Double-Blind Controlled Trial of Azathioprine in Children With Newly Diagnosed Type I Diabetes

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A double-blind controlled trial of azathioprine (2 mg · kg⁻¹ · day⁻¹) was conducted with 49 patients aged 2–20 yr (mean 10.8 yr) who had newly diagnosed type I (insulin-dependent) diabetes. Patients were randomly assigned to receive either azathioprine (n = 24) or placebo (n = 25) for 12 mo, beginning within the 20-day period after diagnosis. Baseline clinical and metabolic characteristics did not differ between the two groups. No patient experienced complete remission, defined as restoration of normal carbohydrate tolerance without other treatment. Partial remission, defined as good metabolic control (hemoglobin A₁c <7.9%, preprandial blood glucose <8 mM with an insulin dose of <0.5 U · kg⁻¹ · day⁻¹), occurred in 10 placebo (40%) and 7 azathioprine (29%) patients at 6 mo and in 4 placebo (16%) and 4 azathioprine (17%) patients at 12 mo (differences not significant). Fasting plasma C-peptide was significantly greater in the azathioprine-treated group at 3 and 6 mo, but this difference was not sustained. C-peptide responses to a standard meal and the frequency of islet cell and insulin antibodies did not differ between the two groups over the 12-mo period. Azathioprine caused no significant side effects. We conclude that in the dosage used, and despite early effects on endogenous insulin secretion, azathioprine alone does not influence the remission phase in children with newly diagnosed type I diabetes. Diabetes 38:779–83, 1989
use it in a larger double-blind controlled trial in newly diagnosed diabetic children.

MATERIALS AND METHODS
Selection of patients. Newly diagnosed patients with type 1 diabetes admitted to the Royal Children's Hospital were selected if they fulfilled the following criteria: 1) satisfied World Health Organization criteria for the diagnosis of diabetes in childhood, 2) had no medical contraindications (including likely pregnancy) or other major chronic system disease, 3) had either a history of measles or had had measles immunization, and 4) had parents who spoke and understood English, were willing and able to participate in regular follow-up, and gave written informed consent. Patients >12 yr old gave separate consent as well. The study was approved by the Royal Children's Hospital Ethics Committee.

Treatment and investigations. All patients were started on a 15-g carbohydrate-exchange diabetic diet and treated with purified porcine insulin, short and intermediate acting (CSL/Novo, Copenhagen), once or twice daily. Self-monitoring of blood glucose (SMBG) was performed before meals, 2–4 times daily, and insulin dosage was adjusted to obtain near-normal glucose levels.

Patients were examined monthly by the same doctor. Hemoglobin A1c (HbA1c; normal <7.9%) was measured monthly by column assay (Bio-Rad, Richmond, CA). ICAs, insulin antibodies, and C-peptide levels (before and 1 h after a standard breakfast, preceded by the usual dosage of insulin) were measured 7–20 days after diagnosis, after diabetes had been stabilized and before azathioprine was begun, and at 3-mo intervals for 1 yr thereafter. ICAs were measured by indirect immunofluorescence on frozen sections of human pancreas obtained from blood group type O organ donors. Insulin antibodies were assayed as previously described (25). C-peptide was measured by radioimmunoassay with a Daiichi kit (Tokyo). The normal fasting range established in 100 healthy subjects aged 18–60 yr is 0.08–0.43 nM, with intra- and intercoefficients of variation of 10 and 15%, respectively. HLA-DR typing was performed according to Terasaki and McClellan (26).

Patients were randomly assigned to receive either azathioprine or a placebo tablet. The participating doctors were not made aware of the code, and the code was not broken for any patient during the 12 mo of treatment. Azathioprine was begun at a conventional dose of 2 mg • kg\(^{-1}\) • day\(^{-1}\) (actual range 1.9–2.1 mg • kg\(^{-1}\) • day\(^{-1}\)), 7–21 days after diagnosis. It was taken once daily, and the dosage was adjusted according to increases in body weight. Fingerstick capillary blood counts were checked before therapy was begun, then at monthly intervals and during any intermittent febrile illness. If the neutrophil count fell below 1500 cells/mm\(^3\), azathioprine was discontinued for 7–10 days until the count returned to normal. Azathioprine was given again at the original dose unless the neutrophil count had fallen below 1000 cells/mm\(^3\), when the dose was reduced to 1.5–1.75 mg • kg\(^{-1}\) • day\(^{-1}\). Liver function tests were performed at 3 and 12 mo. Serum immunoglobulin levels and lymphocyte responses to mitogens (e.g., phytohemagglutinin, concanavalin A, pokeweed mitogen, and mixed lymphocyte culture) were measured, together with neutrophil function (opsonin response, phagocytic index, and chemotaxis) at 3 and 12 mo in 43 patients.

Complete remission was defined as the restoration of normal carbohydrate tolerance without other treatments (either dietary or insulin). Partial remission was defined as good metabolic control (HbA1c ≤7.9%, preprandial fingerstick SMBGs ≤8 mM) with an insulin dose of <0.5 U • kg\(^{-1}\) • day\(^{-1}\).

Statistical analysis. Statistical analyses were performed with a Vax computer with SPSS-X software, BMDP, and various in-house analytical methods. Analyses were performed on absolute and log-transformed data with t tests, Kriskall-Wallis test, and logit tests of proportions. Correlations are indicated by r values and significance by P values.

RESULTS
Fifty hospitalized patients entered the study between December 1984 and March 1986. One patient did not return for follow-up and was withdrawn, leaving 49 patients (26 males, 23 females) aged 2–20 yr (mean age 10.8 ± 0.7 yr) who completed the study. There were no differences between the placebo- (n = 25) and azathioprine-treated groups (n = 24) in terms of baseline clinical characteristics and metabolic control (Table 1). No patient had any other autoimmune disease. One patient in the placebo group had a history of congenital rubella.

No patient had a complete remission. One patient in the placebo group had good metabolic control without insulin between 2 and 4 mo but still required a diabetic diet. Seventeen patients (10 placebo, 7 azathioprine) were in partial remission as defined at 6 mo and 8 patients (4 placebo, 4 azathioprine) at 12 mo, but there was no statistical difference between the two groups. There was no difference between placebo- and azathioprine-treated subjects for mean HbA1c levels and insulin dosage at the 6- and 12-mo follow-up (Table 2). Assessment of SMBGs with a simple coding system was performed blind by one observer and revealed no differences in control between the two groups over 12 mo.

Fasting, unstimulated C-peptide values are shown in Fig. 1. C-peptide did not change over time in the placebo group, whereas there was a significant rise between baseline and

### TABLE 1
Baseline characteristics

<table>
<thead>
<tr>
<th>Patients</th>
<th>n (M/F)</th>
<th>Age (yr)</th>
<th>Duration of symptoms* (days)</th>
<th>Ketoacidosis (n)</th>
<th>Blood glucose* (mM)</th>
<th>Insulin dose† (U • kg⁻¹ • day⁻¹)</th>
<th>HbA₁c (%)</th>
<th>ICA (%)</th>
<th>HLA-DR3/4 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>14/11</td>
<td>9.9</td>
<td>21</td>
<td>6</td>
<td>22.0</td>
<td>0.8 ± 0.07</td>
<td>9.2 ± 0.4</td>
<td>79.2</td>
<td>88.8</td>
</tr>
<tr>
<td>Azathioprine</td>
<td>12/12</td>
<td>11.7</td>
<td>14</td>
<td>3</td>
<td>24.5</td>
<td>0.8 ± 0.06</td>
<td>9.5 ± 0.3</td>
<td>77.3</td>
<td>85.7</td>
</tr>
</tbody>
</table>

HbA₁c, glycosylated hemoglobin A₁c; ICA, islet cell antibodies.

*Median; †mean ± SE.
TABLE 2
Metabolic control

<table>
<thead>
<tr>
<th></th>
<th>6 mo</th>
<th>12 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin A1C (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>6.6 ± 0.2</td>
<td>7.1 ± 0.3</td>
</tr>
<tr>
<td>Azathioprine</td>
<td>7.2 ± 0.4</td>
<td>7.7 ± 0.3</td>
</tr>
<tr>
<td>Insulin dose (U kg⁻¹ day⁻¹)</td>
<td>0.5 ± 0.05</td>
<td>0.7 ± 0.05</td>
</tr>
<tr>
<td>Placebo</td>
<td>0.5 ± 0.05</td>
<td>0.6 ± 0.04</td>
</tr>
<tr>
<td>Azathioprine</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE.

3 mo in the azathioprine group (P < .04). This difference between the two groups was still present at 6 mo, but by 9 and 12 mo, basal C-peptide in the azathioprine group had decreased and was not different from that in the placebo group. When all basal C-peptide values were correlated with fasting blood glucose levels over 12 mo, there was an inverse correlation (r = .324, P < .001) only in the azathioprine group, although mean fasting blood glucose levels did not differ between the azathioprine and placebo groups throughout the period.

A significant C-peptide response to a standard meal was defined as a stimulated value of ≥0.15 nM or a twofold increase over basal. C-peptide responses at baseline were significant in 42 of 47 patients (22 placebo, 20 azathioprine) and at 12 mo in 27 of 49 patients (13 placebo, 14 azathioprine). Of the latter 27 patients, 26 had had a significant response at baseline. Stimulated C-peptide values were correlated at baseline and 12 mo in the azathioprine-treated group (r = .71, P < .001) but not in the placebo group (r = .38, P > .05). Stimulated C-peptide values did not differ between the azathioprine and placebo groups at 3 mo intervals over the 12-mo treatment (Fig. 2). Patients >12 yr old had significantly higher (P < .001) stimulated C-peptide levels at 12 mo, but there was no difference between the placebo and azathioprine groups.

ICAs were detected in 37 of 47 patients (79%) at baseline and in 43 of 49 patients (88%) at 12 mo with no difference between the two groups. Of the 6 patients with undetectable ICAs at 12 mo, 3 had detectable ICAs initially. Azathioprine had no effect on the presence of ICAs over 12 mo. Insulin antibodies were detected in 18 of 48 patients (33%) at baseline and in 42 of 49 patients (87%) at 12 mo, again with no difference between the two groups.

Side effects of azathioprine. There was no statistical difference in the number of infections between the two groups. All infections were mild. However, skin lesions, which included two cases of acne, two of multiple warts, and one of herpes simplex, all occurred in azathioprine-treated patients. Eleven patients (6 placebo, 5 azathioprine) had 1–3 transient episodes of neutropenia (<1500/mm³) associated with intercurrent infections. Four of these 11 patients (3 azathioprine, 1 placebo) each had one episode of neutropenia <1000/mm³ that reversed 7–10 days after withdrawal of the tablets and a dose change to 1.5–1.75 mg · kg⁻¹ · day⁻¹. Only the 3 azathioprine subjects in this group required a reduction in dosage during 12 mo. Hemoglobin levels and platelet counts did not change.

Serum immunoglobulin levels, lymphocyte responses to mitogens, and neutrophil function tests were not different between the two groups at 3 and 12 mo or within the azathioprine group between 3 and 12 mo.

One patient had a minor transient rise in serum aspartate transaminase levels over 2 wk after taking azathioprine for 3 mo, which appeared to be unrelated to therapy. No other side effects related to azathioprine were noted.

DISCUSSION

This trial, which was double-blind for treatment and assessment of the patients, revealed that azathioprine (2 mg · kg⁻¹ · day⁻¹) had no significant effect on the natural history of newly diagnosed diabetes in children. Neither the frequency nor the duration of remissions was influenced by azathioprine therapy: placebo- and azathioprine-treated
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subjects did not differ in insulin dosage required or metabolic control over the 12-mo study. However, over the first 6 mo of the study, azathioprine-treated subjects showed improvement in basal C-peptide secretion. Higher basal C-peptide values were associated with lower fasting blood glucose levels in the azathioprine-treated group. In contrast, there was no difference between groups in meal-stimulated C-peptide secretion throughout the study.

The lack of any clinically significant effect of azathioprine was unexpected in light of results from uncontrolled trials of CsA in young and middle-aged adults (5,6) and our randomized trial of azathioprine in young adults (7). However, a major difference in this trial was the pediatric-age group studied, which had a mean age of 10 yr. Furthermore, reanalysis of the Canadian CsA trial has revealed that children are less likely than adults to have significant clinical remission (27). Conventionally treated younger patients are less likely to retain C-peptide secretion (28), and this was confirmed in our study. Therefore, it is possible that an accelerated process of β-cell destruction in children is less responsive to immunosuppression or, alternatively, that children may require higher dosages of azathioprine for effective therapy.

The early but transient improvement in basal C-peptide secretion in the azathioprine group suggests that immunosuppressive treatment retards but does not prevent ongoing β-cell destruction. The enhanced basal C-peptide secretion in the azathioprine group was probably physiologically important in that there was an inverse correlation with fasting blood glucose levels throughout the study, indicating a contribution from endogenous insulin to overnight glucose control. The lack of apparent effect of azathioprine on stimulated C-peptide values may be related to the design of our experimental protocol, in which the standard breakfast was preceded by the morning insulin injection. Thus, exogenous insulin may have blunted the C-peptide response to the meal, blurring a distinction between the azathioprine and placebo groups.

We chose azathioprine as an immunosuppressive agent because considerable experience with its use in children and kidney-transplantation patients has shown a low incidence of side effects (23). CsA, on the other hand, is associated with risk of kidney damage and minor but undesirable side effects, e.g., hirsutism and changes in facial appearance (29,30), and blood levels must be monitored frequently, making it less acceptable for treatment of children.

The dosage and plasma level of CsA appear to be critical for it to have an effect on newly diagnosed diabetic patients (5,8). We gave the standard maintenance dosage of azathioprine that is prescribed for children with kidney transplantation (31) and that was given to young adults in a previous trial (7). This dosage was adjusted according to increases in body weight over the 12-mo treatment. Although azathioprine compliance is not easily measured because there are no reliable blood or urine assays (22,23), compliance by parental checks and residual tablet counts was further supported by the difference in basal C-peptide values between the two groups. There was no indication from these observations that compliance diminished over time, which might account for the subsequent falloff in basal C-peptide in the azathioprine group. All 49 patients who completed the study appeared to comply with treatment, and all were present for follow-up appointments and investigations.

Our findings of normal immunoglobulin levels and normal lymphocyte responses in vitro in both groups does not necessarily question compliance or the effectiveness of azathioprine because previous studies with patients receiving similar dosages of azathioprine have shown normal immunoglobulin levels (32,33) and normal lymphocyte responses to phytohemagglutinin, mixed lymphocyte culture (32,34), concanavalin A (35), and pokeweed mitogen (35) in the face of suppressed delayed hypersensitivity reactions in vivo (31,34,35). Note also that only patients who were receiving azathioprine developed viral skin lesions or acne, as previously reported (23). Immune function tests were normal in these patients, consistent with the reported disparity between the in vitro and in vivo effect of azathioprine (32–35).

Our patients, both in the placebo and azathioprine groups, had features similar to those noted previously in populations of children with newly diagnosed diabetes. The timing, rate, and duration of remissions were consistent with other reports (28,36). ICAs and insulin antibodies were detected at diagnosis in a similar percentage of patients to that found previously (12–14,37). We also found a direct relationship between age and residual C-peptide at 12 mo, which was consistent with other studies (28,38–40), but we did not find any difference between the azathioprine- and placebo-treated groups.

In the Canadian CsA trial, it appeared that a remission was more likely the earlier CsA was begun, but initial C-peptide levels were not discriminatory (5); in the initial French trial (6), although the length of time before CsA was begun was not analyzed, initial C-peptide levels were higher in those who later had a remission. Despite azathioprine treatment being started relatively early, within 7–20 days of diagnosis, it had no clinically significant effect on the remission phase. Initial C-peptide levels correlated with those at 12 mo, but there was no difference between the azathioprine- and placebo-treated groups.

Thus, in the dosage given, azathioprine did not influence the remission phase in children with newly diagnosed type I diabetes. Whether a higher dosage of azathioprine, other agents, or combinations thereof would be effective remains to be determined. In view of the likelihood that conventional immunosuppression, even if effective, would need to be continued indefinitely (7,9,41) with a risk of long-term side effects (23,24,42), such treatment cannot be considered ideal or routine. There is therefore a need to develop more specific and effective therapies based on knowledge of the mechanisms of β-cell destruction.

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