EFFECTS OF EXPERIMENTAL HYPER- AND HYPOTHYROIDISM ON NUMBERS OF BLOOD MONONUCLEAR CELLS AND IMMUNE FUNCTION IN RATS AND GUINEA-PIGS

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SUMMARY

A possible effect of thyroid hormones on numbers of mononuclear cells and immune reactivity has been studied in hyperthyroid and hypothyroid guinea-pigs and rats. There were no major changes in populations of blood mononuclear cells in hyperthyroid or hypothyroid animals compared with populations in euthyroid animals. Although there was some evidence for depressed cell-mediated responses to an extract of Candida (monilia) albicans in hyperthyroid rats as assessed by skin tests, this was minor, and responses in tuberculin purified protein derivative (PPD) were normal in all groups, whilst production of macrophage migration inhibition factor in response to PPD and Candida was similar in the three groups of animals. Antibody responses to sheep red blood cells, a thymic-dependent antigen, tended to be depressed in hyperthyroid and hypothyroid rats and increased in hyperthyroid and hypothyroid guinea-pigs, although this was significant only for hyperthyroid guinea-pigs 16 days after immunization. Responses to trinitrophenol-Ficoll, a thymic-independent antigen, were similar to the three groups of guinea-pigs. Thus, a major effect of excess or deficiency of thyroid hormone on immune responses to foreign antigens has not been demonstrated, although it is possible that immune reactions against thyroid antigens may be more sensitive to the effect of thyroid hormones than responses to foreign antigens.

INTRODUCTION

There is considerable experimental evidence for effects of thyroxine (T₄) and triiodothyronine (T₃) on immune reactivity. Thus lymphocytes and thymocytes have nuclear receptors for T₄ and T₃ (Tata & Widnell, 1966; Samuels, Tsai, Casanova & Stanley, 1974; DeGroot, Rue, Robertson & Scherberg, 1977), feeding with T₄ induces lymphoid hypoplasia (Warner, 1964) and thymic enlargement (Höhn, 1959) in chickens, and treatment with T₄ prevents steroid-induced involution in chickens (Höhn, 1959). In addition, T₄ and T₃ are slightly mitogenic for human lymphocytes and thymocytes (Bommer & Ritz, 1978). In recent studies Balázs, Leövey, Szabo & Bako (1980) and Chatterjee & Chandel (1980) have shown effects of T₄ and T₃ on immune functions, in vitro and in vivo. The mechanism for the effects of thyroid hormones on lymphocyte function is unclear. In this study possible effects of thyroid hormones on populations of blood mononuclear cells and on immune responses to foreign antigens were investigated in hyperthyroid and hypothyroid animals. No significant effect of either excess or deficiency of thyroid hormones on immune function was demonstrated.

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Adult Wistar rats and Hartley strain female guinea-pigs were used. Animals were made hyperthyroid by treatment with T4 (Sigma Ltd, St Louis, Missouri, U.S.A.; 20µg/1000 g body wt per day, i.p.) or hypothyroid by thyroidectomy followed by treatment with 131I (Amersham Corp., Oakville, Ontario, Canada). Control (euthyroid) animals were treated (i.p.) with 0.9% (w/v) NaCl solution. Blood levels of T4 were monitored at regular intervals. When the appropriate thyroid status (T4 ≥ 10 µg% in hyperthyroid animals and < 0.05 µg% in hypothyroid animals) had been achieved, percentage mononuclear cell populations were determined and the animals were immunized with a variety of foreign antigens. Blood levels of T4 were maintained at preimmunization levels during the course of the experiments by altering the dose of T4. Delayed hypersensitivity skin tests and tests for production of leukocyte migration inhibition factor (MIF) were carried out before the animals were killed.

Rats were immunized with sheep red blood cells (0.3 ml 2% solution, i.p.) and blood was drawn on days 0, 3, 5, 7, 12 and 15. They were next immunized with Freund's complete adjuvant (FCA) mixed with an equal volume of human thyroid extract (0.2 ml, s.c.) and then with an extract of Candida (monilia) albicans (Hollister-Stier Ltd, Mississauga, Ontario, Canada; 0.4 ml 10% solution, intradermally). Delayed hypersensitivity skin tests with tuberculin purified protein derivative (Mantoux) (PPD; Connaught Labs Ltd, Willowdale, Ontario, Canada) and Candida were carried out on day 14: footpad swelling was measured 24 and 48 h after injection of Candida (0.1 ml 10% solution) and PPD (0.1 ml containing 5 tuberculin units (T.U.)). Production of leukocyte MIF in response to PPD, Candida and thyroid extract was measured on day 16.

Guinea-pigs were immunized with trinitrophenol (TNP)-Ficoll (Pharmacia Ltd, Dorval, Quebec, Canada), a thymic (T) -independent antigen (20 µg/animal, i.p.), and blood was drawn on days 0, 6, 8, 10, 12 and 17. They were next immunized with sheep red blood cells (0.25 ml 10% solution, i.p.) and bled on days 0, 6, 10, 12, 14, 16 and 30. Four weeks later they were injected with FCA (0.4 ml, s.c.) and delayed skin tests with PPD (25 T.U.) were carried out after 4 weeks. Three days before being killed animals were injected with paraffin oil. Peritoneal exudate cells were collected at autopsy.

Peripheral blood leukocytes were harvested using a Hypaque-Ficoll gradient (Winthrop Labs, Aurora, Ontario, Canada; Pharmacia Ltd, Dorval, Quebec, Canada) (Böyum, 1968). This population consists of 80–90% lymphocytes, 10–20% monocytes and <1% neutrophils.

**Rosette tests**

Guinea-pig peripheral blood T lymphocytes have receptors for rabbit red blood cells. Total T cells, 'activated' T cells (those T cells which form rosettes with rabbit red blood cells during 5 min incubation; Wybran & Fudenberg, 1973), mononuclear cells with Fc receptors for immunoglobulin G(IgG) (bursa-derived (B) lymphocytes and monocytes) and Tγ cells were enumerated using standard rosette tests (Hallberg, Gurner & Coombs, 1973; Wybran & Fudenberg, 1973; Special Technical Report, 1974; Wall, Gray & Greenwood, 1977; Gupta, Fernandes, Nair & Good, 1978; Platoucas, Good & Gupta, 1979; Chartier, Bandy & Wall, 1981; Wall & Chartier, 1981). In all rosette tests mononuclear cells surrounded by three or more red blood cells were considered positive and the percentage of mononuclear cells forming rosettes was determined from 200 cells. Duplicate tests were set up.

**Antibody tests**

Serum antibodies against sheep red blood cells, TNP-Ficoll and thyroglobulin were measured using standard haemagglutination techniques (Böydén, 1951). Delayed hypersensitivity skin tests were carried out by injecting 0.1 ml antigen into the footpads and
measuring increased footpad swelling at 24 and 48 h. Control animals were injected with 0.9% NaCl solution into one footpad.

Production of leukocyte MIF in response to Candida (3 and 5%), PPD (10 and 100µg/ml), and thyroid extract (100 and 200µg/ml) was determined in rats using the agarose micro-droplet technique (Harrington, Stastney & Stastney, 1973). Control tests used 0.9% NaCl solution instead of antigen. Migration was measured after 18 h using a projection microscope. The area of migration was calculated and results were expressed as migration indices, i.e. area of migration in response to antigen/area of migration in control tests.

**Macrophage function**

Macrophage function was assessed by injecting sterile paraffin oil into guinea-pigs 3 days before killing. At autopsy peritoneal exudate cells were collected, washed, and tested for (a) migration across a glass surface from a capillary tube, (b) inhibition of migration by a standard preparation of lymphokines containing MIF and (c) ability to engulf paraffin oil as determined by the percentage of macrophages in peritoneal exudates and the percentage of macrophages containing oil droplets.

**Antigen preparation**

Human thyroid extract was prepared from surgically obtained normal tissue following the method of Schneider & Hogeboom (1950). Briefly, the cleaned tissue was minced, homogenized in 0.25 M-sucrose and extracted overnight in phosphate-buffered 0.9% NaCl solution (pH 7.4). This was then centrifuged at 1500 g for 20 min. The protein concentration of the supernatant fraction was adjusted to 100 and 200 µg/ml and samples were stored at −20°C.

**RESULTS**

The mean total blood lymphocyte count in hyperthyroid rats (2488 ± 213 (s.e.m.))/mm³, n = 10) was significantly (P<0.05, Student’s t-test) increased compared with that in euthyroid rats (1785 ± 105/mm³, n = 10). Levels of total blood lymphocytes and percentages of lymphocytes, T cells, Fc receptor positive mononuclear cells and Tₜ cells in euthyroid, hyperthyroid and hypothyroid guinea-pigs are summarized in Table 1. There were

<table>
<thead>
<tr>
<th>Group</th>
<th>Total lymphocyte counts (×10⁶)</th>
<th>Lymphocytes (%)</th>
<th>T Lymphocytes (%)</th>
<th>Tₜ Cells (%)</th>
<th>Fc Receptor positive cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euthyroid</td>
<td>4507 ± 384 (16)</td>
<td>58.0 ± 3.4 (16)</td>
<td>31.0 ± 2.2 (14)</td>
<td>20.7 ± 2.4 (13)</td>
<td>11.0 ± 1.3 (13)</td>
</tr>
<tr>
<td>Hyperthyroid</td>
<td>5279 ± 550 (11)</td>
<td>57.0 ± 4.0 (11)</td>
<td>35.5 ± 2.4 (17)</td>
<td>16.8 ± 3.3 (9)</td>
<td>11.0 ± 1.0 (18)</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>NS</td>
<td>NS</td>
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<td>NS</td>
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NS, Values are not significantly different from values for euthyroid animals (Student’s t-test).
essentially no differences between the three groups of animals. In four hyperthyroid and four euthyroid animals the percentage of 'activated' T cells was measured before and after immunization with PPD to determine whether this population increased after induction of cell-mediated immunity. Whilst the percentage of 'activated' T cells increased in all animals there was no significant difference between the two groups.

Mean sheep red blood cell antibody titres, particularly in the early (IgG) phase, were decreased in both hyperthyroid and hypothyroid rats compared with those in euthyroid rats, although the differences just failed to reach significance (Fig. 1a). On the other hand, sheep red blood cell antibody titres were slightly increased in hyperthyroid and hypothyroid guinea-pigs compared with titres in euthyroid animals, although this was significant only for hyperthyroid animals on day 16 ($P<0.05$, Student's $t$-test) (Fig. 1b). The TNP-Ficoll antibody titres were similar in the three groups of guinea-pigs on all days tested (Fig. 1c).

Fig. 1. Serum antibody titres in euthyroid (△), hyperthyroid (○), and hypothyroid (●) rats and guinea-pigs expressed as mean (+ s.e.m.) ln titre$^{-1}$, after primary immunization (i.p.) with (a) sheep red blood cells (rats), (b) sheep red blood cells (guinea-pigs) and (c) trinitrophenol-Ficoll (guinea-pigs). Haemagglutinating antibodies were tested for on unabsorbed, unheated sera. *$P<0.05$ compared with value for euthyroid animals (Student’s $t$-test).
Mean increase in footpad thickness in response to Candida in euthyroid rats was 0.55 ± 0.15 mm (n = 10) which was greater than in hyperthyroid rats (0.10 ± 0.02 mm, n = 10, P < 0.05) but less than in hypothyroid rats (0.79 ± 0.11 mm, n = 10), although this latter difference was not significant. Mean responses to PPD (0.31 ± 0.15, 0.16 ± 0.01 and 0.28 ± 0.02 mm, n = 10 for all groups, for euthyroid, hyperthyroid and hypothyroid rats respectively) were not, however, significantly different (Fig. 2). Mean increases in footpad swelling in response to PPD in hyperthyroid and hypothyroid guinea-pigs (0.88 ± 0.17 mm, n = 21 and 0.61 ± 0.15 mm, n = 6 respectively) were not significantly different from that in euthyroid animals (0.89 ± 0.10 mm, n = 38). Production of MIF in response to Candida was slightly increased in hypothyroid compared with that in euthyroid rats, with mean migration indices of 0.73 ± 0.06 (n = 9) and 0.9 ± 0.02 mm (n = 10) respectively, although the difference was not significant. Responses to Candida were similar in hyperthyroid and euthyroid rats and responses to PPD were similar in all three groups. Migration inhibition factor production in response to human thyroid extract was not demonstrated and mean migration indices for the three groups were not significantly different. Macrophage function, as assessed by migration, response to lymphokines, and phagocytosis of oil, was similar in the three groups of guinea-pigs.

![Graph showing footpad swelling](image)

**Fig. 2. Delayed hypersensitivity skin test responses to an extract of *Candida (monilia) albicans* (open bar) and tuberculin purified protein derivative (shaded bar) in euthyroid, hyperthyroid, and hypothyroid rats expressed as mean (± S.E.M.) increase in footpad thickness. *P < 0.05 compared with value for euthyroid rats (Student's *t*-test).**

**DISCUSSION**

To summarize, the main findings from this study were that there were no major changes in populations of blood mononuclear cells in hyperthyroid or hypothyroid animals and although depressed cell-mediated immunity to *Candida (monilia) albicans* extract in hyperthyroid rats, as assessed by delayed hypersensitivity skin tests, was demonstrated, this was minor, and T cell responses were generally normal in hyperthyroid and hypothyroid animals. There was a tendency for sheep red blood cell antibody titres to be lower in hyperthyroid and hypothyroid rats and higher in hyperthyroid and hypothyroid guinea-pigs compared with those in euthyroid animals, although this was significant only for hyperthyroid rats 16 days after immunization. Responses to the T-independent antigen, TNP-Ficoll, were similar in the three groups of guinea-pigs. Macrophage function was similar in hyperthyroid, hypothyroid, and euthyroid animals.
Our findings contrast with those of Balázs et al. (1980), who demonstrated a dose-related effect of T₃ on a variety of immune functions including peripheral blood lymphocyte transformation response to phytohaemagglutinin, antibody-dependent cellular cytotoxicity and polymorphonuclear function both in vitro and in vivo, and of Chatterjee & Chandel (1980) who showed that B and T cell responses to Salmonella typhi H and A antigens were enhanced in hyperthyroid rats whilst responses were depressed in hypothyroid animals. Concentrations of T₃ used by Balázs et al. (1980) were higher than those found in the serum of patients with thyrotoxicosis, whilst in our studies serum T₄ (and presumably T₃) levels in hyperthyroid animals were comparable to those found in patients with Graves’s hyperthyroidism. Chatterjee & Chandel (1980) tested only one antigen, hence it is difficult to compare results. Moreover, it seems unlikely that their animals would have been hyperthyroid after only 2 weeks of treatment with T₄ or T₃.

Despite our failure to demonstrate a significant effect of hyper- or hypothyroidism on immune responses to extrinsic antigens, there is clinical evidence for an effect of thyroid hormone excess or deficiency on immune response to thyroid antigens in patients with autoimmune thyroid disorders. Thus, whilst remission in patients with Graves’s hyperthyroidism treated with anti-thyroid drugs may be due to an immunosuppressive effect of the drugs (Wall, Lee Manwar, Greenwood & Walters, 1976; McGregor, Petersen, McLachlan, Rooke, Smith & Hall, 1980), an effect of lowering of blood levels of T₄ appears more likely. Treatment with T₄ in patients with Hashimoto’s thyroiditis is associated with a fall in antibody titres and reduction of lymphoid infiltration (LeBoeuf & Ducharme, 1966). Moreover, there is some evidence to suggest that T₄ treatment of predisposed subjects such as relatives of patients with Graves’s disease or of patients with Hashimoto’s thyroiditis may precipitate Graves’s hyperthyroidism (Sung & McDougall, 1978; Easton & Wall, 1981). It also seems possible that ophthalmopathy may develop in patients with Graves’s hyperthyroidism due to an effect of hyperthyroxinaemia on abnormal regulatory cells. We are presently testing the latter hypothesis by immunizing hyperthyroid and euthyroid guinea-pigs with orbital antigens and comparing clinical and immunological parameters of eye disease in the two groups.

Immune function and peripheral blood cell population are essentially normal in patients with Graves’s disease (Mulaisha, Abdou & Utiger, 1975; Calder, Irvine, Davidson & Wu, 1976; Lundell, Wasserman, Granberg & Blomgren, 1976; Volpé, 1978; Wall & Chartier, 1981) despite the fact that the patients may be severely hyperthyroid. It would appear to be more appropriate, therefore, to induce hyper- and hypothyroidism in experimental animals prone to develop thyroid autoimmune disease, such as obese strain Leghorn chickens, and to determine the frequency, time of onset, and severity of autoimmune phenomena in these animals.

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REFERENCES


Immune function in experimental thyroid disease


