Effect of thalidomide on the incidence of iodine-induced and spontaneous lymphocytic thyroiditis and spontaneous diabetes mellitus in the BB/Wor rat

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Abstract. Thalidomide, a derivative of glutamic acid, has immunosuppressive effects and suppresses graft-vs-host disease in the rat and following bone marrow transplantation in man. It is effectively used in the treatment of erythema nodosum leprosum and has a potential therapeutic effect in a variety of autoimmune diseases. In view of these observations, we evaluated the effect of thalidomide on the incidence of spontaneous and iodine-induced lymphocytic thyroiditis and spontaneous insulin dependent diabetes mellitus in the BB/Wor rat. Thalidomide did not suppress the incidence of lymphocytic thyroiditis and serum anti-thyroglobulin antibodies or affect the serum concentrations of T₄, T₃ and TSH in this rat model. Thalidomide also did not affect the incidence of insulin dependent diabetes mellitus. In contrast to preliminary studies in man and rat demonstrating efficacy in the therapy of autoimmune diseases, thalidomide did not prevent or suppress autoimmune lymphocytic thyroiditis or insulin-dependent diabetes mellitus in the BB/Wor rat.

Thalidomide, a derivative of glutamic acid, was originally introduced as a sedative and hypnotic drug (1). It achieved widespread use because of its apparently low toxicity. Unfortunately, the drug was teratogenic and was withdrawn from general use (2). However, it was later found to have an immunosuppressive effect. Sheskin first noted that thalidomide was beneficial in the treatment of erythema nodosum leprosum (3). After this initial report, many case reports and two randomized studies demonstrated that thalidomide was efficacious in lepromatous leprosy and in a number of other inflammatory and autoimmune diseases (4). Recently, thalidomide was shown to be effective in treating and preventing graft-vs-host disease in a rat model (5) and after bone marrow transplantation in man (GW Santos: Johns Hopkins Medical School, Baltimore, MD. Personal communication). These data suggest that thalidomide is an immunosuppressive agent. There is no agreement on the mechanism of action of the drug, but it is believed that thalidomide influences T cell regulatory functions, leading to increased suppressor T cells (6) and decreased helper T cells (7). Furthermore, it may inhibit neutrophilic chemotaxis (8), phagocytosis, and nonspecific inflammatory chemical mediators (9).

Lymphocytic thyroiditis is an autoimmune thyroid disease which occurs with an incidence of approximately 50% in the insulin-dependent diabetes mellitus prone BB/Wor rat (10). Excess iodine ingestion markedly increases the incidence of lymphocytic thyroiditis (11). This animal model has been extensively used to study the immunopathogenesis of diabetes mellitus and lymphocytic thyroiditis. Therefore, it also serves as a model to study the immunosuppressive effects of various drugs and immunologic manoeuvres on the occurrence of diabetes mellitus and lymphocytic thyroiditis. For example, lymphocytic transfusion, macrophage depletion, and antibody to class II major histocompatibility complex (MHC) gene products have all been shown to decrease the incidence of
both diabetes mellitus and lymphocytic thyroiditis (12-15). Both cyclosporin-A and silica decrease the incidence of diabetes mellitus in this animal model (16,17), and methimazole decreases the incidence of lymphocytic thyroiditis (18).

The present studies were carried out to investigate the effect of thalidomide on the incidence of lymphocytic thyroiditis and diabetes mellitus in the BB/Wor rat.

Material and Methods

Male and female insulin-dependent diabetes mellitus prone BB/Wor rats, 25-30 days old, were obtained from the colony maintained at the University of Massachusetts Medical Center, Worcester, MA, in accordance with the NIH guidelines of 1985. The BB/Wor rats have a cumulative incidence of diabetes mellitus of approximately 60% by 120 days of age. More than 85% of diabetic rats develop diabetes mellitus between 60 and 120 days of age (10). The cumulative incidence of lymphocytic thyroiditis in these rats is approximately 40% by 90 days of age and doubles in the presence of excess iodine ingestion (11). Thus, in experiment 1, BB/Wor rats received iodine in their drinking water to enhance the incidence of lymphocytic thyroiditis. We have very recently observed that 2 sublines of BB/Wor rats, BA and NB lines, have a cumulative incidence of spontaneous lymphocytic thyroiditis of approximately 70% by 90 days of age (19). These 2 sublines were, therefore, used in experiment 2 and iodine was not given since they already have such a high incidence of spontaneous lymphocytic thyroiditis. There are no sex-related differences in the incidence of either diabetes mellitus or lymphocytic thyroiditis in the BB/Wor rats.

Experimental protocols

Experiment 1: Twenty-nine male and female BB/Wor rats were divided into 2 groups: group 1 received thalidomide, 50 mg/kg in vegetable oil by gavage daily from 42 to 102 days of age, and group 2 received 0.38 ml/100 g body weight of the vegetable oil diluent by gavage daily. All rats received 0.05% iodine in their drinking water from 30 days of age. Thalidomide was kindly supplied by Dr. Jao Romaldini, Sao Paulo, Brazil. It was homogenized in vegetable oil (20 kg/l) and freshly prepared each week.

Experiment 2. Forty-nine male and female BA and NB line BB/Wor rats were divided into 2 groups: group 1 received thalidomide, 75 mg/kg in vegetable oil by gavage daily from 30 to 100 days of age, and group 2 received 0.38 ml/100 g of the vegetable oil diluent by gavage daily. All rats in both experiments were weighed and tested for glycosuria three times weekly beginning at 60 days of age. Rats that developed diabetes mellitus were treated daily with sc protamine zinc insulin (PZI) for the remainder of the experiment. In addition, rats with ketonuria were treated with parenteral sodium bicarbonate and lactated Ringer’s solutions. Rats were killed at 102 days of age in experiment 1, and at 100 days of age in experiment 2. Blood was obtained at sacrifice and serum was assayed for T₄, T₃, TSH and antithyroglobulin (anti-Tg) antibody concentrations. Thyroids were removed from all animals and fixed in Bouin’s solution. Paraffin sections (4 μm) obtained from the equatorial portion of each thyroid were stained with hematoxylin and eosin. The thyroids were examined for the presence and severity of lymphocytic thyroiditis by a pathologist (MCA) who was unaware of the treatment status of the animals.

Assays

All sera were assayed by RIA in the same assay, in random order, and in duplicate for T₄, T₃ and TSH concentrations (11). Reagents for the TSH assay were kindly supplied by the National Pituitary Agency, NIH. Serum anti-Tg antibody titres were measured in triplicate in the same assay by a modification of an enzyme-linked immunosorbent assay (11). Briefly, rat Tg at a concentration of 4.8 nmol/l in 50 mmol/l carbonate buffer, pH 9.6, was coated on 96 well microplates (Costar Cambridge, MA). After a 1-h incubation at 36°C, the microplates were incubated at 4°C for 18 h. The microplates were then washed twice with 200 μl 10 mmol/l PBS in 0.05% Tween-20 buffer (PBS-Tween). Aliquots (200 μl) of the diluted sera (1:160 in PBS-Tween buffer) were added to the Tg-coated wells. After incubation for 3 h at room temperature, the wells were washed three times with 200 μl PBS-Tween buffer; 200 μl of a 1:1000 dilution of goat anti-rat immunoglobulin alkaline phosphatase conjugate (Sigma, St. Louis, MO) in PBS buffer was added to each well and allowed to incubate for 18 h at 4°C. The wells were washed three times with 200 μl PBS-Tween buffer. After this final wash, 200 μl of a 2.7 mmol/l solution of p-nitrophenylphosphate-alkaline phosphatase substrate in 0.1 mol/l cold glycine buffer, pH 10.4, was added to the wells. The reaction was stopped after 30 min by the addition of 50 μl 3 mol/l NaOH. The absorbency was read at 405 nm. Results are expressed as absorbency minus the reagent blank.

Statistical analyses

All parametric data are presented as the mean ± SEM. Student’s t-test was used to compare the two means. Non-parametric data sets were analysed using the Fisher exact statistic (for 2 × 2 tables). For the purposes of analysis, thyroiditis was scored as present or absent, without regard to the severity of the lesion.

Results

Experiment 1

As shown in Table 1, thalidomide administration
Table 1.
Effect of thalidomide administration from 42 to 102 days of age on the incidence of lymphocytic thyroiditis, diabetes mellitus and serum T₄ and TSH concentrations in BB/Wor rats.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Thalidomide&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of rats</td>
<td>16</td>
<td>13</td>
</tr>
<tr>
<td>Lymphocytic thyroiditis</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>% lymphocytic thyroiditis</td>
<td>62</td>
<td>70</td>
</tr>
<tr>
<td>Serum TSH (mU/l)</td>
<td>256±72&lt;sup&gt;b&lt;/sup&gt;</td>
<td>249±93</td>
</tr>
<tr>
<td>Serum T₄ (nmol/l)</td>
<td>39.9±3.9</td>
<td>47.6±5.2</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td>% Diabetes mellitus</td>
<td>100</td>
<td>77</td>
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</tbody>
</table>

<sup>a</sup>50 mg thalidomide/kg by gavage daily  
<sup>b</sup>Mean ± SEM

(50 mg/kg) did not affect serum thyroid hormone concentrations. There were no statistically significant differences in the serum concentrations of TSH and T₄ between the control and thalidomide-treated groups. The mean serum TSH concentrations were above the values in experiment 2, probably owing to iodine-induced hypothyroidism in the presence of lymphocytic thyroiditis. There were no statistically significant differences in the incidence of lymphocytic thyroiditis (thalidomide, 70% vs control, 62%) or diabetes mellitus (thalidomide, 77% vs control, 100%) between the 2 groups.

Experiment 2
As shown in Table 2, there were no statistically significant differences in the serum concentrations of TSH, T₄, and T₃ between the control and thalidomide-treated groups. Although the incidence of lymphocytic thyroiditis in the thalidomide-treated rats was slightly lower than in the diluent-treated control rats, this difference was not statistically significant. There was no statistically significant difference in anti-Tg antibody levels between the two groups (thalidomide, 0.34 ± 0.08 OD vs control, 0.26 ± 0.04). All thalidomide- and diluent-treated control rats developed diabetes mellitus.

Discussion
Thalidomide was first synthesized in 1954 by Kunz et al. and was used as a sedative and hypnotic drug (1). Shortly thereafter, it was also used as a tranquilizer and anti-emetic drug during pregnancy. A few years later, in 1961, thalidomide was withdrawn from the market because of its teratogenic effect and interest in the drug diminished (2). However, in 1964, Sheskin (3) made interesting observations on the possible immunosuppressant effects of thalidomide, since it was beneficial in the treatment of erythema nodosum leprosum. Marked improvement in this disorder occurred in patients who were taking thalidomide as a sedative. A multicenter study confirmed this observation (20). Thalidomide has been reported to be beneficial in small numbers of patients with diverse immunologic or inflammatory disorders, including discoid lupus erythematosus, subacute cutaneous lupus erythematosus, aphtous ulcers, Behcet's syndrome, Weber-Christian disease, ulcerative colitis, pyoderma gangrenosum, actinic prurigo, prurigo nodularis, and rheumatoid arthritis (4,21,22). Larger trials of thalidomide in patients with lepromatous leprosy and actinic prurigo have been conducted and the drug has proven to be efficacious in these two disorders (4,21). Recently, thalidomide was reported to be effective in treating and preventing graft-vs-host disease after bone marrow transplantation in a rat model (5) and showed promise as an

Table 2.
Effect of thalidomide administration from 30 to 100 days of age on the incidence of lymphocytic thyroiditis, diabetes mellitus and serum T₄, T₃, TSH and anti-Tg antibody concentrations in BA and NB line BB/Wor rats.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Thalidomide&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of rats</td>
<td>25</td>
<td>24</td>
</tr>
<tr>
<td>Lymphocytic thyroiditis</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>% lymphocytic thyroiditis</td>
<td>60</td>
<td>42</td>
</tr>
<tr>
<td>Serum TSH (mU/l)</td>
<td>43.2±4.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>42.3±4.4</td>
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<tr>
<td>Serum T₄ (nmol/l)</td>
<td>61.8±1.3</td>
<td>59.2±1.3</td>
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<tr>
<td>Serum T₃ (nmol/l)</td>
<td>1.3±.04</td>
<td>1.2±.03</td>
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<tr>
<td>Anti-Tg Antibody (OD)</td>
<td>0.26±0.04</td>
<td>0.34±0.08</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>25</td>
<td>24</td>
</tr>
<tr>
<td>% Diabetes mellitus</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

<sup>a</sup>75 mg thalidomide/kg by gavage daily  
<sup>b</sup>Mean ± SEM
adjunctive immunosuppressant in preventing chronic graft vs host disease in patients receiving bone marrow transplantation (GW Santos: Personal communication). All of these data supported the immunosuppressive effect of thalidomide. In contrast, thalidomide has not proven to be effective in other immunologic diseases in animals such as experimental allergic encephalomyelitis (23), experimental allergic neuritis in guinea pigs (23) and rats (24), and experimental arthus and anaphylactic reactions in guinea pigs (25). Furthermore, the effect of thalidomide on the survival of skin grafts was inconsistent. Hellman et al. (26) reported that thalidomide prolonged the survival of skin homografts in mice. However, this finding was not consistently found by other investigators (27-29). Neither renal transplantation in dogs (30) nor renal allotransplantation in baboons (31) were affected by thalidomide.

The mechanism of action of thalidomide is unclear. Diseases in which thalidomide has been proven to be useful do not share any apparent common immune abnormality which could help elucidate the drug’s immunosuppressant effect. Thalidomide may act as an immunomodulating agent on T cell subsets (32). Studies by Tutschka et al. in humans suggested that thalidomide might generate suppressor T cells (6), and thalidomide has been shown to decrease helper T cells in the peripheral blood of healthy male subjects (7). Furthermore, the drug may act on other parameters of the immune response such as an inhibition of the IgM but not the IgG antibody response (33) and inhibit neutrophilic chemotaxis (8) and phagocytosis (9). Finally, thalidomide might oppose the effect of chemical mediators at the level of nonspecific inflammatory responses (9).

In the present study, thalidomide had no effect on the serum concentrations of TSH, T4, and T3 in the BB/Wor rat. This strongly suggests that the drug does not have an antithyroid action. No significant difference in the incidence of lymphocytic thyroiditis, as assessed histologically and by the occurrence of anti-Tg antibodies, or diabetes mellitus, evident by glycosuria and insulin requirements, were observed in either experiment. Conventional microscopic examination failed to note significant differences in the intensity of thyroiditis or the mononuclear cell composition of thalidomide vs control animals. The doses of thalidomide employed in both experiments (50 mg/kg and 75 mg/kg) were much higher than the usual human dose (2.5 mg/kg) and similar to those employed in preventing acute graft-vs-host disease in the rat (5). Thus, the failure of thalidomide to decrease the incidence of lymphocytic thyroiditis and diabetes mellitus or to decrease serum anti-Tg antibody levels was probably not due to an insufficient dose of the drug.

The pathogenesis of lymphocytic thyroiditis and insulin-dependent diabetes mellitus in man and experimental animal models has not been entirely defined but is probably due to the combination of cell-mediated and humoral factors in genetically susceptible hosts. Since, as discussed earlier, thalidomide appears to have modest suppressive effects on both cell-mediated and antibody associated autoimmune disorders, the failure of the drug to alter the incidence of lymphocytic thyroiditis and diabetes mellitus in the BB/Wor rat might be due to either its marginal immunosuppressive effect or to its failure to suppress the complex immunologic factors responsible for the occurrence of both disorders in this rat model.

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