Polyclonal Anti-T-Cell Therapy for Type 1 Diabetes Mellitus of Recent Onset

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Abstract

The destruction of pancreatic β-cells in type 1 diabetes mellitus is mediated by autoreactive T-lymphocyte clones. We initiated a prospective randomized controlled trial of polyclonal rabbit anti-T-cell globulin (ATG) in patients with type 1 diabetes within 4 weeks of diagnosis and with residual post-glucagon C-peptide levels still over 0.3 nmol/l. ATG was administered as an initial bolus of 9 mg/kg followed by 3 consecutive doses of 3 mg/kg. An interim analysis was performed to establish whether any significant changes in C-peptide production and insulin requirement had occurred that would justify the continuation of this pilot study. By May 2004, 11 subjects were assigned to treatment with ATG along with intensified insulin therapy and 6 to intensified insulin therapy with placebo, and were followed for a period of at least 6 months. During the first 12 months a significant difference in the insulin dose trends was found between the groups (p = 0.010) with a lower insulin dosage in the ATG group. There was also a difference in the glucagon stimulated C-peptide level trends of marginal significance (p = 0.068). Compared to values at screening, stimulated C-peptide levels significantly improved in the ATG group (p = 0.012) but not in the placebo group. Complete diabetes remission occurred in 2 patients in the ATG and in none of the placebo group. Glycosylated hemoglobin at 12 months tended to be lower in the ATG group (p = 0.088). Significant adverse effects of ATG treatment, mainly transient fever and moderate symptoms of serum sickness (7 and 6 subjects, respectively) were observed during the first month only. The interim analysis of this ongoing study suggests that short-term ATG therapy in type 1 diabetes of recent onset contributes to the preservation of residual C-peptide production and to lower insulin requirements in the first year following diagnosis.

Keywords: Type 1 diabetes · immune intervention · antithymocyte globulin · ATG · insulin · C-peptide

Introduction

Type 1 diabetes mellitus (T1DM) is primarily an autoimmune disease characterized by selective destruction of pancreatic β-cells. Interaction of genetic and hitherto unknown environmental triggers results in a loss of β-cell tolerance and the development of an immune cascade, which involves a series of inflammatory cellular and humoral factors leading finally to β-cell death [1, 2]. Although the exact pathogenesis of this process has not been fully elucidated, extensive evidence supports the hypothesis that a central role is played by autoreactive T cells which are not deleted, become activated and clonally expand [3].

Since the immunological nature of T1DM was recognized, various immunotherapeutic approaches have been tried with limited success [4]. In already established clinical disease, the goal of immune intervention is restricted to the preservation of surviving β-cell mass. This could be potentially achieved through a selective deletion of the β-cell antigen-specific T cell clone(s) and restoration of immune tolerance to β-cells using a safe short-term procedure before irreversible β-cell destruction occurs [5, 6].

Based on promising results in animal models of diabetes, several attempts have been performed in humans. Anti-CD5 immunoconjugate coupled to the A-chain of ricin toxin was given as a short course to 15
subjects with recent diabetes with promising results [7]. A combination of cyclosporine A with the initial therapy of an anti-interleukine-2 receptor antibody showed better results in remission induction than therapy with cyclosporine alone [8, 9]. Recently, humanized non-mitogenic anti-CD3 antibodies were reported to mitigate the deterioration of insulin production T1DM of recent onset [10].

The identification and specific targeting of the autoreactive T cell clone(s) in diabetes is not yet possible [3]. Moreover, the deletion of only one T cell subtype involved in the autoimmune process may not be sufficient to restore self-tolerance in humans. From this point of view, therapy with polyclonal anti-T-cell antibodies, which display multiple anti-T-cell specificities [11, 12], seems to be worth testing. Surprisingly, experience in only 10 subjects receiving anti-thymocyte globulin (ATG) to slow down the progression of β-cell loss has been reported [13, 14]. Despite some encouraging results, these studies were not continued, probably due to side-effects of animal immunoglobulin.

In this report we describe the first results of a randomized controlled trial of a polyclonal rabbit anti-thymocyte globulin initiated in patients with new-onset T1DM. The drug has previously proved to be highly efficient in the prevention and treatment of allograft (including pancreas) rejection [15, 16] and in inducing the remission of autoimmune diseases such as aplastic anemia, systemic sclerosis [17] and inclusion body myositis [18].

The primary objective of the study is to compare the effect of ATG treatment, together with intensified insulin therapy (ATG group), on the rate of complete diabetes remission with that of intensified insulin therapy only (placebo group) in T1DM of recent onset. Additional objectives are to compare the insulin doses between the two groups, to compare the course of the specific humoral markers of autoimmunity between the groups and to assess the safety of ATG treatment in T1DM.

The aim of this interim analysis was to evaluate if any significant effects could be demonstrated after inclusion of the first 17 subjects and whether concomitant adverse effects would not preclude further continuation of the study.

Methods

Study design

This is a prospective randomized single-blind controlled pilot study that comprises a total of 30 subjects with T1DM of recent onset. Subjects with a typical clinical diagnosis of T1DM of not more than 1 month duration, aged 18-35 yr, treated with up to 40 units of insulin per day for 1 month, were evaluated as potential study participants. Additional inclusion criteria were: body mass index up to 32 kg/m², exclusion of pregnancy in women, C-peptide level ≥ 0.3 pmol/ml following iv. administration of 1 ml glucagon (mean of the 4 and 6 min. post injection values) and positivity of at least one of the immunological markers of autoimmune diabetes. The immunological evaluation included anti-GAD autoantibodies, anti-insulin antibodies (AIA; in subjects treated with insulin for up to two weeks), anti-tyrosine phosphatase (IA2) antibodies and islet cell antibodies (ICA). Subjects with previous immunosuppressive therapy, concurrent severe infection, granulocyte count ≤ 2 x 10⁹/l and platelet count ≤ 120 x 10⁹/l were excluded from study enrollment.

The study was approved by the local ethics committee and by the State Institute for Drug Control of the Czech Republic and was undertaken in accordance with the Declaration of Helsinki principles. Written informed consent was obtained from all participants.

After inclusion, the subjects were randomly assigned either to ATG or placebo treatment. Randomization envelopes were prepared for 30 subjects at an ATG/placebo ratio of 1:1. A first ATG dose (ATG-Fresenius (S), Germany) of 9 mg/kg in 1500 ml saline was administered over a period of approximately 6 h into the antecubital vein through an indwelling teflon catheter. The treatment was preceded by an intradermal test with 0.05 ml of this solution, which was performed 60 min. before intravenous ATG administration. On the following 3 days, additional doses of 3 mg/kg of ATG were infused in 1000 ml saline. Subjects assigned to placebo received saline only. In all subjects, diabetes was treated with an intensified insulin regimen (4 daily doses of human insulin, frequent blood glucose self-monitoring, comprehensive diabetes education) with the aim of achieving near-normal blood glucose levels. All subjects stayed in hospital for 4-10 days depending on their metabolic control and clinical condition.

Standard biochemistry, blood count, lymphocyte populations (using flow cytometry) were measured at screening, before treatment start, on days 1, 4, 7 and 10 and thereafter at 1, 3, 6, 12, 18 and 24 months. Fasting and post-glucagon C-peptide levels were tested at screening, 1, 6, 12 and 24 months. HbA1c and immunological markers were assessed at screening, at day 10 and at 1, 3, 6, 9, 12, 18 and 24 months.
Clinical Chemistry with an upper limit of normal analyzer, Bio-Rad, USA) using the standard calibration by high performance liquid chromatography (Variant 2, Norway) [20].

Norway) [20]. C-peptide was detected using an immuno-cytocchemical method (CMV-vue Kit, DiaSorin, USA).

Early cytomegalovirus antigen (pp65) in peripheral leukocytes was detected using an immuno-cytocchemical method (CMV-vue Kit, DiaSorin, USA).

Statistical analysis

For the comparison of single values between the ATG and placebo groups, the Mann-Whitney 2 sample test and Fisher’s exact test were applied. For the comparison of trends in insulin requirements and C-peptide levels, the model of analysis of variance (ANOVA) with repeated measures and a grouping factor was used. Wilcoxon’s paired test was used to compare changes within individual groups. P values <0.05 were considered statistically significant. With regard to the low number of subjects included in the study, p values >0.05 and <0.1 were considered as marginally significant.

Results

Study subjects

Between November 2000 and January 2004, 36 subjects with T1DM of recent onset, fulfilling the age and duration of diabetes criteria, were screened and 17 of them complied with the C-peptide and autoantibody criteria, and agreed to participate in the study. Eleven subjects were randomized to treatment with ATG and 6 to placebo. By June 2004, all were followed for a minimum of 6 months and 11 of them for 1 year (7 in the ATG group and 4 in the placebo group). In one patient, only the first dose of ATG was administered, because she refused additional treatment.

Basic characteristics of both groups are shown in Table 1. At least 1 autoantibody assay was positive in all subjects. Two or more tests were positive in 5 patients in the ATG group and in 3 in the placebo group.

Table 1. Basic characteristics at study entry

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ATG group (n = 11)</th>
<th>Placebo group (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female (n)</td>
<td>9 / 2</td>
<td>4 / 2</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>28.80 ± 6.20</td>
<td>26</td>
</tr>
<tr>
<td>Median</td>
<td>21 - 35</td>
<td>21 - 34</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.40 ± 4.50</td>
<td>21.90 ± 2.90</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>9.22 ± 2.20</td>
<td>8.64 ± 1.80</td>
</tr>
<tr>
<td>C-peptide (nmol/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>0.37 ± 0.17</td>
<td>0.30 ± 0.06</td>
</tr>
<tr>
<td>Post glucagon</td>
<td>0.55 ± 0.20</td>
<td>0.46 ± 0.14</td>
</tr>
<tr>
<td>Diabetes diag. (days)¹</td>
<td>10.90 ± 9.46</td>
<td>8.40 ± 9.50</td>
</tr>
<tr>
<td>Insulin dose (units/kg/day)</td>
<td>0.48 ± 0.27</td>
<td>0.50 ± 0.19</td>
</tr>
<tr>
<td>Anti-GAD65 positive (n)</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Anti-insulin positive (n)</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>IA2 positive (n)</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>ICA positive (n)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>At least 1 HLA-DQ susceptible haplotype (n)²</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>At least 1 HLA-DQ resistant haplotype (n)³</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

Legend: Data are mean ± SD. For all parameters p > 0.1 was found. n: number of subjects. ¹ Time since diabetes diagnosis in days (mean ± SD). ² Diabetes-susceptible haplotypes included DQB1*0201 - DRB1*03 and DQB1*0302 - DRD1*04. ³ Diabetes-resistant haplotype was DQB1*0602 - DRB1*15.
Metabolic control

There was no difference between the 2 groups in the insulin dose, C-peptide levels and glycosylated hemoglobin at study entry (Table 1). All participants were treated with 4 daily doses of human insulin. Mean (± SD) glycosylated hemoglobin values in the ATG and placebo groups were 4.00 (± 1.53) and 4.72 ± 1.36 % at 6 months, respectively (p > 0.1) and 3.86 ± 0.63 and 4.54 ± 0.72 at 12 months, respectively (p = 0.088, Mann-Whitney test). In 2 subjects from the ATG group and in none in the placebo group, complete remission of clinical diabetes was achieved (from 3 to 7 months after study entry in one and from 11 months for more than 24 months in the other). Three months after remission start, glycosylated hemoglobin in both subjects was within the normal range (up to 4%).

Mean insulin doses in the course of 12 months are shown in Figure 1. The curves are statistically different (p = 0.010, ANOVA with repeated measures). While in the ATG group the change in insulin dose between study entry and 6 months was marginally significant (p = 0.058), it did not change significantly in the placebo group (p > 0.1; Wilcoxon’s paired test; Figure 2).

Although the trends of the mean fasting (Figure 3) and the post glucagon (Figure 4) C-peptide levels during the 12-month period suggest an increase in the ATG group, they are not statistically significant for the fasting levels (p > 0.1) and only marginally significant for the post glucagon C-peptide levels (p = 0.068, ANOVA with repeated measures). However, the Wilcoxon’s paired test comparing the entry and 6-month data proved a significant increase of the post glucagon levels in the ATG group (p = 0.012), while no change was observed in the placebo group (p > 0.1; Figure 5).
Figure 3. Mean ± SD of C-peptide levels during 12 months post study start. SDs are shown as vertical lines in one direction only. The trends of the curves are not significantly different (p > 0.1; ANOVA with repeated measures).

Figure 4. Mean ± SD of C-peptide levels following glucagon administration during 12 months post study start. SDs are shown as vertical lines in one direction only. The trends of the curves are marginally statistically different (p = 0.068; ANOVA with repeated measures).
Lymphocyte populations

As expected in the ATG group, the total lymphocyte count decreased rapidly following the first ATG dose to a mean value of 0.280 ± 0.140 x 10^9/l, then started to increase at day 4 and returned to pre-study values at 2 months. The CD19+ cell count did not change significantly. Figure 6 shows the CD4+/CD8+ cell ratio, which was comparable at study entry in both groups but became and remained inverted in the ATG group throughout the study period. ANOVA with repeated measures demonstrated a significant difference in the trend of the CD4+/CD8+ ratio curves during the 12-month period (p = 0.028). In comparison with the values at screening, the ratio significantly decreased in the ATG group at 1, 3 and 12 months (p = 0.020, 0.001 and 0.002, respectively; Wilcoxon’s pair test). The mean platelet count fell in the ATG group to a nadir of 135 ± 32 x 10^9/l with the lowest individual value of 95 x 10^9/l and returned to normal in all subjects from day 7.

Figure 5. Individual changes in C-peptide levels following glucagon administration between the study start and 6 months of treatment (p = 0.012 for the ATG group and p > 0.1 for the placebo group; Wilcoxon’s paired test). Mean ± SD C-peptide levels at study start and after 6 months in the ATG group were 0.59 ± 0.16 and 1.02 ± 0.31 nmol/l, respectively and in the placebo group 0.49 ± 0.06 and 0.51 ± 0.24 nmol/l, respectively.

Adverse effects

ATG administration caused a rise in body temperature in all treated subjects and exceeded 38°C in 7 of them. Chills were present following the first dose in 7 subjects and disappeared in all of them by day 3. Phlebitis of the antecubital vein developed in 2 subjects and resolved in 5 days. Six subjects in the ATG group reported elevated body temperature and arthralgia attributable to serum sickness 9-11 days after the first ATG dose. The symptoms resolved in all of them within 4 days. Transient lymphadenopathy was observed in 1 patient in the ATG group and 1 in the placebo group 2 weeks after study entry. Low-grade fever occurred in 1 subject from the placebo group.

No adverse event attributable to the study treatment occurred in any patient later than one month after study entry. The CMV early antigen status did not change to positive in any patient following study entry.

Discussion

Though the optimal time for immune intervention to prevent T1DM should ideally precede the beginning of immune mediated β-cell destruction, most clinical studies using protocols with a potential risk of adverse effects have been performed in patients with already failing insulin production. Thus the aims have been restricted to the prevention or at least delay of further β-cell loss. Therapy should be only temporary and with minimal risks of long-term complications.

The first results of our prospective randomized intervention study in T1DM of recent onset suggest that 4 doses of polyclonal anti-thymocyte globulin may preserve residual C-peptide production and contribute to excellent metabolic control with the use of lower doses of exogenous insulin during the first year of clinical disease when compared to non-treated sub-
bjects. In particular, glucagon-stimulated C-peptide levels after 6 months improved significantly in the ATG group and, in comparison to the placebo group, their trend was more favorable throughout the 12-month period. The trend toward a lower need of insulin was apparent in the ATG group from 3 months after study start and lasted for at least 12 months. Moreover, in 2 subjects from the ATG group, clinical remission of diabetes was achieved.

Significant adverse events occurred in the ATG treated group and included fever with chills in 7 of 11 subjects and symptoms of serum sickness in 6. Preventive measures, such as antihistamine and glucocorticoid administration, which are commonly used in transplant patients before ATG administration, were not undertaken, as they were not a part of the study protocol. Nevertheless, the incidence of serum sickness symptoms was surprisingly high. In our previous study, no single case of serum sickness following ATG treatment was observed in 24 patients undergoing simultaneous pancreas and kidney transplantation [16]. This difference may be explained by the absence of additional systemic immune suppression or by a special predisposition in patients with active autoimmune disease. In patients with recent-onset diffuse scleroderma, serum sickness was reported in 7 of 13 patients treated with ATG [17]. Elevated temperature represents a common side-effect of treatment with anti-lymphocyte preparations and has been attributed to cytokine release following stimulation of the Fc receptor on the monocytes and macrophages [21]. Elevated temperature, however, also developed in most T1DM patients treated with humanized monoclonal anti-CD3 antibodies with modified Fc region [22]. In our study, no important infectious complication occurred and no adverse events attributable to ATG treatment were observed after 4 weeks from the study start.

Experience with ATG therapy was previously described by Eisenbarth et al. [14], who compared the effects of short-term prednisone (9 subjects), prednisone plus equine ATG (5 subjects evaluated) or placebo (3 subjects) in patients with clinical T1DM of 5-133 days duration. The study entry criteria were not based on C-peptide levels and only 2 subjects treated with ATG plus prednisone entered the randomized part of the study. Despite a tendency to a lower insulin requirement in the ATG treated group, the study was stopped due to side-effects of the ATG, thrombocytopenia being the most important. In contrast to the report by Eisenbarth et al., the entry criteria are prospectively defined in our study, the study is random-ized and we use a different type of anti-thymocyte preparation without any additional immunologically active drug. Though some side-effects of the therapy observed up to this point have also been significant, in our opinion they do not outweigh the potential therapeutic benefit. In particular, clinically important thrombocytopenia did not occur in any subject.

Long-term enhanced suppressor T-lymphocyte activity in 4 newly diagnosed T1DM patients treated with rabbit ATG and prednisone were described by Schatz et al. [13]. However, a lack of beneficial clinical response and potentially serious side-effects in 3 of 4 subjects were also observed.

The active mechanisms of polyclonal anti-lymphocyte and anti-T-lymphocyte immune sera are thought to be cytolysis of activated lymphocytes mediated by the binding of the complement, blocking of signal transduction and opsonisation of activated cells with subsequent sequestration in the reticuloendothelial system [15, 23, 24]. Apart from antibodies to a wide range of lymphocyte surface antigens, presence of anti-LFA1, anti-ICAM-1 and antibodies to integrins and chemokines may be responsible for anti-adhesive properties [11]. Recently, antibodies against CTLA-4 and other co-stimulatory molecules were found as active components of ATG [12]. In contrast to other immunosuppressants whose activity depends on T cell activation, polyclonal antibodies can eliminate preactivated nonproliferating lymphocytes. This could be important for arresting the autoimmune process. However, a single dominant active mechanism has not been defined and considerable differences may exist between products from individual suppliers who use various tissues for immunization.

It has been speculated that interaction of anti-T-cell antibodies with the immune system may, apart from immune suppression, lead to the stimulation of regulatory cells that could inhibit the autoimmune process in diabetes [6, 22]. In clinical transplantation, the possible tolerogenic effect of anti-T-cell antibodies may be inhibited by concomitant long-term immunosuppression. Recently, Starzl et al. [25, 26] demonstrated an excellent short-term outcome with an extremely low need for systemic immunosuppressants in various organ recipients pre-treated with rabbit polyclonal T cell antibodies. In the intervention study with humanized non-Fc receptor binding anti-CD3 antibodies, Herold et al. observed an inverted CD4+/CD8+ cell ratio 3 months following drug administration [22] which may support the concept of a durable tolerogenic effect. This inversion was also clearly apparent in
our study and remained present for at least 12 months.

Based on our preliminary data, we speculate that ATG administration in T1DM (autoimmune type) of recent onset may slow down the autoimmune process by deletion of several different clones of activated T cells and impair signal transduction from the activated cells at different levels. While not affecting the activity of suppressor cells, the treatment may offer the immune system the chance to receive sufficient inhibitory signals aiding the destruction of the remaining autoreactive T cells.

Of course, this approach could be more effective in earlier stages of the disease. However, at present it would hardly be acceptable to investigate a new treatment method with a potent immunosuppressive drug in subjects who are merely at high risk of diabetes development but do not yet suffer from the disease. In addition, even a partial effect, leading to transitory remission of diabetes or higher residual insulin secretion, might be of clinical importance for the patient [27] and contribute to a better understanding of the autoimmune process. In our study, the entry criterion for C-peptide level (≥ 0.3 nmol/l) was set rather high and led to the exclusion of a high proportion of potential study participants. On the other hand, the preservation of such a C-peptide level would certainly represent a significant modification of the natural course of the disease. The recently published guidelines for intervention trials in subjects with newly diagnosed T1DM suggest a limit for C-peptide concentration of 0.2 nmol/l [28].

In our study, the most important side-effects of ATG treatment were the cytokine release syndrome and serum sickness, which were more frequent than in organ recipients on multiple immunosuppressive therapy. These side-effects could possibly be mitigated by preventive measures such as temporary corticosteroid and/or antihistamine administration or with the use of tumor necrosis factor alpha antagonists. In conclusion, short-term ATG therapy in type 1 diabetes mellitus of recent onset contributes to the preservation of residual C-peptide production and to a lower insulin requirement in the first year following diagnosis. In our opinion, the encouraging results of this pilot prospective randomized study justify its further continuation in a larger patient group. Should our approach prove to be successful, additional modifications mitigating the adverse events and perhaps preserving the immunological effect with an acceptable long-term pharmacological immune suppression could follow.

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References


