IA-2 Antibody–Negative Status Predicts Remission and Recovery of C-Peptide Levels in Type 1 Diabetic Patients Treated With Cyclosporin

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OBJECTIVE — The use of cyclosporin in recent-onset type 1 diabetes has demonstrated the potential for immune intervention in the treatment and prevention of the disease. However, a proportion of patients failed to respond to cyclosporin treatment. Indicators of resistance to immune intervention would be valuable for the most effective use of such therapies in disease prevention. The aim of this study was to determine whether presence of IA-2 antibodies is such a marker.

RESEARCH DESIGN AND METHODS — IA-2 antibodies were determined by radioligand binding assay in sera from patients recruited into the Canadian-European cyclosporin trial. Insulin dose requirements and glucagon-stimulated C-peptide secretion were analyzed in patients grouped according to IA-2 antibody status at entry.

RESULTS — Cyclosporin treatment had no significant effect on frequency of IA-2 antibodies during the 1 year of treatment. Cyclosporin caused significant reduction in insulin requirements and significant increases in C-peptide secretion mainly in patients negative for IA-2 antibodies. Analysis of GAD antibodies in combination with antibodies to IA-2 indicated that the group most resistant to cyclosporin were IA-2 antibody positive, GAD antibody negative.

CONCLUSIONS — The results demonstrate that IA-2 antibody analysis is valuable in identifying individuals for whom immunosuppressive treatment would be most effective.

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Type 1 diabetes is caused by the specific loss of insulin-secreting pancreatic β-cells by a mechanism in which autoimmunity to pancreatic islets is likely to play a key role (1). Procedures to block islet autoimmunity have been proposed as effective means to prevent disease in individuals identified as at risk. Clinical trials of general immunosuppressive agents such as cyclosporin have demonstrated that immunosuppression can reduce insulin requirements and enhance endogenous β-cell function in patients with recent-onset type 1 diabetes (2,3). However, not all diabetic patients were found to benefit from the immunosuppressive treatment, and therefore, some individuals may be resistant to immune intervention. The identification of markers that can predict a successful outcome would greatly assist future effective use of immune intervention in the prevention of diabetes.

Studies of diabetic patients treated with cyclosporin have shown that early immune intervention is a critical factor in the success of the treatment in sustaining endogenous β-cell function (2,3). Genetic markers such as HLA or the presence of the immune markers islet cell antibodies (ICAs), insulin autoantibodies (IAAs), or GAD antibodies (GADAs) at onset of disease are not, by themselves, useful in predicting study outcome (2–6). Interestingly, cyclosporin was found to have different effects on the antibody responses to these target antigens after initiation of treatment. Thus, cyclosporin caused a rapid decrease in ICA titers during the first year of treatment and inhibited antibody responses to exogenous insulin while having no significant effect on GADA (3–6). A fourth antibody marker, the tyrosine phosphatase–like molecule IA-2, is now established as being associated with young age of onset and rapid progression to type 1 diabetes in a number of populations (7–9) and, therefore, may identify individuals with particularly aggressive β-cell destruction. The aim of this study was to use serum samples available from the Canadian-European Cyclosporin Trial to investigate whether the presence of IA-2 antibodies (IA-2As) can aid in identification of individuals for whom immunosuppression would be most effective and whether the immunosuppressive treatment is able to influence the immune response to IA-2.

RESEARCH DESIGN AND METHODS

— The subjects of this study were patients with recent-onset

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Abbreviations: GADA, GAD antibody; IA-2A, IA-2 antibody; IAA, insulin autoantibody; ICA, islet cell antibody; RR, relative risk.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Characteristics of patients at entry*

	Cyclosporin-treated	Placebo
n	45	52
Age (years)	20.8 ± 6.4	21.2 ± 5.7
Weight (kg)	61.5 ± 12.7	62.0 ± 10.6
Percentage of women	20	28.8
Duration of symptoms (weeks)	5.6 ± 2.8	6.0 ± 2.7
HbA_{1c} (%)	10.4 ± 2.0	9.7 ± 2.2
Stimulated C-peptide (nmol/l)	0.35 ± 1.15	0.41 ± 0.24
Insulin dose ($IU \cdot kg^{-1} \cdot day^{-1}$)	0.52 ± 0.3	0.47 ± 0.2
ICA positive (%)	64.4	66.6
Median ICA titer (JDF units)	40	40
HLA DR 3/4 (%)	29	25
HLA DR 4/non-DR3 (%)	40	44
HLA DR 3/nonDR4 (%)	24	23
HLA (non-DR3/non-DR4) (%)	7	4

*Data are means ± SE unless otherwise indicated. JDF, Juvenile Diabetes Foundation.

type 1 diabetes who participated in a multicenter placebo-controlled, double-blind, randomized prospective trial of cyclosporin immunosuppression for 12 months. The main results of this intent-to-treat study with regard to the effects of cyclosporin treatment on insulin requirement and β-cell function and an account of the adverse effects have been reported elsewhere (2,4,6). Informed consent from all subjects was obtained after the nature of the experimental procedures had been explained to them.

In this study, we focused on *1*) the effect of 12 months of cyclosporin treatment on the prevalence and levels of IA-2A and 2) the possible predictive value of IA-2A status at entry on the course and 12-month outcome with regard to non–insulin-requiring remission, insulin dose, and C-peptide levels in both the natural history (placebo group) and during immunosuppression (cyclosporin group). Therefore, we analyzed IA-2A in all available sera from patients with at least 12 months of compliant follow-up and with complete data sets for the selected study variables. Of the 188 patients originally recruited, 1 patient was irretrievably lost to follow-up and 45 patients discontinued drug treatment before 1 year. Of the remaining 142 patients, 97 (45 treated with cyclosporin and 52 with placebo) met the inclusion criteria described above. The entry characteristics of the patients are shown in Table 1. The 97 patients that were included were comparable to the 188 originally recruited with regard to these entry characteristics (2).

Antibodies to IA-2A were analyzed by radioligand binding assays (7). cDNA representing the cytosolic domain (amino acids 603–979; IA-2ic) of IA-2 was cloned into the pSP64 polyA in vitro transcription vector (Promega, Madison, WI) and translated in vitro using commercial kits (Promega) in the presence of [^{35}S]methionine (Amersham, U.K.). Incorporated radioactivity was determined by precipitation with 10% trichloroacetic acid and scintillation counting. Aliquots (20 μl) containing 20,000 cpm of in vitro–translated protein in immunoprecipitation buffer (10 mmol/l HEPES, pH 7.4, 150 mmol/l NaCl, 0.5% Triton X-100, 10 mmol/l benzamidine, 0.5 mg/ml BSA, and 5 mmol/l methionine) were incubated with 5 μl of test serum for 5 h at 4°C. Immune complexes were isolated on 5 μl of protein A-Sepharose, and immunoprecipitates were washed in filter plates (Millipore, Bedford, MA) with immunoprecipitation buffer and once with water. Radioactivity precipitated was measured by scintillation counting and expressed relative to an antibody-positive serum, arbitrarily assigned a value of 100 units. Sera with antibody levels >80 units were reanalyzed at a dilution of 1:40. Sera were considered positive if antibody levels were higher than the 99th percentile of antibody levels in sera from 217 healthy subjects (aged 1–43 years) with no family history of type 1 diabetes, of whom 137 were <15 years of age. The threshold for positivity for antibodies to IA-2 was 5 units. The interassay coefficient of variation around the threshold of positivity was 9.9%, and the assay achieved 81% sensitivity and 97% specificity in the 1995 Combined Autoantibody Workshop (10). Analyses of ICA, IAA, GADA, and C-peptide concentrations in the patients have been described elsewhere (4–6).

Statistical methods

The titers or levels of different antibodies among the patients were compared in a hierarchical log-linear model (11) to assess whether occurrence of antibodies were correlated. Insulin-free remission was modeled by a discrete-time proportional hazards model (Cox-model) for the remission rates, with treatment and antibody status as covariates (12). Treatment and other effects are reported as relative risks (RRs). A similar model was used for the rates of recurrence of insulin treatment. C-peptide levels and insulin dose in patient groups were modeled in a mixed normal error model (variance component model) with random effect of patient and fixed effects of treatment, time since diagnosis, and antibody status (13). We also applied models with random patient-effect on the slope of the response by time. Effects of antibody status and insulin treatment were modeled as differences in time-trends of the response. Effects are reported as differences between groups of patients in C-peptide levels (or insulin dose) per month. The estimated SDs of the between-patient and within-patient variations are given. All estimates are given with 95% CIs or prediction intervals.

RESULTS

Effect of cyclosporin on the prevalence and levels of IA-2As

Serum samples from 45 type 1 diabetic patients treated with cyclosporin and 52 patients in the placebo group were analyzed for IA-2A using a radioligand binding assay (7) both at entry into the study and after 12 months of treatment (Fig. 1). At entry, 31 (69%) of the cyclosporin-treated patients and 29 (56%) of the patients in the placebo group were positive for IA-2A with mean (± SEM) antibody levels of 48.1 ± 7.1 and 43.2 ± 7.2 units, respectively. After 12 months of the study, there were no significant changes in the prevalence of IA-2A in either group. Thus, at 12 months, 29 (64%) of the cyclosporin-treated patients and 31 (60%)

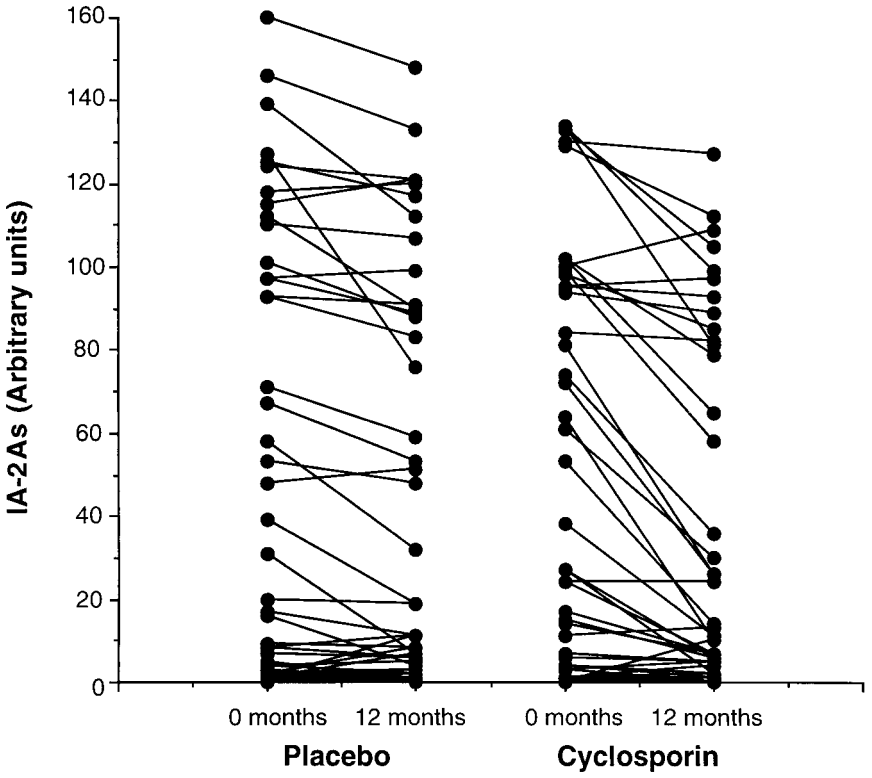

Figure 1—*IA-2A levels in individual placebo- or cyclosporin-treated diabetic patients at study entry and after 12 months of treatment.*

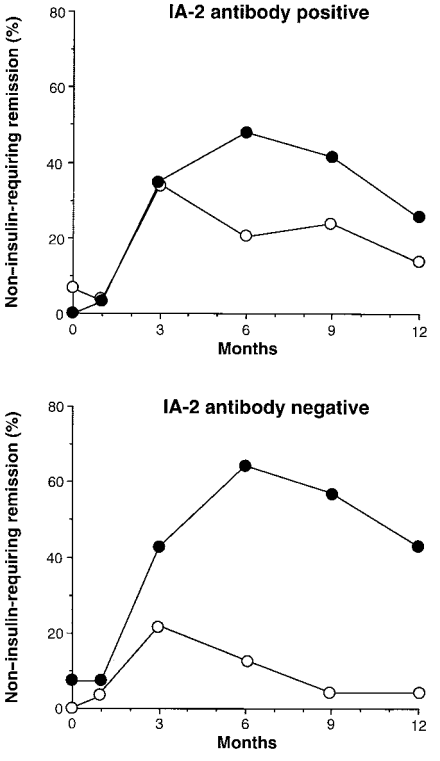

Figure 2—*Frequency of non–insulin-requiring remission in type 1 diabetic patients during 12 months of treatment with cyclosporin (●) or placebo (○). Patients were grouped according to IA-2A status at study entry. Cyclosporin-treated: IA-2A–positive,* n = *31; IA-2A–negative,* n = *14. Placebo: IA-2A–positive,* n = *29; IA-2A–negative,* n = *23).*

of those given placebo were positive for IA-2 antibodies with mean antibody levels of 34.3 ± 6.1 and 38.1 ± 6.6, respectively. There was no significant difference in IA-2A levels between the cyclosporin- and placebo-treated patients at either time point. Therefore, in marked contrast to its effect on ICA and insulin antibodies (5,6), but similar to GADA (4), cyclosporin treatment has little influence on the IA-2A response in type 1 diabetic patients during the first year of diabetes.

Effect of IA-2A positivity on insulin requirement and β-cell in cyclosporin-treated and untreated patients

Of the 97 patients, insulin-free remission developed in 42 patients during the 12-month follow-up period. Among the placebo-treated patients, there was no significant difference between the remission rates in IA-2A–positive and IA-2A–negative subjects. The effect of cyclosporin treatment on remission rate was significantly different between IA-2A–positive and IA-2A–negative patients (Fig. 2). For the IA-2A–negative patients, treatment elevated remission rates by a factor 4.2 (95% CI 1.4–12.4), whereas cyclosporin had little effect on remission rates in the IA-2A–positive group (RR 1.4, 95% CI 0.64–3.0).

Of the 42 patients in whom insulin-free remission developed, 24 patients had recurrence of insulin dependence within the follow-up period. Insulin recurrence was analyzed by taking treatment, antibody status, time of insulin-free remission, and time since insulin-free remission into account. There was a significant effect of time of remission on the rate of recurrence to insulin dependence; the later the remission, the higher the recurrence rates (RR 1.5/month, 95% CI 1.4–2.1). Therefore, the earlier remission started, the longer it lasted. Combined with the results for insulin-free remission, we observed that cyclosporin treatment had an elevating effect on remission rate and a depressing effect on rate of recurrence among the IA-2A–negative patients but no significant effect on IA-2A–positive patients.

There was no significant difference in insulin dose between IA-2A–positive and IA-2A–negative patients on placebo treatment (Fig. 3). However, in the cyclosporin-treated group, IA-2A–negative patients required lower insulin doses than IA-2A–positive patients. This can be expressed as a treatment effect of 15 ± 3.6 mU/kg per month in IA-2A–positive patients and 27 ± 4.6 mU/kg per month in the IA-2A–negative patients ($P < 0.05$).

The time course of glucagon-stimulated C-peptide levels are shown in Fig. 4. At entry into the study, there was no significant difference in stimulated C-peptide levels between cyclosporin-treated and nontreated patients or between IA-2A–positive and IA-2A–negative patients. Cyclosporin treatment had different effects on the course of C-peptide responses in IA-2A–positive and IA-2A–negative patients; relatively higher levels of C-peptide secretion were observed in IA-2A–negative patients (7.0 ± 3.2 pmol/l per month) than in IA-2A–positive patients (17.8 ± 4.1 pmol/l per month; $P < 0.05$).

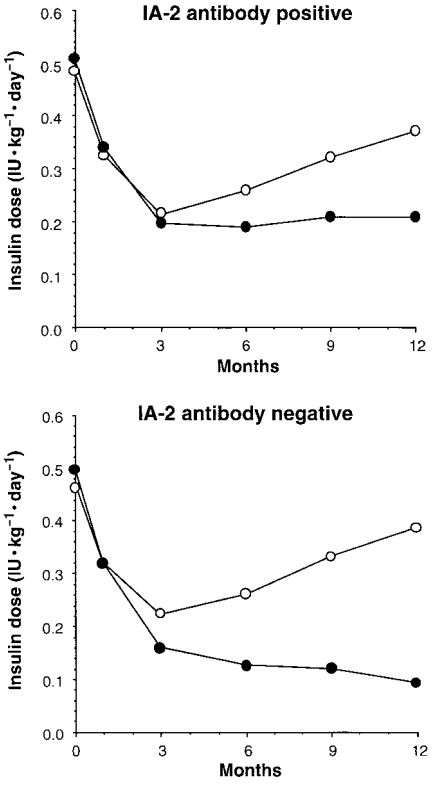

Figure 3—*Estimated mean insulin dose requirements of type 1 diabetic patients, positive or negative for IA-2A at study entry, during 12 months of treatment with cyclosporin (●) or placebo (○). The between-patient variation, calculated as the SD of the difference between two randomly chosen patients, was 0.227 IU · kg^{-1} · day^{-1}, and the within-patient variation, calculated as the SE of the difference between two measurements from the same patient at the same time, was 0.216 IU · kg^{-1} · day^{-1}.*

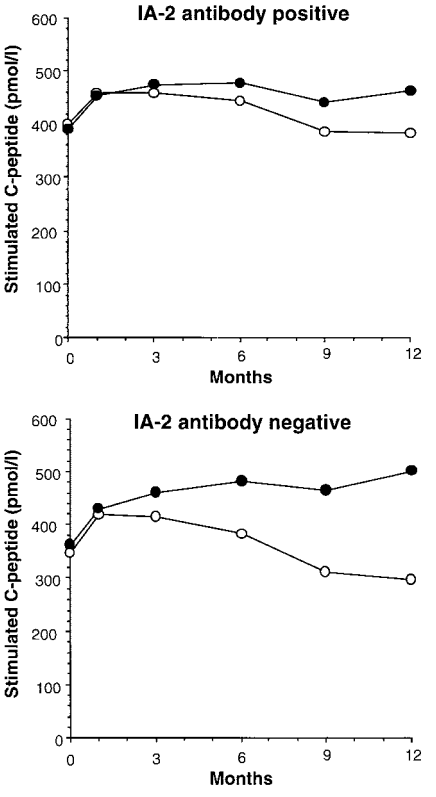

Figure 4—*Estimated mean glucagon-stimulated C-peptide levels of type 1 diabetic patients, positive or negative for IA-2A at study entry, during 12 months of treatment with cyclosporin (●) or placebo (○). The between-patient variation was 230 pmol/l and the within-patient variation was 192 pmol/l.*

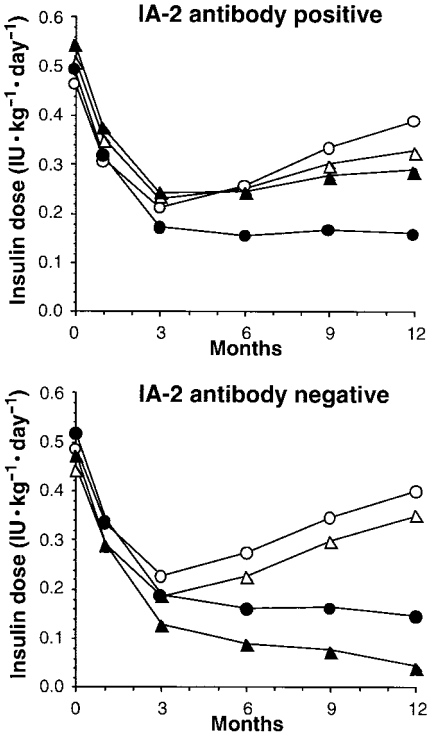

Figure 5—*Influence of GADA status on estimated mean insulin dose requirements of type 1 diabetic patients, positive or negative for IA-2A at study entry, during 12 months of treatment with cyclosporin (closed symbols) or placebo (open symbols). GADA-positive patients are represented by circles, and GADA-negative patients are represented by triangles.*

Effect of other antibodies

Models similar to the ones described above were fitted, using ICA, IAA, and GADA as covariates. No differential effects were observed on partitioning the results by ICA or IAA, but there were differences according to GADA. Consequently, models of the form as above were fitted, but combining the status of GADA and IA-2A as covariate. A cyclosporin-mediated effect on insulin dose and C-peptide secretion was present, and of the same order of magnitude, in three of the groups divided according to antibody status (decrease of 23.6 ± 3.3 mU per month) but was virtually absent (5.6 ± 5.6 mU per month) in IA-2A–positive/GADA-negative patients ($P < 0.05$, Fig. 5). For stimulated C-peptide, the treatment effect was 16.1 ± 2.9 pmol/l per month, except for the GADA-negative/IA-2A–positive group, in which the treatment effect was −7.1 ± 4.9 pmol/l per month ($P < 0.05$).

CONCLUSIONS — A variety of procedures are currently being evaluated for effectiveness either in blocking progression of diabetes in individuals identified as at risk or in ameliorating disease in patients with recently diagnosed disease. Previous clinical trials to achieve these aims have included cyclosporin treatment, which had beneficial effects on remission rate and β-cell function in many patients with newly diagnosed diabetes (2,3). However, immunosuppression was not universally effective. Clearly, markers that can predict or monitor effectiveness of immune intervention would be valuable for future prevention trials. Although further use of cyclosporin for diabetes therapy is unlikely, owing to potential toxic effects, valuable lessons can be learned from the results of the earlier cyclosporin trials that may be applicable to other procedures for immune intervention. In this study, we have analyzed samples available from the European-Canadian cyclosporin trial to determine the value of IA-2A in predicting or monitoring the outcome of immunosuppressive treatment.

Cyclosporin was shown to have little effect on the levels of IA-2A during the 1-year period of treatment. These results are similar to the effect of cyclosporin on GADA status in the same group of patients but contrast with inhibitory effects of the agent both on ICA titers and on insulin antibodies that develop after initiation of insulin therapy (3–6). Therefore, humoral immune responses to two major islet cell autoantigens in type 1 diabetes, GAD and IA-2, are resistant to cyclosporin inhibition, despite the recovery of β-cell function in the treated subjects. The results of these studies also demonstrate that the effects of cyclosporin on GADA and IA-2A are different from those

on ICA levels. The dissociation of the effects of the immunosuppressant on ICA and GADA/IA-2A underlines that, although both GAD and IA-2 can contribute to ICA staining on pancreatic sections (14,15), the major target of the ICA reactivity is a different, possibly unidentified, antigen.

Although cyclosporin was found to inhibit ICA and insulin antibodies, neither the presence nor level of these antibodies, nor those of GADA, were found to predict the effectiveness of cyclosporin treatment on insulin requirements or endogenous β-cell function (3–6). The presence of IA-2A is a major marker of diabetes development that was not investigated in these earlier studies. IA-2A is well established as being associated with rapid progression to diabetes development and is found predominantly in diabetic patients of younger age at onset. These characteristics suggest that the presence of this marker may be indicative of more aggressive autoimmunity, which may be difficult to block by immunosuppressive regimens. Consistent with this, the presence of IA-2A is reported to be associated with lower serum C-peptide levels and higher insulin dose requirements 2 years after diagnosis (16), although this was not confirmed by other studies (17,18). In our study, IA-2A–positive patients given placebo did not show a more rapid loss of C-peptide secretion during the 1 year of study. Nevertheless, subjects who were IA-2A–positive were resistant to cyclosporin-mediated increases in the rate of insulin-free remission and reduction of insulin dose over this period. Furthermore, cyclosporin increased glucagon-stimulated C-peptide secretion, an indicator of endogenous β-cell function, only in IA-2A–negative patients. Although GADA status alone was previously found not to influence insulin requirements or β-cell function in response to cyclosporin treatment, when analyzed in combination with IA-2A, this marker does provide additional information on who will most benefit from the immunosuppressive therapy. Thus, cyclosporin had virtually no effect on insulin dose and C-peptide secretion in patients who were positive for IA-2A but negative for GADA, whereas the drug had beneficial effects in subjects with all other combinations of the two markers. Therefore, analysis of antibody combinations may be most valuable in determining effectiveness of immune intervention.

The results of this study demonstrate that, despite previous indications, the analysis of islet autoantibody markers, particularly IA-2A, is valuable in determining the outcome of immunosuppression. Although IA-2A is associated with the HLA-DR4/DQB1*0302 haplotype (19,20), it is unlikely that our findings in relation to autoantibodies are secondary to genetic effects at the HLA locus, because no significant association of cyclosporin-mediated remission with HLA-DR was observed in previous analyses (6). It is not possible from this study to determine the reasons for IA-2A–linked resistance to immunosuppression; we speculate that this might be related to the more rapid progression to diabetes in IA-2A–positive individuals. It may therefore be necessary to adjust the degree of immunosuppression according to the antibody status of the subject. Although our results are necessarily restricted to the effects of cyclosporin treatment in recent-onset diabetes, they may clearly have implications for other protocols of immune intervention and it will be of interest to perform similar analyses as ongoing trials are completed.

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