Cyclosporin A Suppresses Insulin Autoantibodies and Heterologous Insulin Antibodies in Type I Diabetic Children

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We report that cyclosporin A (CsA) suppresses the insulin autoantibodies that are present before insulin therapy in the sera of one-third of studied type I (insulin-dependent) diabetic children. CsA also reversibly blocks the production of antibodies after exogenous insulin injection, whereas high titers of heterologous insulin antibody are observed in all type I patients not receiving CsA. *Diabetes* 37:1049–52, 1988

Insulin autoantibodies (IAAs) are present in the sera of 16–36% of recently diagnosed type I (insulin-dependent) diabetic adults before exogenous insulin therapy (1,2). They are detected in a greater proportion of type I diabetic children, their occurrence being inversely related to age (3–5). The role of IAA in the pathogenesis of type I diabetes is still obscure. These autoantibodies can be found in the sera of future type I diabetic patients several years before the onset of the diabetic syndrome (6,7). When present at this prehyperglycemic phase of the disease, IAAs show no tendency to spontaneously disappear and instead increase progressively until diagnosis (7). Once insulin therapy is started, the evolution of IAAs cannot be followed, because they are indistinguishable from the heterologous insulin antibodies (HIAs) produced by diabetic subjects in response to insulin injections. We report that cyclosporin A (CsA) blocks both IAA and HIA production in children with type I diabetes of recent onset.

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MATERIALS AND METHODS

Patients. The subjects were children with type I diabetes. All were initially admitted to our unit, where serum samples were collected before the subjects received their first insulin dose. A group of patients was enrolled in a 12-mo trial of CsA. We selected 38 of these patients for our study. Fifteen were randomly chosen from the CsA-treated diabetic subjects who were able to stop insulin therapy 1–3 mo after inclusion into the trial (group CsA-NIR) and who thereafter maintained near-normoglycemic levels with CsA alone. Fifteen other patients were randomly chosen from the CsA-treated patients who had to maintain insulin therapy at doses >0.20 U · kg^{-1} · day^{-1} (group CsA-IR; failures). We added 10 patients to the study who interrupted CsA abruptly after 4–6 mo of immunosuppression. We also included 2 diabetic patients treated with CsA alone. Both were first-degree relatives of patients with type I diabetes, were HLA-DR3/4, had elevated titers of islet cell antibodies (ICAs), and had unstimulated insulin secretion. At the time of inclusion into the CsA trial, both consistently had fasting blood glucose >160 mg/dl, with peak values at 210 and 205 mg/dl. Their recorded maximal glucose levels were 340 and 280 mg/dl, respectively. Neither of them, however, had diabetes symptoms. Both experienced rapid glycemic normalization 2–3 wk after the onset of CsA treatment. Twenty-five other diabetic children did not receive CsA and were thus treated with insulin alone (group INS). In addition, we selected 5 type I diabetic patients aged 8–15 yr who could spontaneously interrupt insulin therapy after 2–8 wk of treatment. They were studied after 1–2 mo of a honeymoon period. All were back to insulin 4–11 wk later. The clinical characteristics of the patients are presented in Table 1.

Treatment. CsA was started concomitantly with insulin therapy at a dose of 7.5 mg · kg^{-1} · day^{-1} given orally. The dose was later adjusted to maintain trough levels of CsA between 150 and 350 ng/ml blood (Table 2), evaluated by standard immunoassay (Sandoz, Basel).

Blood glucose was maintained within near-normal limits by twice-daily injections of a mixture of regular and inter-

TABLE 1
Clinical characteristics of patients

	With cyclosporin A		
	NIR	IR	Insulin alone
n	15	15	25
Age (yr)	11 ± 2	11 ± 3	9.5 ± 4
Sex (M/F)	5/10	10/5	10/15
Duration of symptoms (days)	27 ± 5	48 ± 7	54 ± 9

NIR, subjects who could stop therapy 1–3 mo after inclusion into the trial; IR, subjects who had to maintain insulin therapy at doses >0.20 U · kg^{-1} · day^{-1}.

mediate-acting insulin (Actrapid HM and Monotard HM, Novo, Bagsvaerd, Denmark). The daily insulin dose was adjusted according to blood glucose monitoring. Glycosylated hemoglobin (GHb) was measured every 3 mo.

Patients of group CsA-NIR stopped insulin 62 ± 7 days after starting CsA. They were maintained without insulin as long as fasting and postprandial blood glucose was <140 and <200 ng/ml, respectively, and GHb remained <7.5% (normal mean + 4SD) (Table 2).

Methods. Insulin binding was measured in serum samples by a sensitive radioassay derived from the method described by Palmer et al. (1) and modified by acid-charcoal dextran extraction of serum insulin (8). Briefly, after acid-charcoal extraction, 80 μl of supernatant is incubated with mono-^{125}I-labeled (Tyr-A14)–human insulin (20,000 cpm/tube, 0.011 pmol/tube) (Novo) in 0.04 M phosphate buffer (pH 7.4). The labeled antibody complex is precipitated with polyethylene glycol diluted in 0.05 M barbital buffer (pH 8.6) with 0.1% Tween 20. We verified that concentrations up to 2000 ng/ml of CsA had no effect on insulin-binding measurement. Insulin antibodies are considered present when the percentage of labeled insulin bound by the serum exceeds the mean percentage of labeled insulin bound by the sera of 80 nondiabetic children (1 ± 0.19%) by ≥5SD, i.e., insulin binding ≥2%, and when this binding is displaceable by unlabeled insulin. The intra-assay coefficients of variation were 4% in the range of 1–2% insulin binding and 3% in the range of 30–50% insulin binding. The corresponding inter-assay coefficients of variation were 14 and 10%.

ICAs were determined by indirect immunofluorescence on frozen sections of a human group 0 pancreas with a fluoresceinated anti-human IgG serum (Wellcome, Dartford, UK; 9). Complement-fixing ICAs (CF-ICAs) were determined on similar sections via fluoresceinated anti-C3 serum (Dakopatts, Glostrup, Denmark; 10). Antibody titers were determined by serial $\log_2$ dilutions. The precision and accuracy of ICA determination with the same pancreas used in this study were previously evaluated by the standard curve under the code number 4 as part of the first international workshop on the standardization of cytoplasmic ICAs (11). Intra-assay coefficient of variation was 95.6% when determining ICA titers of 22 samples studied blind twice. The specificity and sensitivity of ICA determination were 98.75 and 69%, respectively.

RESULTS

Before the initiation of therapy, IAAs were present with a similar frequency and at a comparable titer in the different groups of patients (Table 3). All patients receiving insulin alone exhibited a rapid increase in serum insulin binding (Fig. 1). In contrast, CsA-treated patients did not show HIA production after exogenous insulin administration. There is evidence that this is not due to the diminution or cessation of insulin therapy in the CsA-treated patients: *1*) the difference in HIA production was already established at 3 mo, although the CsA-treated diabetic subjects had been exposed to rather large insulin doses (Table 2); *2*) insulin binding is similarly suppressed in the sera of both the CsA-treated patients still receiving insulin and those who have stopped; *3*) although 6 patients of group CsA-IR received >0.5 U insulin · kg^{-1} · day^{-1} (mean dose 0.7 ± 0.1) throughout the period of CsA treatment, their insulin binding averaged only 1.6 ± 0.1% at 3 mo and 1.5 ± 0.07% at 9–12 mo; and *4*) serum insulin binding remained elevated (19.1 ± 7%, range 8.5–46%) in the 5 patients who spontaneously interrupted insulin therapy (honeymoon patients). In all the patients who abruptly interrupted CsA, we observed a prompt and large

TABLE 2
Characteristics of treatment

	With cyclosporin A		
	NIR	IR	Insulin alone
Insulin dose (U · kg^{-1} · day^{-1})			
0–3 mo	0.22 ± 0.10	0.50 ± 0.25	0.81 ± 0.20
3–6 mo		0.41 ± 0.22	0.80 ± 0.16
6–12 mo		0.35 ± 0.12	0.82 ± 0.20
Glycosylated hemoglobin (%)*			
0–3 mo	6.14 ± 0.16	6.5 ± 0.2	7.1 ± 0.3
3–6 mo	6.40 ± 0.20	6.9 ± 0.2	7.8 ± 0.2
6–12 mo	6.40 ± 0.20	6.8 ± 0.2	7.4 ± 0.2
Cyclosporin A dose (mg · kg^{-1} · day^{-1})	8.1 ± 0.2	8.0 ± 0.1	
Cyclosporin A trough level (ng/ml)	235 ± 29	275 ± 19	

NIR, subjects who could stop therapy 1–3 mo after inclusion into the trial; IR, subjects who had to maintain insulin therapy at doses >0.20 U · kg^{-1} · day^{-1}.
*Normal mean ± SD 4.7 ± 0.7.

TABLE 3
Insulin-binding values in sera of studied patients

	With cyclosporin A		Insulin alone
	NIR (n = 15)	IR (n = 15)	(n = 25)
Before therapy			
Insulin binding (%)	2.7 ± 0.8	2.4 ± 0.5	3.5 ± 1.2
Patients with insulin binding >2%*	6 (40%)	4 (26%)	7 (28%)
Insulin binding in positive patients	5 ± 1.6	2.9 ± 0.3	8.0 ± 2.3
At 3 mo			
Insulin binding (%)	2.1 ± 0.5	1.6 ± 0.1	27 ± 5†
Patients with insulin binding >2%	2 (13%)	2 (13%)	25 (100%)†
Insulin binding in positive patients	3.2 ± 0.5	2.3 ± 0.2	27 ± 5†
At 9–12 mo			
Insulin binding (%)	1.7 ± 0.1	1.6 ± 0.1	38 ± 6†
Patients with insulin binding >2%	2 (13%)	0 (0%)	25 (100%)†
Insulin binding in positive patients	3.2 ± 0.4		38 ± 6†

NIR, subjects who could stop therapy 1–3 mo after inclusion into the trial; IR, subjects who had to maintain insulin therapy at doses >0.20 $U \cdot kg^{-1} \cdot day^{-1}$.
*Reflects presence of insulin autoantibodies.
†$P < .001$.

increase of serum insulin binding from 1.9 ± 0.1 to 16 ± 4% ($P < .01$). The effects of CsA on IAA could be studied only in the 2 diabetic patients who did not receive insulin injections. In both, CsA suppressed IAA production, which became undetectable after 3 mo of immunosuppression (Fig. 2). In addition, in the 10 CsA-treated diabetic patients who had IAA at inclusion, insulin binding decreased from 4.4 ± 1 to 2.2 ± 0.2% at 3 mo ($P < .05$; Fig. 2). A slight decrease in ICA and CF-ICA titers was frequently observed during the 12-mo study both in the CsA-treated patients and in those treated with insulin alone (Table 4), without detectable differences between the two groups.

DISCUSSION

Our study first indicates that CsA completely suppresses the production of insulin antibodies usually formed by diabetic children in response to insulin therapy. This effect is observed whether CsA is given concomitantly with the first

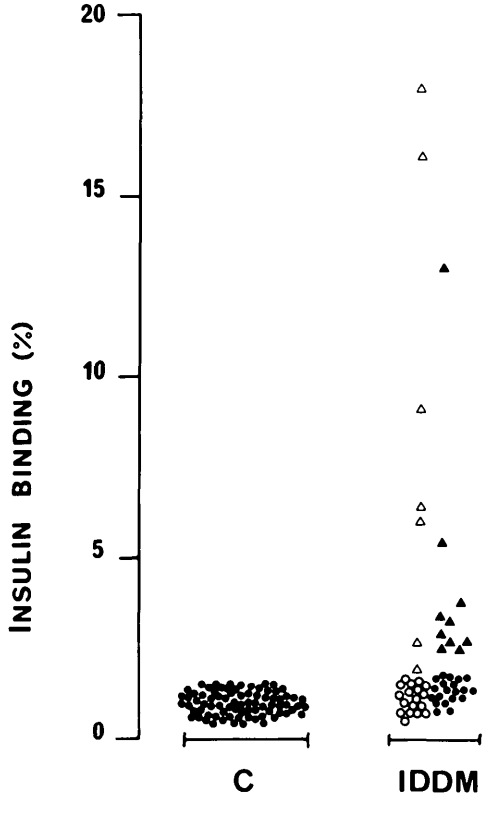

FIG. 1. Insulin binding in serum of 80 normal children (C) and of 30 type I diabetic (IDDM) patients before therapy onset. ●, insulin autoantibody–negative (IAA^-) patients to be treated with cyclosporin A (CsA); ○, untreated IAA^- patients; ▲, IAA^+ patients to be treated with CsA; △, untreated IAA^+ patients.

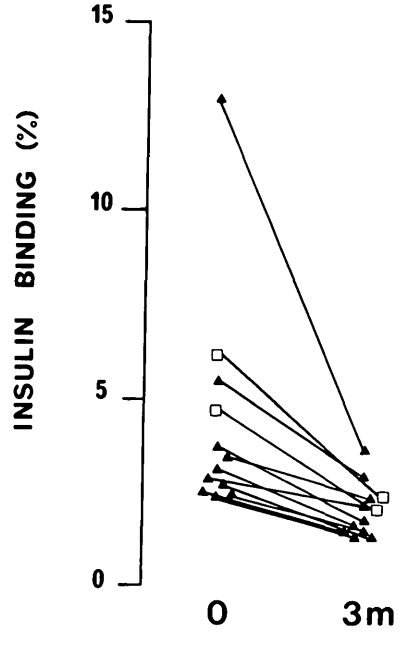

FIG. 2. Suppression of serum insulin binding in patients positive for insulin autoantibodies treated with cyclosporin A plus insulin (▲) or with cyclosporin A only (□).

TABLE 4
Islet cell antibodies and complement-fixing islet cell antibodies in cyclosporin A–treated patients and control group

	With cyclosporin A (n = 30)		Insulin alone (n = 25)	
	0 mo	12 mo	0 mo	12 mo
ICAs				
Number positive	22 (73%)	19 (63%)	18 (72%)	17 (68%)
Titer (log_2)	4.4 ± 0.4	3.6 ± 0.4*	4.2 ± 0.4	3.5 ± 0.5*
Decreasing titer (n)	19 of 22 (86%)		9 of 18 (50%)	
CF-ICAs				
Number positive	13 (43%)	11 (36%)	13 (52%)	10 (40%)
Titer (log_2)	3.85 ± 0.5	2.7 ± 0.5*	2.85 ± 0.3	2.1 ± 0.3*
Decreasing titer (n)	13 of 13 (100%)		10 of 13 (77%)	

ICAs, islet cell antibodies; CF-ICAs, complement-fixing islet cell antibodies.
*$P < .05$.

insulin injections, as in most of our patients, or after 3–5 wk of insulin therapy, as observed in two of the patients and as we reported recently in a large series of diabetic adults (12). In fact, the blockade of HIA was not entirely unexpected, because it is known from several animal models that CsA affects antibody formation if given before or at the time of the antigenic challenge (13–15). HIAs have no spontaneous tendency to disappear in diabetic subjects, even when insulin injections were discontinued, as observed in the five children with a spontaneous honeymoon period. As reported in adults, the effect of CsA on HIA is completely reversible when immunosuppression is interrupted (12).

The effect of CsA on the IAAs present in the sera of ~30% of the diabetic children before insulin therapy was started is more original and interesting. We know of no other demonstration of CsA effects on autoantibodies associated with other human autoimmune diseases. IAAs were first recognized in the sera of untreated newly diagnosed type I diabetic patients in 1983 (1), and their production was later found to be more abundant in children (3–5). About 75% of our patients were also ICA positive. Although both ICAs and IAAs are known to be associated with type I diabetes, whether they are pathophysiologically involved in the β-cell destructive process remains unknown. It is therefore interesting that only IAAs were affected by CsA in association with the short-term favorable effect of the drug on the course of the disease. As observed herein, CsA has only minor effects on the production of ICA and CF-ICA (12,16,17; Table 4).

If IAAs are only markers of the β-cell destructive process, the blockade of their production might be a secondary result of the CsA-induced diminution of the β-cell lysis. If IAAs are directly involved in the autoimmune β-cell destruction of type I diabetes, our observations support a search for a relation between the favorable effect of CsA in type I diabetes and its ability to specifically alter the production of IAAs.

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