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# Experimental evidence pointing to the bidirectional interaction between the immune system and the thyroid axis

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**Abstract**

Among the many examples of neuroendocrine–immune system interactions the relationship between the thyroid axis and the immune function has yet to be clearly established. Here we studied the influence of thyroid hormones on the course of an alloimmune response. Murine $T_3$ and $T_4$ levels were found to be increased a few days after the immunization of mice with allogeneic lymphoid cells. Besides in vivo treatment with $T_4$ was shown to increase alloantibody titers during the early stages of alloimmunization and to enforce lymphoid proliferation in vitro in a mixed lymphocyte reaction. Conversely, lowering thyroid hormone seric levels by propylthiouracil treatment, negatively modulates the humoral and cellular alloimmune responses. The evidence here points to the existence of a bidirectional communication between both systems. The possibility that the antigenic challenge would increase the thyroid gland activity thus leading to a positive modulatory action upon the immune response is also discussed. 

*Keywords:* Alloimmunization; Immune system; Thyroxine; Triiodothyronine; Alloantibodies; Mixed lymphocyte reaction

## 1. Introduction

Several efficient autoregulatory mechanisms confer a certain degree of autonomy to the immune system. However, increasing evidence shows that immune processes operate in a coordinated way with other body systems. Moreover, the existence of a circuit between the neuroendocrine and the immune systems that operates in both directions has been well

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documented [2,24]. Bidirectional regulations involve shared usage of common signal molecules and their receptors [5,24].

In fact, there are clear examples that immune cells can be influenced by hormones [16], neurotransmitters [6,14] and neuropeptides [4] and also by brain function alterations. Conversely immune-derived products such as lymphokines and monokines can affect endocrine, autonomic and central mechanisms [24].

Among these interactions the immune system can be directly modulated by the hypothalamus–pituitary axis and the corresponding target endocrine glands (namely thyroid, gonads, adrenal) [1,24].

The interactions of thyroid hormones in the immune development and function has been demonstrated in both mammalian [8,12] and avian systems [7,20,21]. The association of thyroidectomy [12], hypophysectomy [19], hypothyroidism [8,23] and hypopituitarism [8,13] with thymic growth depression and with a decrease in circulating lymphocyte number, has been demonstrated. Conversely, in vivo supplementation with triiodothyronine ($T_3$) or thyroxine ($T_4$) reverses these effects and stimulates thymic growth and the mitotic cell index of cortical thymocytes [8,12,17]. Also the production of a thyrotropin (TSH)-like molecule induced by mitogenic activation of human peripheral leukocytes [25] and the enhancement of an in vitro antibody response by TSH [3] has been shown. However the role that interactions between thyroid hormones and the immune system play in vivo, as well as their mutual modulation have not yet been well defined. Thus, the aim of the present work was to study the mutually regulatory mechanisms exerted by the immune system and the thyroid axis that would confirm the existence of a bidirectional communication circuit between both systems. For this purpose seric levels of thyroid hormones during the course of an alloimmune response as well as the variation in alloantibody titers by in vivo administration of thyroid hormones or antithyroid agents were studied.

## 2. Experimental procedures

### 2.1. Mice

Inbred BALB/c (H-$2^d$) and C3H (H-$2^k$) mice were obtained from the Instituto de Oncología "A. H. Roffo". All animals were used at age 60–70 days.

### 2.2. In vivo thyroid hormones and antithyroid agent treatments

Thyroxine hormone ($T_4$) treatment: BALB/c mice ($n = 20$) received an intraperitoneal injection of 40 μg thyroxine ($T_4$) (purchased from SIGMA) (dissolved in 0.1 N NaOH, diluted 1:10 with phosphate-buffered saline (PBS), pH 7.2, and Millipore filtered) five times per week for one month. Mice injected with the vehicle alone were used as controls.

Propylthiouracil (PTU) treatment: to promote low circulating levels of thyroid hormones in BALB/c mice ($n = 40$), PTU was given in the drinking water (0.5 mg/ml) for 18 days as previously reported [10,22]. The daily ingested amount of PTU was approximately 6 mg/100 g body weight (bw)/day.

Propylthiouracil + triiodothyronine (PTU + $T_3$) treatment: half of the animals treated with PTU also received 20 μg/100 g bw/day of $T_3$ (dissolved in the same vehicle used for thyroxine) intraperitoneally for the last six days of treatment. Control animals received the vehicle of the hormone alone.

### 2.3. Alloimmunizations

BALB/c mice were immunized with C3H lymphoid cells. All immunizations were carried out between animals of the same sex (female) to avoid immune responses directed to the sex related minor histocompatibility complex antigen (H-Y), according to the following schedule: one i.d. injection of $1 \times 10^7$ lymphoid cells (pooled spleen, lymph node and thymus suspension), followed at seven-day intervals by one or more immunizations with $3 \times 10^7$ lymphoid cells i.p. Unless specified, mice were killed four or five

days after the last booster. Non-immunized mice were administered with the same volumes of RPMI 1640 medium alone. This schedule was also performed in untreated mice ($n = 20$), and in $T_4$, PTU or PTU + $T_3$ treated mice ($n = 20$ in all treatments).

### 2.4. Cell suspensions

Lymphoid organs were removed and softly homogenized in RPMI 1640 medium with a loosely fitting teflon glass homogenizer; the cell suspension was filtered through a metal mesh and then through needles, washed three times in RPMI 1640, checked for viability by trypan blue exclusion test and resuspended in the same medium at the desired concentration.

### 2.5. Antibody titers

Sera were titered for cytotoxic antibodies using a trypan blue exclusion test in a two step, complement-dependent microcytotoxic assay as described [9]. Briefly, C3H lymph node cells were incubated with 1:2 serial dilutions of the test serum; guinea pig serum (Gibco Co.) was added as a source of complement and 50% lysis above negative controls (cells alone, cells plus normal serum or cells plus complement alone) was used as the titration point.

Also, alloantibody titers were determined by enzyme-linked immunosorbent assay (ELISA) over C3H fixed cells on 96 flat-bottomed wells (Falcon 3912, Becton Dickinson), with 0.5 glutaraldehyde in PBS. A goat anti-mouse IgG phosphatase alkaline conjugated (Sigma Chemical Co.) and p-nitrophenylphosphate (Sigma Chemical Co.) as substrate were used for developing coloration that was read at 405 nm. It is worth noting that all the dilutions of sera were made in PBS containing 1 mM Na azide and 15% of normal goat sera to manage the blockade of Fc receptors on C3H fixed cells. Staining were considered positive when the optical density (O.D.) values were above the mean value plus 2 S.D. of normal sera (sera from non-immunized and/or vehicle alone injected mice give non-statistical differences among them).

### 2.6. Hormone determinations

Control non-immunized or alloimmunized animals were bled at the times indicated in the Results. The blood was collected into plain tubes (without anticoagulant). Seric $T_3$ and $T_4$ levels were determined using highly sensitive double antibody radioimmunoassay kits (Diagnostic Products Corporation, USA).

### 2.7. Mixed lymphocyte reactions

Mitomycin C-treated lymph node cells from C3H mice were used as allogeneic stimulators of one way mixed lymphocyte reactions and BALB/c lymphocytes from control, $T_4$, PTU or PTU + $T_3$ treated mice were used as the responding cell populations. All cell suspensions were cultured at a concentration of $1 \times 10^6$ cells/ml in a final volume of 0.2 ml of RPMI 1640 medium (Gibco Co.) supplemented with 10% heat-inactivated fetal bovine serum, 2 mM glutamine and antibiotics in 96-well flat-bottomed microtiter plates (Corning, NY) following standard procedures [26]. They were kept at 37°C in a 5% $CO_2$ atmosphere for the culture times indicated in results.

### 2.8. [$^3$H]Thymidine incorporation

Proliferation was tested by [$^3$H]thymidine ([$^3$H]TdR, INC, Irvine, CA, USA; 15 Ci/mmol) incorporation. Sixteen hours before the end of culture incubation, microcultures were pulsed with 1 μCi of [$^3$H]TdR. The cells were harvested on filter paper with an automatic cell harvester (Micromate 196, Packard) and the radioactivity was counted by liquid scintillation. Results were expressed as stimulation index (SI) calculated as the rate between dpm values in experimental cultures and dpm from control values obtained with unstimulated cells.

### 2.9. Statistical analysis

Student's *t*-test for unpaired values was used to determine the level of significance. When multiple comparisons were necessary, after analysis

of variance, the Student–Newman–Keuls test was applied. Differences between means were considered significant if $P \leq 0.05$.

## 3. Results

### 3.1. Variation of $T_3$ and $T_4$ serum levels during the course of an alloimmunization

In order to analyze if the immune response is able to modulate seric thyroid hormone levels, BALB/c mice alloimmunized with C3H lymphoid cells were bled at different times after the beginning of an alloimmunization schedule.

Results demonstrated that levels of both $T_3$ (Fig. 1) and $T_4$ (Fig. 2) were significantly higher than those obtained on non-immunized controls (basal). By the second week, $T_4$ values remained increased, while $T_3$ concentration began to decrease. Therefore, under these experimental conditions, thyroid gland activity was enhanced at early stages of the immune response.

### 3.2. Influence of thyroid hormones on antibody response

To further characterize the bidirectional interaction between the thyroid axis and the immune system, the effect of seric thyroid hormones levels on the degree of an alloimmune response was evaluated. For this purpose up or down regulation of thyroid hormone levels on alloantibody production were analyzed with respect to control untreated alloimmunized mice.

First, $T_4$ or PTU treated BALB/c mice were alloimmunized with C3H lymphoid cells and cytotoxic titers of alloantibody were tested. As shown in (Fig. 3), cytotoxic titers in $T_4$-treated animals were higher than those found in control alloimmunized mice, particularly at the beginning of the alloimmunization schedule. Conversely in

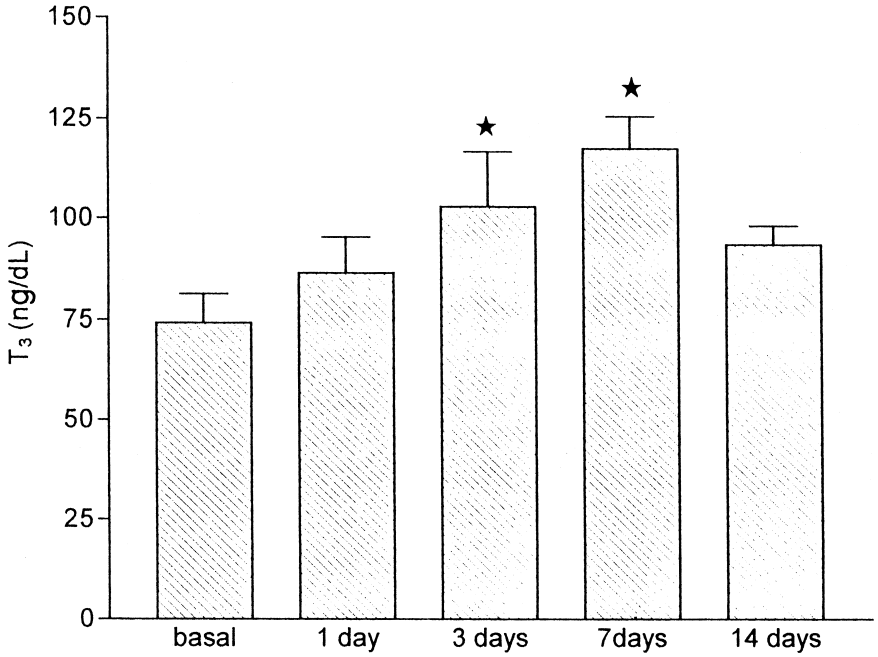



Fig. 1. $T_3$ serum levels during allogeneic immunization on BALB/c mice. Animals were immunized with C3H lymphoid cells according the schedule described in Experimental procedures. At the indicated times blood samples were collected and serum levels of $T_3$ were measured by RIA. Data shown are the mean $\pm$ SE of $n = 3$ experiments performed in duplicate. *Differ significantly from control values with $P < 0.05$.

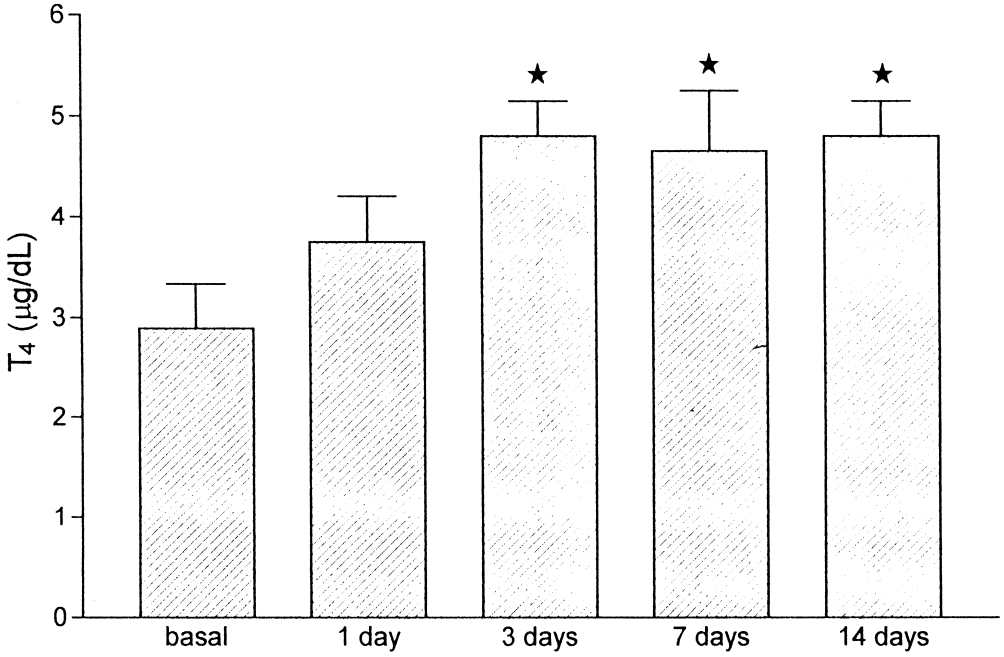



Fig. 2. BALB/c seric levels of $T_4$ during allogeneic immunization. BALB/c mice were immunized twice with C3H lymphoid cells and their blood samples were assayed by RIA for $T_4$ determination. Results shown are the mean ± SE of $n = 3$ experiments performed in duplicate. *Differ significantly from control animals with $P < 0.05$.

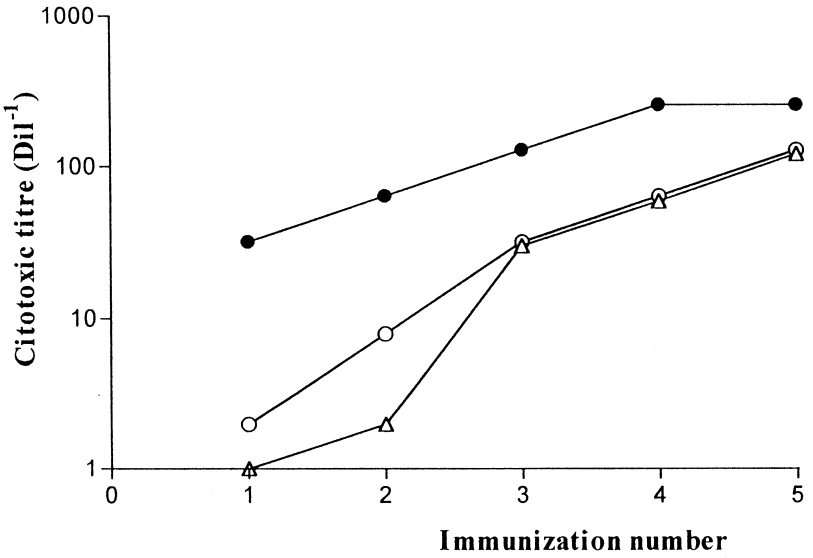
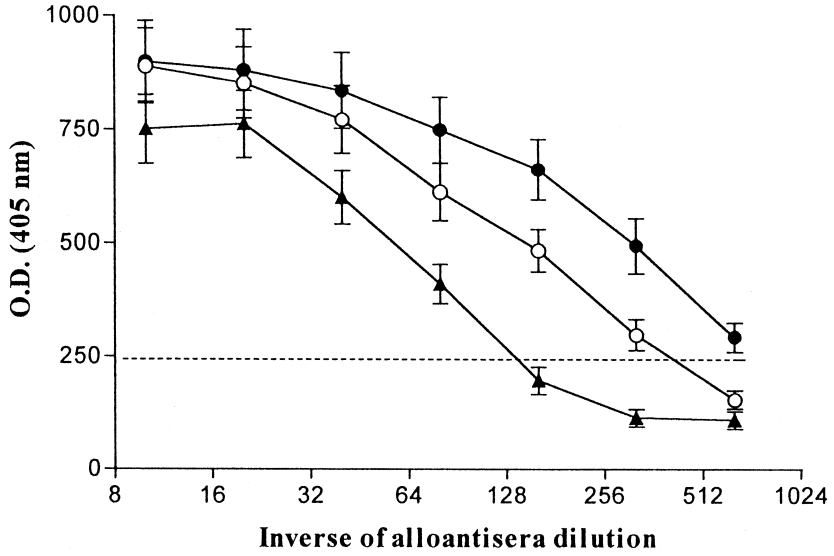



Fig. 3. Thyroid hormone modulation during the course of alloimmunization, evaluated through alloantibody cytotoxic titers. Control (○—○), in vivo treated with $T_4$ (●—●) or in vivo treated with PTU (△—△). BALB/c mice were alloimmunized as indicated in Experimental procedures. Data show a representative experiment of seric alloantibody cytotoxic titers determined on samples collected 4–6 days after the last booster and calculated as described before.

PTU treated animals no detectable alloantibody production was found after the first week of immunization and lower cytotoxic titers were evident in the second week. To further confirm this decrease in alloantibody response, titration of second week alloantisera was also evaluated by the more sensitive ELISA assay with C3H fixed cells as coating antigen. Results from control and PTU-treated mice are shown in (Fig. 4) demonstrating that alloantisera from control mice were positively reactive for two higher dilutions than those from PTU-treated animals. It is worth noting that in vivo administration of $T_3$ reverted this situation although to a lesser value than that obtained on animals who had only received $T_3$ (data tabulated in Fig. 4) whose




| HIGHER POSITIVE DILUTION OF SECOND WEEK ALLOANTISERA | | | |
|---|---|---|---|
| CONTROL | PTU | PTU + $T_3$ | $T_3$ |
| 1/320 | 1/80 | 1/640 | 1/1280 |

Fig. 4. Evaluation of alloantibody reactivity through ELISA assays. Control (○—○), PTU (▲—▲) or PTU + $T_3$ (●—●) treated BALB/c mice from second week alloimmunization were serially diluted and tested following the ELISA technique described in Experimental procedures over C3H fixed cells as coating antigen. Positive reactivity was considered when O.D. obtained at 405 nm were above the mean value ± 2 S.D. of normal sera (sera from non-immunized and/or vehicle alone injected mice). Data shown are the mean value of four sera ± S.D. The second week alloantisera higher dilution giving positive reactivity for each treatment and, additionally that corresponding to mice treated only with $T_3$, are also depicted in the Table 1.

Table 1
Seric thyroid hormone levels from control, $T_4$ or PTU treated animals during the course of alloimmunization

| Treatment[a] | *n* | $T_3$ (ng/dl)[b] | $T_4$ (μg/dl)[b] |
|---|---|---|---|
| Control | 20 | $73.6 \pm 6.2$ | $3.2 \pm 0.4$ |
| $T_4$ | 20 | $842.7 \pm 13.7$[c] | $63.6 \pm 5.1$[c] |
| PTU | 20 | $47.1 \pm 3.5$[c] | $1.4 \pm 0.2$[c] |
| $T_4$ + 1st week alloimmunization | 4 | $876.0 \pm 19.9$ | $72.2 \pm 8.7$ |
| $T_4$ + 2nd week alloimmunization | 4 | $845.9 \pm 8.8$ | $68.3 \pm 4.4$ |
| $T_4$ + 3rd–5th week alloimmunization | 12 | $834.7 \pm 20.2$ | $63.2 \pm 2.8$ |
| PTU + 1st week alloimmunization | 4 | $67.8 \pm 4.3$[d] | $2.3 \pm 0.2$[d] |
| PTU + 2nd week alloimmunization | 4 | $56.5 \pm 3.8$ | $2.4 \pm 0.2$[d] |
| PTU + 3rd–5th week alloimmunization | 12 | $48.0 \pm 4.3$ | $1.9 \pm 0.2$ |

[a] BALB/c mice were treated in vivo with $T_4$, PTU or vehicle alone (control) as indicated in Experimental procedures and, where indicated were also immunized for 1–5 weeks with allogenic C3H cells following the schedule described before.

[b] Seric thyroid hormone levels were determined by RIA as indicated. Results shown are the mean ± SE of the *n* indicated sera. It is worth noting that data from 3rd to 5th weeks alloimmunization correspond to four animals from each corresponding week.

[c] Statistical comparison was performed among the treated animals and controls, giving both treatments significant differences from control values with $P < 0.01$.

[d] Thyroid hormone levels in PTU-treated alloimmunized animals were statistically different from the corresponding non-immunized PTU-treated mice with at least $P < 0.05$.

titers were even higher than those found in control mice.

Additionally, thyroid hormone levels from control and alloimmunized mice after $T_4$ or PTU treatment are shown in Table 1. $T_4$ treated animals had high levels of both thyroid hormones while PTU-treated mice showed low levels as expected. Moreover, for $T_4$ treated animals no statistical differences were found in thyroid hormone levels during the course of immunization. However the allogeneic stimulus was able to induce higher levels of $T_3$ and $T_4$ after the first week and of $T_4$ after the second week of alloimmunization. No differences were found after three to five boosters, showing the same variations in $T_3$ and $T_4$ levels than those observed previously for untreated animals.

### *3.3. Effect of thyroxine on a one way mixed lymphocyte reaction (MLR)*

To determine if thyroid hormone levels could also play a role in an in vitro induced alloimmune response, $T_4$ or PTU treated BALB/c mice cells were cultured in a MLR with C3H lymphoid cells as an allogeneic stimulus. The stimulation index (SI) calculated as explained in experimental procedures, were compared to those obtained with control lymphocytes from BALB/c mice that have received only the vehicle. Table 2 summarizes the SI in both cases. $T_4$ treated mice cells showed SI significantly higher than control cells at day six of culture, while lower SI values were obtained with lymphocytes from PTU-treated animals.

Table 2
$T_4$ and PTU action on MLR induced lymphocyte proliferation

| Time (days) | SI control[a] | SI $T_4$[a] | SI PTU[a] |
|---|---|---|---|
| 2 | $1.16 \pm 0.05$ | $1.21 \pm 0.05$ | $1.12 \pm 0.03$ |
| 4 | $1.72 \pm 0.08$ | $2.24 \pm 0.11$ | $1.36 \pm 0.06$ |
| 6 | $4.20 \pm 0.21$ | $7.68 \pm 0.06$[b] | $2.27 \pm 0.08$[b] |
| 8 | $1.53 \pm 0.07$ | $2.00 \pm 0.09$ | $1.45 \pm 0.07$ |

[a] Lymph node cells from control or in vivo $T_4$ or PTU treated BALB/c mice were cultured for the indicated days in MLR using as allogeneic stimulus mytomicine C-treated C3H lymphocytes, following the experimental conditions already described. Stimulation Indexes (SI) were calculated as indicated before and are the mean ± SE of $n = 5$ experiments.

[b] Differ significantly from SI control value with $P < 0.01$ and $P < 0.05$, for $T_4$ and PTU respectively.

## 4. Discussion

The interactions between the immune system and the hypothalamus–pituitary–adrenal axis would play an important role in the maintenance of homeostasis during the course of infections, inflammatory and neoplastic processes. The existence of interactions between the thyroid axis and the immune function has been documented but the bidirectional integration of both biological circuits, as well as its physiological significance have not yet been well defined. In the present work we try to determine the mutual regulation of both systems in an immune, namely an allogeneic, response.

First we analyze if the immune response was able to modulate seric levels of thyroid hormones. We found an early increase, three days after the beginning of alloimmunization, of both $T_3$ and $T_4$ hormones. To further analyze if this increment of thyroid hormones would have any implication for the specific immune response, we investigated if an in vivo treatment with thyroid hormones would modulate humoral and cellular responses.

For inducing high levels of thyroid hormones, mice were treated in vivo with thyroxine for two reasons: thyroxine is usually used in hormone replacement therapies in hypothyroid patients and the half-life of thyroxine is higher than that of $T_3$, allowing a constant potency and prolonged action being converted to $T_3$ in peripheral tissues. We found that thyroxine was able to increase significantly alloantibody titers in comparison to antibody levels found in normal control animals. This increment was mainly observed during the early alloimmunization (1–3 weeks) arriving at a plateau of constant titers tending to reach almost equal levels compared to control mice. Accordingly Blalock et al. [3] found that TSH is a potent enhancer of the in vitro antibody response of murine spleen cells, increasing the number of cells producing antibodies to sheep erythrocytes. Also, the presence of nuclear receptors for thyroid hormones in lymphocytes has been described [27,28], pointing to a possible direct effect of these hormones on lymphocyte function. In contrast, [29] found that the rise in circulating thyroid hormones had no significant effect on peak antibody titers and that reducing the levels of hormones slowed the decline in antibody titer. This controversy would reflect differences in the experimental conditions, namely in thyroid hormone levels. In fact it was shown that daily administration of 60 mg $T_4$ for 10 weeks increased responses against a thymus dependent antigen in chickens [18], but serum hormone concentrations were not reported by these authors. Thyroid hormone levels were determined in mice sera after $T_4$ treatment showing an approximate 10-fold increase above basal conditions during all the experimental schedules. Furthermore, this increment was higher and more persistent than that obtained by Williamson et al. and no differences between immune and control animals could be seen in this condition. Additionally, lowering thyroid hormone levels with the antithyroid agent PTU, induced both a decrease in cytotoxic alloantibody titers and in thyroid hormone levels. This was also confirmed by using a more sensitive ELISA assay with C3H fixed cells as coating antigens. In this case in vivo administration of $T_3$ was not only able to revert the decrease in the humoral response, but was also able to induce higher levels of alloantibodies in a similar way to that obtained after $T_4$ administration. We used $T_3$ to revert PTU actions as this antithyroid agent besides blocking thyroid hormone synthesis also inhibits the peripheric deiodination of $T_4$ to $T_3$ as well. Fabris [11] has also described a decrease in the antibody response to either sheep or chicken red blood cells in newborn young adult rats deprived of thyroid gland. Also with regard to the humoral immunity, a depression of the primary humoral immune response has been reported in hypothyroid chickens [15].

Taking together these results it could be assumed that the increase in thyroid hormone levels at the beginning of the antigenic challenge would positively modulate the immune response and would perhaps be an early stimulus for getting efficient levels of alloantibodies. In fact, the major differences in antibody titers were obtained in the first weeks of alloimmunization not at peak antibody titers. Furthermore, no modification in specific antibody titers were found

when thyroid hormones were given after the antigenic challenge [30].

When studying the effect of thyroid hormone levels on cellular responses we found that in vivo $T_4$ treatment was able to increase while PTU treatment induced the decrease of the SI of lymphocytes proliferating in a MLR. It is worth noting that $T_3$ replacement in PTU-treated animals reverted PTU effects on the cellular response in the MLR (data not shown). Other authors have shown an increase in thymic incorporation of radiolabelled thymidine after injection of thyroxine in mice [31]. Also it was shown that in vivo thyroid hormone treatment increases the expression of interleukin 2 (IL-2) receptor on avian splenocytes [7], and that patients with Grave's disease showed an increased circulating concentration of soluble IL-2 receptor, a marker of T lymphocyte activation [32]. On the contrary, a decrease in the proliferative response to T cell mitogens such as phytohemagglutinin or concavalin A has been reported in splenic and peripheral blood lymphocytes from hypothyroid animals [11,15].

Experimental evidence presented in this paper indicates a bidirectional modulation of thyroid axis and the immune response. The level of interaction seemed to be important during the early course of the immune response and would provide sound basis for future research about the mechanisms involved in the mutual physiological integration of both systems.

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## References

[1] Besedovsky HO, Del Rey A. Feed-back interactions between immunological cells and the hypothalamus–pituitary–adrenal axis. Neth J Med 1991;39:274–80.

[2] Blalock JE. The syntax of immune–neuroendocrine communication. Immunology Today 1994;15:504–11.

[3] Blalock JE, Johnson HM, Smith EM, Torres BA. Enhancement of the in vitro antibody response by thyrotropin. Biochem Biophys Res Comm 1984;125:30–4.

[4] Carr DJJ. Neuroendocrine peptide receptors on cells of the immune system. FEBS Lett 1992;52:84–105.

[5] Cavagnaro J, Lewis RM. Bidirectional regulatory circuit between the immune and neuroendocrine systems. In: Cruse JM, Lewis Jr RE, editors. The year in immunology 1988 — cellular, molecular and clinical aspects, 4. Basel: Karger, 1989. p. 241–52.

[6] Cazaux CA, Sterin-Borda L, Gorelik G, Cremaschi GA. Down-regulation of β-adrenergic receptors induced by mitogen activation of intracellular signaling events in lymphocytes. FEBS Lett 1995;364:120–4.

[7] Chandratilleke D, Hàla K, Marsh JA. Effects of in vivo thyroid hormone treatment on the expression of interleukin-2 receptors on avian splenocytes. Int J Immunopharmacol 1996;18:203–10.

[8] Comsa J, Leonhardt H, Ozminski K. Hormonal influences on the secretion of the thymus. Thymus 1979;1:81–93.

[9] Cremaschi GA, Sterin-Borda L, Genaro AM, Sterin-Borda E, Braun M. Pharmacologic effects on mouse isolated atria of immunoglobulins directed against class I histocompatibility antigens. J Immunol 1984;133:2681–5.

[10] Denereaz N, Lemarchand-Beraud T. Severe but not mild alterations of thyroid hormone function modulate the density of thyroid-stimulating hormone receptors in the rat thyroid gland. Endocrinology 1995;136:1694–700.

[11] Fabris N. Immunodepression in thyroid-deprived animals. Clin Exp Immunol 1973;15:601–9.

[12] Fabris N. Influence of thyroid hormones on the immune system. In: Hesch RD, editor. Low $T_3$ syndrome. Serono Symphosium N° 40, London: Academic Press, 1981. p. 199–207.

[13] Fabris N, Pierpaoli W, Sorkin E. Hormones and immunological capacity IV: restorative effects of developmental hormones or of lymphocytes on the immunodeficiency syndrome of the dwarf mouse. Clin Exp Immunol 1971;9:227–40.

[14] Genaro AM, Cremaschi GA, Borda ES. Muscarinic cholinergic receptors on murine lymphocyte subpopulations — selective interactions with second messenger response system upon pharmacological stimulation. Immunopharmacology 1993;26:21–9.

[15] Jam D, Heller D, Snapir N. The effect of thyroidal state on the immunological state of the chicken. Dev Comp Immunol 1981;5:483–90.

[16] Johnson HM, Downs MO, Pontzer CH. Neuroendocrine peptide hormone regulation of immunity. In: Blalock JE, editor. Neuroimmunoendocrinology. Chemical Immunology, 52. Basel: Karger, 1992. p. 49–83.

[17] Johnson BE, Marsh JA, King DB, Lillehoj HS, Scanes SG. Effects of $T_3$ on expression of T-cell markers and

immune function in thyroidectomized white Leghorn chickens. Proc Soc Exp Biol Med 1992;199:104–13.

[18] Kim SB, Lee CS. Studies on histological changes in the bursa of Fabricius in chicks treated with thyroxine. II Effect of thyroxine on antibody production. III Electronmicroscopic observations on the bursa of Fabricius after thyroxine or propylthiouracil administration. Korean J Vet Res 1980;20:99–104.

[19] King DB, Scanes CG. Effect of mammalian growth hormone and prolactin on the growth of hypophysectomized chickens. Proc Soc Exp Biol Med 1986;182:201–7.

[20] Marsh JA, Gause WC, Sandhu S, Scanes CG. Enhanced growth and immune development in dwarf chickens treated with mammalian growth hormone and thyroxine. Proc Soc Exp Biol Med 1984;175:351–60.

[21] Marsh JA, Lauterio TJ, Scanes CG. Effects of triiodothyronine treatments on body and organ growth and the development of immune function in dwarf chickens. Proc Soc Exp Biol Med 1984;177:82–91.

[22] Nishida M, Kawada J. Hormonal control of manganese transport in the mouse thyroid. Experientia 1992;48:262–5.

[23] Pierpaoli W, Baroni C, Fabris N, Sorkin E. Hormones and immunological capacity II: Reconstitution of antibody production in hormonally deficient mice by somatotropic hormone, thyrotropic hormone and thyroxine. Immunology 1969;16:217–30.

[24] Savino W, Dardenne M. Immune–neuroendocrine interactions. Immunology Today 1995;16:318–22.

[25] Smith EM, Phan M, Coppenharer D, Kruger TE, Blalock JE. Human lymphocyte production of immunoreactive thyrotropin. Proc Natl Acad Sci USA 1983;80:6010–3.

[26] Waithe WI, Hirschom K. Lymphocyte response to activators. In: Weir DM, editor. Handbook of experimental immunology–cellular immunology, vol. 2. Oxford, London: Blackwell Scientific Publications, 1978 chapter 26.

[27] Tsai JS, Samuels HM. Thyroid hormone action: Demonstration of putative nuclear receptors on human lymphocytes. Clin Endocr Metab 1974;38:919–22.

[28] Lemarchand-Beraud T, Holm AC, Scazzia BR. Triiodothyronine and thyroxine nuclear receptors in lymphocytes from normal, hyper and hypothyroid subjects. Acta Endocr 1977;85:44–54.

[29] Williamson RA, Davison TF, Payne LN. Effects of thyroid hormones on humoral and cell-mediated immune responses in the domestic fowl. Dev Comp Immunol 1990;14:305–18.

[30] Cole NA, Gallaran RH, Rodriguez SL, Purdy CW. Influence of triiodothyronine injections on calf immune response to an infectious bovine rhino tracheitis virus challenge and nitrogen balance of lambs. J Anim Sci 1994;72:1263–73.

[31] Aoki N, Wakisaka G, Nagata I. Effects of thyroxine on T-cell counts and tumor cell rejection in mice. Acta Endocrinol 1976;81:104–9.

[32] Mariotti S, Caturegli P, Barbesino G, Marino M, Del Prete GF, Chiovata L, Tonacchera M, De Carli M, Pinchera A. Thyroid function and thyroid autoimmunity independently modulate serum concentration of soluble interleukin 2 receptor in thyroid diseases. Clin Endocrinol Oxf 1992;37:415–22.