5.
24 Harris LVD, Albrink MJ, Van Eck WF, Man EV, Peters JP. Serum lipids in diabetic acidosis. Metabolism 1953; **2**:120–32.
25 Fulop M, Howard E. Plasma triglyceride and cholesterol in diabetic ketosis. Arch Intern Med 1989; **149**:1997–2002.
26 Shaw SC, Malone JI, Simpson NE. A randomized trial of intensive insulin therapy in newly diagnosed insulin-dependent diabetes mellitus. N Engl J Med 1989; **320**:550–4.
27 Atkinson MA, Maclaren NK, Luchetta R. Insulitis and diabetes in NOD mice reduced by prophylactic insulin therapy. Diabetes 1990; **39**:933–7.
28 Calne RY, Collier D St J, Lim S, Pollard SG, Samaan A, White DJG, Thiru S. Rapamycin for immunosuppression in organ allografting. Lancet 1989; **2**:227.
29 Collier D St J, Calne R, Thiru S *et al.* Rapamycin in experimental renal allografts in dogs and pigs. Transpl Proc 1990; **22**:1674–5.
30 Morris RE, Meiser BM, Wu J, Shorthouse R, Wang J. Use of rapamycin for the suppression of alloimmune reactions *in vivo*: schedule dependence, tolerance induction, synergy with cyclosporine and fk506, and effect on host-*versus*-graft and graft-*versus*-host reactions. Transpl Proc 1991; **23**:521–4.
31 Maclaren NK. How, when and why to predict IDDM. Diabetes 1988; **37**:1591–4.