Inhibition of Insulin Release In Vitro Mediated by Mononuclear Cells From Diabetic Patients Treated With Cyclosporin A or Placebo

MONIQUE DEBRAY-SACHS, PIERRE SAI, GILLES FEUTREN, FRANÇOIS LANG, DIDIER MAUGENDRE, CHRISTIAN BOITARD, JACQUES HORS, AND JEAN-FRANÇOIS BACH

Anti-β-cell–specific cell-mediated immunity was studied over a 12-mo period in 65 recently diagnosed diabetic patients randomly receiving either cyclosporin or placebo. Anti-β-cell cellular immunity was assessed by an in vitro test based on the inhibition of insulin release from cultured rat islet cells by patients' mononuclear cells. This β-cell–suppressive effect disappeared in cyclosporin A–treated patients within 1 mo and did not reappear during 12 mo of follow-up. Conversely, the suppressive effect persisted unchanged in placebo-treated patients during 12 mo of follow-up. These changes were predictive neither of cyclosporin A–induced remission nor of relapses. Results of the insulin-release inhibition test were not correlated to islet cell autoantibodies or HLA phenotype. *Diabetes* 37:873–77, 1988

There is clear evidence to suggest that human insulin-dependent diabetes mellitus (IDDM) is a genetically programmed autoimmune disease (1). Although humoral immunity has been extensively studied in IDDM (2,3), the autoimmune reaction probably predominantly involves T-lymphocytes. Such a possibility is suggested by the presence of inflammatory cells invading islets (insulitis) (3), and especially the high proportion of T-lymphocytes among them (4), and the capacity of purified T-lymphocytes to inhibit insulin release from islet cells in vitro (5,6). Studies in spontaneously diabetic animals (BB rats and NOD mice) clearly support the hypothesis of a pathogenic role of T-lymphocytes in the destruction of the insulin secretory cell; diabetes can be prevented by neonatal thymectomy (7,8) or treatment by anti-T-lymphocyte monoclonal antibodies (9) and can be transferred to nondiabetic littermates by purified T-lymphocytes (10).

Cyclosporin A (CsA) is a powerful immunosuppressive agent responsible for relatively selective inhibition of T-lymphocyte–mediated immunity, particularly of the production of lymphokines by helper T-lymphocytes (11). Because the pathogenesis of IDDM may be T-lymphocyte mediated, such T-lymphocyte selectivity renders CsA a good candidate for immunotherapy in this disease.

Given the favorable results obtained with CsA in the BB rat and NOD mouse models of diabetes (12,13)—its powerful immunosuppressive activity compared with classic treatment in organ transplantation (14) and its T-lymphocyte selectivity—clinical trials of CsA have been initiated in human IDDM (15).

We recently reported that CsA significantly increases the rate and length of remissions in patients with recent-onset IDDM (16). In this double-blind trial, the rate of complete remission after 9 mo of CsA treatment was 24.1% in the CsA group and 5.8% in the placebo group. Interestingly, a dose-effect relationship was observed; the rate of remission increased to 37% in patients who initially had efficient blood CsA levels (≥300 ng/ml).

The aim of this study was to investigate the effect of CsA treatment on lymphocyte-mediated anti-β-cell–specific cellular immunity in diabetic patients and to assess the predictive value of this test for remissions induced by CsA treatment compared to other immunologic parameters [e.g., islet cell antibodies (ICAs)]. Cellular immunity against β-cells was evaluated by an in vitro test based on the inhibition of insulin release from cultured rat cells by patients' mononuclear cells (MNCs). This inhibition is known to be specific for IDDM and for insulin secretion (17) and to be mediated by T-lymphocytes, especially $CD3^+$ $CD4^-$ cells (6).

MATERIALS AND METHODS

Patients. Sixty-five patients (44 males and 21 females) aged 15–40 yr (mean 25.7 ± 0.8 yr) with recent-onset IDDM were studied. In all cases, first symptoms dated from <6 mo

From the Service d'Immunologie Clinique, Hôpital Necker, Paris, the Laboratoire de Diabétologie, Faculté de Médecine et Ecole Vétérinaire, Nantes, and the Hôpital Saint Louis, Paris, France.

Address correspondence and reprint requests to Monique Debray-Sachs, Immunologie Clinique, Hôpital Necker, 161, rue de Sevres, 75743 Paris Cedex 15, France.

Received for publication 2 June 1987 and accepted in revised form 7 December 1987.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/37/7/873/356489/37-7-873.pdf by guest on 19 April 2024

TABLE 1
Baseline characteristics of diabetic patients

	Cyclosporin group	Placebo group
Sex (M/F)	22/10	22/11
Age (yr)*	24.8 ± 1.3	26.6 ± 1.2
Duration of symptoms (mo)*	2.1 ± 0.3	2.5 ± 0.3
Duration of insulin therapy (days)*	27.5 ± 3.7	22.7 ± 2.1
Islet cell antibody positive	12/26	13/30
DR3/4	5/24	7/29
Non-DR3 or 4	1/24	3/29
Insulin-release index*	1.15 ± 0.05	1.04 ± 0.02

*Means ± SE.

(mean 2.3 ± 0.2 mo), and insulin therapy had been instituted since <2 mo (mean 25.1 ± 2.1 days). Diagnosis of IDDM was based on the 1985 World Health Organization definition (18). At the start of the trial, CsA (n = 32) or placebo (n = 33) was added to conventional insulin treatment. CsA was initially given at 7.5 mg · kg^{-1} · day^{-1} and was increased to 10 mg · kg^{-1} · day^{-1} after 3 mo in case of clinical inefficacy. CsA was stopped at 6 mo in case of failure. In all other cases, the treatment was continued during the 12-mo follow-up period. Control subjects were 102 normal blood donors.

Study of insulin secretion by islet cell suspension in vitro in presence of MNCs. A 15-ml venous heparinized blood sample was taken just before drug intake, when CsA blood level would be lowest. Peripheral blood MNCs were then prepared with a Ficoll-Hypaque density gradient.

Preparation of dispersed islet cells. Langerhans islets were obtained from collagenase (Boehringer, Mannheim, FRG)-treated adult Wistar rat (Iffa-Credo, L'Arbresle, France) pancreases with the method of Lacy and Kostianovsky (19), modified as previously described (5). To obtain islet cell suspension, freshly isolated islets were subjected to EDTA and dispase (Boehringer) as described by Ono et al. (20). The islet cell suspension was incubated in 96-well flat-bottomed Falcon microtest plates (Becton Dickinson, Oxnard, CA; 5 × 10^3 cells/well) for 12 h in 100 μl basal medium: minimal essential medium with Earle's salts (Gibco, Paisley, Scotland) supplemented with 10% fetal calf serum, 2 mM glutamine, 1 mM sodium pyruvate, 0.814 mg/L nonessential amino acids (Gibco), 100 μg/ml streptomycin, and 100 μU penicillin (4 × 10^6 cells/ml). Either control or diabetic MNCs were added to the islet suspension at a concentration of 4 × 10^5 cells/well in 100 μl (5 wells for each lymphocyte population).

After an 18-h incubation the cells were washed, and the medium was replaced by 200 μl basal medium containing 5.5 mM glucose or 200 μl stimulatory medium (basal medium plus 10 mM theophylline and 20 mM arginine) for a 10-min period. Aliquots of the incubation media were collected in each well immediately before and at the end of the 10-min incubation period. Aliquots collected for insulin determination were immediately frozen at −20°C. Insulin was determined in culture supernatants by radioimmunoassay.

Expression of results. Islet cell function was assessed by the net insulin release during the 10-min test period in the presence of the basal or the stimulatory media. It was expressed as an insulin release (IR) index

$$\text{IR index} = \frac{\text{stimulated release}}{\text{basal release}}$$

ICA determination. ICAs were determined by indirect immunofluorescence on frozen sections of a human group O pancreas with a fluoresceinated anti-human IgG serum (Wellcome, Dartford, UK) as described by Bottazzo et al. (2). Antibody titers were determined by twofold serial dilutions.

HLA-DR typing and blood CsA-level evaluation. All patients were typed for class II (DR) MHC antigens as previously described (21). Whole-blood CsA levels were evaluated by radioimmunoassay with the Sandoz kit on blood taken immediately before drug intake (trough level).

Clinical effect of CsA. Complete remission was defined as persisting good metabolic control with fasting blood glucose <7.8 mM (140 mg/dl), postprandial blood glucose <11.1 mM (200 mg/dl), and HbA_{1c} ≤7.5% in the absence of insulin treatment. Partial remission was defined by the same metabolic status obtained with <0.25 U · kg^{-1} · day^{-1} insulin. Patients receiving >0.25 U · kg^{-1} · day^{-1} insulin were considered treatment failures.

Statistics. Results are expressed as means ± SE. The statistical evaluation of nonsignificant (NS) and significant (P value) differences between studied groups was performed via the unpaired Student's t test.

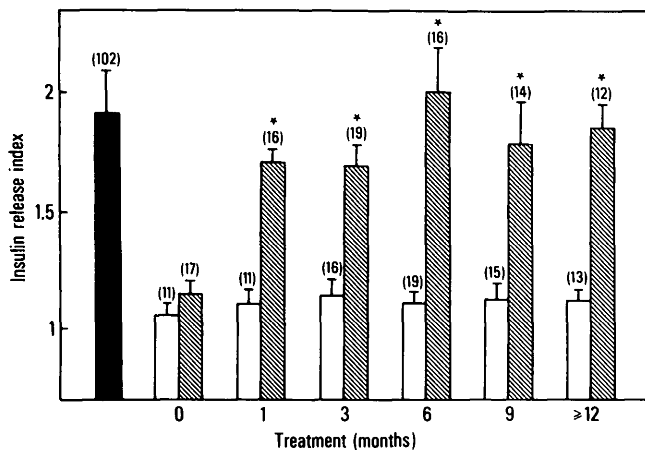

FIG. 1. Evolution of insulin release index from stimulated rat islet cells in presence of mononuclear cells from cyclosporin (*hatched bar*)- or placebo (*open bar*)-treated diabetic patients or from nondiabetic control subjects (*solid bar*). Results are means ± SE with number of patients in parentheses (*P < .001 vs. placebo).

TABLE 2
Correlation between insulin release index and initial cyclosporin blood level

	Cyclosporin A <300 ng/ml* (n = 4)	Cyclosporin A ≥300 ng/ml* (n = 11)	P
Before treatment	1.26 ± 0.16†	1.08 ± 0.07	NS
1 mo	1.36 ± 0.12	1.84 ± 0.06	NS
3 mo	1.26 ± 0.26	1.78 ± 0.09	<.05

*Cyclosporin blood trough level by radioimmunoassay determination (Sandoz kit).
†Mean value during the first 3 mo of treatment.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/37/7/873/356489/37-7-873.pdf by guest on 19 April 2024

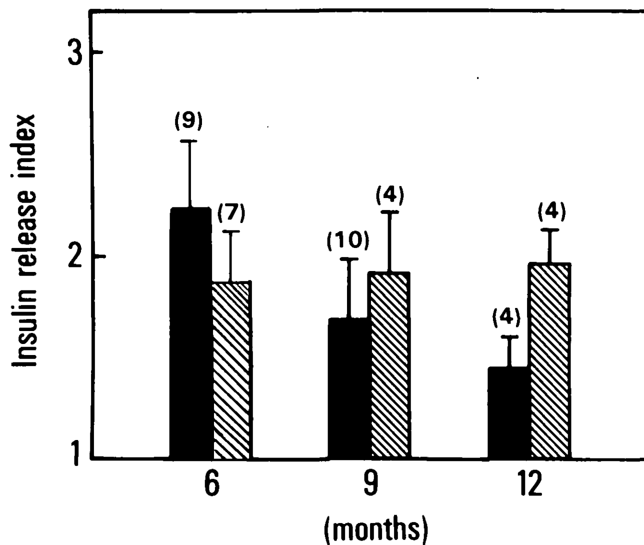

FIG. 2. Evolution of insulin release index from stimulated rat islet cells in presence of mononuclear cells from cyclosporin-treated diabetic patients. Results are expressed according to occurrence of remission (*hatched bar*) or clinical failure (*solid bar*) of treatment (NS). In case of failure, cyclosporin was stopped at 6 mo. Means ± SE with number of patients in parentheses.

RESULTS

Basal IR index before CsA treatment. The initial mean IR index in the group of patients receiving CsA or placebo was 1.11 ± 0.03, a value that appeared to be significantly lower than that in the control nondiabetic subjects (1.90 ± 0.18; $P < .001$). The presence of ICAs was studied in 56 of 65 patients; 53 patients were HLA typed. Anti-ICAs were present in 44% of patients studied. Twenty-three percent of the HLA-typed patients were HLA-DR3/4. Forty-three percent were HLA-DR3/X, and 26% were HLA-DR4/X. No significant difference was noted with regard to initial clinical and immunological parameters between the placebo and the CsA groups (Table 1).

Remission predictive value of initial IR index. In CsA-treated patients the initial IR index was not predictive of the effect of CsA on the clinical course of diabetes. Patients who were ultimately considered as failures of treatment at 6 mo ($n = 7$) had a mean initial IR index of 1.08 ± 0.08, whereas patients who had a complete remission at 6 mo ($n = 3$) or partial remissions ($n = 5$) did not show initial IR indexes significantly different from those of failures (1.26 ± 0.13 and 1.17 ± 0.08, respectively). In the placebo group, only one patient had a complete remission at 6 mo. His initial IR index was 1.09, compared to 1.04 for the whole group of placebo-treated patients.

Evolution of IR index under CsA therapy. In the placebo group the mean IR index remained unchanged throughout the follow-up period (Fig. 1), reflecting the persistence of cell-mediated anti-β-cell immunity. IR index in this group was always significantly ($P < .01$) lower than that of control subjects (Fig. 1).

In contrast, in the patients receiving CsA treatment the mean IR index rapidly increased, becoming significantly higher at 1 mo than initial values ($P < .001$) and values observed in the placebo group ($P < .001$), reaching the level of normal subjects. This effect was maintained during the 12-mo of follow-up. The improvement was more intense after 1 mo in the patients who had CsA blood trough levels >300 ng/ml during the first 3 mo of treatment ($n = 11$), with a mean IR index of 1.84 ± 0.06, compared to 1.36 ± 0.12 ($P < .01$) in patients whose CsA level was <300 ng/ml ($n = 4$) (Table 2). This difference is in keeping with the observation that the subgroups of patients with CsA blood levels >300 ng/ml had higher rates of remission at 6 mo (52%) than those showing CsA blood levels <300 ng/ml (14%).

No correlation was observed between the clinical course of diabetes (remission or failure) and evolution of IR index at 6 mo (Fig. 2). However, anti-β-cell cellular immunity tended to reappear after interruption of CsA treatment at 6 mo in case of clinical failure, as reflected by a drop in IR index, a difference not reaching, however, the level of significance in this limited series of patients.

Four relapses were observed in 12 CsA-treated remission patients when the CsA dosage was reduced to doses providing a trough level <200 ng/ml. In these subjects the IR index was 1.92 ± 0.30 before relapse (NS compared with the CsA-remission group) and decreased to 1.63 ± 0.14 in the 3 mo after relapse. However, this decrease was not significantly different from prerelapse values, indicating that IR index is not of predictive value for the relapse of insulin dependency.

Correlation with HLA-DR phenotype. The IR index was not different in 12 of 53 patients who were HLA-DR3/4$^+$ (1.04 ± 0.03) compared with other subjects (1.06 ± 0.03). Nor was there any difference of IR index during the course of CsA treatment between DR3/4$^+$ patients and other patients: at 6 mo, DR3/4$^+$ subjects had an index of 1.73 ± 0.13

TABLE 3
Relationship between initial and 6-mo insulin release index and HLA-DR typing or presence of islet cell antibodies

	Before	At 6 mo	
		Placebo	Cyclosporin
DR3/4			
+	1.04 ± 0.03 (5)	1.09 ± 0.01 (4)	1.75 ± 0.13 (4)
−	1.06 ± 0.03 (15)	1.10 ± 0.03 (13)	2.24 ± 0.27 (11)
Islet cell antibody			
+	1.15 ± 0.10 (7)	1.10 ± 0.05 (6)	1.91 ± 0.24 (7)
−	1.06 ± 0.03 (15)	1.12 ± 0.06 (13)	2.19 ± 0.31 (9)

Values are means ± SE with numbers of patients in parentheses.
DR3/4 positive vs. negative, NS.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/37/7/873/356489/37-7-873.pdf by guest on 19 April 2024

(n = 4) compared to 2.24 ± 0.27 in other patients. The same was true for the placebo group, where the IR index was 1.09 ± 0.01 for HLA-DR3/4$^+$ patients (n = 4) compared to 1.10 ± 0.03 in other subjects (n = 13) (NS; Table 3).

ICA detection. ICAs were initially detected in 25 of 56 patients (titer 1/1 to 1/64). There was no difference in initial IR index according to the presence or absence of ICAs: 1.15 ± 0.10 vs. 1.06 ± 0.03. The presence of ICAs was not associated with a particular trend in the evolution of IR index in either the CsA or the placebo group (Table 3).

At variance with the rapid normalization of the IR index, no consistent change was observed in the CsA group in the evolution of ICA titers, which did not differ significantly from the placebo group at 6 and 12 mo (21a).

DISCUSSION

These results show that CsA treatment reverses anti-β-cell–specific mediated immunity as assessed by the lymphocyte-mediated inhibition of insulin release by rat islet cells that is consistently observed at the onset of diabetes (5). Previous studies have indicated that inhibition of insulin release is performed by T-lymphocytes (with the CD3$^+$ CD4$^-$ CD8$^+$ phenotype; 6). The inhibition is not affected by the presence of antibody, complement, or aggregated IgG, and it is β-cell specific (glucagon release is not affected; 16)—all arguments that indicate it is a β-cell–specific T-lymphocyte–mediated phenomenon with the intriguing feature of the absence of conventional MHC restriction (human T-lymphocytes and rat islet cells). In the placebo group, this inhibition persisted unchanged for the 12-mo follow-up regardless of the clinical course of diabetes. Conversely, in the CsA-treated patients the inhibition was rapidly reversed. The difference between the two groups was already significant at 6 mo. This CsA-induced reversal of anti-β-cell–specific cellular immunity was lower in patients who had initially low CsA blood levels and consequently showed a lower rate of remission. However, interestingly, the evolution of cell-mediated anti-β-cell immunity was not correlated with the clinical course of diabetes; CsA-treated patients in whom exogenous insulin requirement persisted (treatment failure) showed an improvement in the IR index (disappearance of cell-mediated immunity) similar to that noted in cases with remission. This observation suggests a predominant role for nonimmunologic parameters in the absence of response to CsA treatment, especially a very low residual β-cell mass, a hypothesis substantiated by several strong arguments described elsewhere (1). Note also that the spontaneous remissions occurring in the placebo group with a low frequency were not associated with an increase in IR index. These data suggest that the spontaneous remissions probably reflect a particular metabolic status rather than an improvement in the underlying anti-islet immune autoreactivity.

Cellular immunity against β-cells was no longer detected within 1 mo of onset of CsA therapy. The decrease was more clear-cut when efficacious CsA blood trough levels were reached (>300 ng/ml) (22). Disappearance of anti-β-cell cellular immunity was delayed in patients who had lower CsA blood levels, but IR index was similar after 6 mo of treatment in both subgroups of patients. This delay may explain the lower clinical efficacy of CsA at low CsA blood levels (15).

To date, no study of CsA-induced modifications of cell-mediated organ-specific immune responses has been reported in human autoimmune diseases other than diabetes. In experimental models of CsA treatment in autoimmune diseases, the study of cell-mediated immunity has only been performed in S-retinal-antigen–induced uveitis in the rat (23,24), showing inhibition of S-antigen–stimulated proliferation of lymphocytes from CsA-treated animals concomitant with prevention of the disease.

The rapid abrogation of the capacity of the diabetic patient's lymphocytes to inhibit insulin release during CsA therapy may be interpreted in several ways. CsA could act directly at the effector stage (on already differentiated T-lymphocytes). This is in opposition to its known relative lack of effect on cytotoxic T-lymphocytes (25). It cannot be excluded, however, that CsA interferes with the synthesis of various lymphokines (26,27) that are directly responsible for the inhibition of β-cell function or indirectly activate β-cell–toxic T-lymphocytes (26–28) or other cell types, e.g., macrophages, that release toxic monokines (as recently demonstrated for interleukin 1; 29). Alternatively, CsA could act at an earlier stage, preventing islet cell reactive sensitization.

The discrepancy between the persistence of anti-ICAs and the disappearance of cellular immunity against β-cells during CsA therapy suggests a predominant role of cell-mediated immunity in the pathogenesis of diabetes, because the clinical status of the patients was improved by treatment in parallel to an increase in IR index. These opposite results are in agreement with the absence of effect of CsA on established antibody production (30).

In conclusion, this study points to a major role of cell-mediated immunity in the pathogenesis of human IDDM, as detected by the insulin-release inhibition test. Abnormalities detected by this test are completely reversed by CsA, whereas anti-ICA production persists. CsA-induced reversal of anti-β-cell–specific cellular immunity was observed in every patient, including those who had no clinical remission of diabetes. These results suggest that an increased remission rate will not be achieved by increased immunosuppression but rather by taking into account nonimmunologic parameters, i.e., residual β-cell mass.

ACKNOWLEDGMENTS

We thank H. Cohen, M. Lecuyer, L. Gomez, S. Pogy, and M. Ouary for excellent technical assistance and Dr. Binion of the Centre National de Transfusion, Nantes. We are deeply indebted to Dr. R. Assan, Dr. B. Charbonnel, Dr. Du Rostu, Dr. Ph. Passa, Dr. M. Pehuet-Figoni, Dr. G. Tchobroutsky, and Dr. P. Vexiau, who are involved in the CDF study as participating clinicians.

REFERENCES

1. Eisenbarth GS: Type I diabetes mellitus: a chronic autoimmune disease. *N Engl J Med* 314:1360–68, 1986
2. Bottazzo GF, Florin-Christensen A, Doniach D: Islet cell antibodies in diabetes mellitus associated with autoimmune polyendocrine deficiencies. *Lancet* 2:1279–83, 1974
3. Bottazzo GF, Dean RM, McNally JH, MacDay EH, Swift PGF, Gamble DR: In situ characterization of autoimmune phenomena and expression of HLA molecules in the pancreas in diabetic insulitis. *N Engl J Med* 313:353–60, 1985
4. Gepts W: Pathologic anatomy of the pancreas in juvenile diabetes mellitus. *Diabetes* 14:619–33, 1965
5. Boitard C, Debray-Sachs M, Pouplard A, Assan R, Hamburger J: Lymphocytes from diabetics suppress insulin release in vitro. *Diabetologia* 21:41–46, 1981
6. Boitard C, Chatenoud LM, Debray-Sachs M: In vitro inhibition of pan-

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/37/7/873/356489/37-7-873.pdf by guest on 19 April 2024

creatic β cell function by lymphocytes from diabetics with associated autoimmune diseases: a T cell phenomenon. *J Immunol* 129:2529–31, 1982

7. Like AA, Kislauski E, Williams RM, Rossini AA: Neonatal thymectomy prevents spontaneous diabetes mellitus in the BB/W rat. *Science* 216:644–46, 1982
8. Ogawa M, Maruyama T, Hasegawa T, Kavaya T, Kobayashi F, Tochino Y, Uda H: The inhibitory effect of neonatal thymectomy on the incidence of insulitis in nonobese (NOD) mice. *Biomed Res* 2:103–108, 1985
9. Weringer EJ, Byman K, Biggs V, Guberski DL, Like AA: Monoclonal antibodies to rat 0X-8 and 0X-19 reactive T cells prevent BB/W diabetes. *Diabetes Res Clin Pract* S1:S599, 1985
10. Wicker LS, Miller BJ, Mullen Y: Transfer of autoimmune diabetes mellitus with splenocytes from nonobese diabetic (NOD) mice. *Diabetes* 35:855–60, 1986
11. Bach JF, Strom T. (Eds.): Cyclosporine. In *The Mode of Action of Immunosuppressive Agents*. Amsterdam, Elsevier/North-Holland, 1985, p. 241–70
12. Laupacis A, Gardell C, Dupré J, Stiller CR, Keown P, Wallace AC, Thibert P: Cyclosporine prevents diabetes in BB Wistar rats. *Lancet* 1:10–12, 1983
13. Mori Y, Suko M, Okudaira H, Matsuba I, Tsuruoka A, Sasaki A, Yokoyama H, Tanase T, Shida T, Nishimura M, Terada E, Ikeda Y: Preventive effect of cyclosporin on diabetes in NOD mice. *Diabetologia* 129:244–47, 1986
14. Cohen DJ, Lorstscher R, Rubin MF, Tilney NL, Carpenter CB, Strom TB: Cyclosporin: a new immunosuppressive agent for organ transplantation. *Ann Intern Med* 101:667–82, 1984
15. Stiller CR, Dupré J, Gent M, Jenner MR, Keown PA, Laupacis A, Martell R, Rodger NW, Graffenried BV, Wolfe BMJ: Effects of cyclosporine immunosuppression in insulin-dependent diabetes mellitus of recent onset. *Science* 223:1362–67, 1984
16. Feutren G, Papoz L, Assan R, Vialettes B, Karsenty G, Vexiau P, Du Rostu H, Rodier M, Sirmai J, Lallemand A, Bach JF: Cyclosporin induces remissions in recently diagnosed insulin-dependent diabetics. *Lancet* 2:119–24, 1986
17. Boitard C, Sai P, Debray-Sachs M, Assan R, Hamburger J: Antipancreatic immunity: in vitro studies of cellular and humoral immune reactions directed toward pancreatic islet. *Clin Exp Immunol* 55:571–80, 1984
18. WHO Study Group: *Diabetes Mellitus*. Geneva, World Health Org., 1985 (Tech. Rep. Ser. 727)
19. Lacy PE, Kostianovsky M: Method for the isolation of intact islets of Langerhans from the rat pancreas. *Diabetes* 16:35–39, 1967
20. Ono J, Takaki R, Tukuma M: Preparation of single cells from pancreatic islets of adult rat by the use of dispase. *Endocrinol Jpn* 24:265–70, 1977
21. Deschamps I, Lestradet H, Bonaïti C, Schmitt MM, Busson A, Benajam-Marcelli-Barge A, Hors J: HLA genotype studies in juvenile insulin-dependent diabetes. *Diabetologia* 19:189–93, 1980

21a. Boitard C, Feutren G, Castano L, Debray-Sachs M, Assan R, Hors J, Sach, JF: Effect of cyclosporin A treatment on the production of antibody in insulin-dependent (type I) diabetic patients. *J Clin Invest* 80:1607–12, 1987

22. Kahan BD: Individualization of cyclosporine therapy using pharmacokinetics and pharmacodynamic parameters. *Transplantation* 40:457–76, 1985
23. Nussenblatt RB, Rodrigues MM, Wacker WB, Cevario SJ, Salinas-Carmona MC, Gery I: Cyclosporin A inhibition of experimental autoimmune uveitis in Lewis rats. *J Clin Invest* 67:1228–31, 1981
24. Nussenblatt RB, Carmona MS, Waksman BH, Gery I: Cyclosporin A: alterations of the cellular immune response in S-antigen–induced experimental autoimmune uveitis. *Int Arch Allergy Appl Immunol* 70:289–94, 1983
25. Hess AD, Tutschka PJ: Effect of cyclosporin A on human lymphocyte responses in vitro. I. CsA allows for the expression of alloantigen activated suppressor cells while preferentially inhibiting the induction of cytolytic effector lymphocytes in MLR. *J Immunol* 124:2601–608, 1980
26. Eliott JF, Lin Y, Mizel SB, Bleackley RC, Haemish DG, Paetkau V: Induction of interleukin-2 messenger RNA inhibited by cyclosporin A. *Science* 226:1439–41, 1984
27. Granelli-Pipermo A, Inaba K, Steinman RM: Stimulation of lymphokine release from T lymphoblasts: requirement for mRNA synthesis and inhibition by cyclosporin A. *J Exp Med* 160:1792–802, 1984
28. Bunjes D, Hardt C, Rollinghoff M, Wagner H: Cyclosporin A mediates immunosuppression of primary cytotoxic T cell responses by impairing the release of interleukin 1 and interleukin 2. *Eur J Immunol* 11:657–61, 1981
29. Bendtzen K, Mandrup-Poulsen T, Nerup J, Nielsen JH, Dinarello CA, Svenson M: Cytotoxicity of human pI 7 interleukin-1 for pancreatic islets of Langerhans. *Science* 232:1545–47, 1986
30. Kunkl A, Klaus GGB: Selective effects of cyclosporin A on functional B cell subsets. *J Immunol* 125:2526–31, 1980

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/37/7/873/356489/37-7-873.pdf by guest on 19 April 2024