Triiodothyronine Enhances Expression of the Interleukin-2 Receptor Alpha Chain

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Abstract. The effect of thyroid hormone on immune function is unclear. The influence of L-triiodothyronine on expression of the interleukin-2 receptor alpha chain by peripheral blood mononuclear cells from healthy volunteers and YT cells (an interleukin-2 independent natural killer-like cell line) was examined. Concanavalin A stimulation significantly ($p < 0.05$ and $p < 0.01$) increased soluble interleukin-2 receptor alpha chain production when mononuclear cells were cultured with triiodothyronine (1–100 nmol/l) for 3 days. The stimulatory effect of triiodothyronine on interleukin-2 receptor alpha chain expression was greater in the presence of concanavalin A (5 μ g/ml) plus interleukin-2 (1 U/ml) than in the presence of concanavalin A alone. Triiodothyronine also significantly ($p < 0.01$) increased interleukin-2 receptor alpha chain expression when YT cells were cultured for 2 days with interleukin-2 (1 U/ml), but did not influence receptor expression when YT cells were cultured with forskolin or 12-O-tetradecanoyl phorbol 13-acetate, potent activators of signal transduction. In conclusion, triiodothyronine may have an immunomodulatory effect by enhancing expression of the interleukin-2 receptor alpha chain on peripheral blood mononuclear cells in the presence of interleukin-2.

Key words: Interleukin-2 receptor alpha chain, Soluble interleukin-2 receptor, L-triiodothyronine
(*Endocrine Journal* 46: 437–442, 1999)

THYROID hormone has various actions related to the morphogenesis of the central nervous system, amphibian metamorphosis, metabolism, and protein synthesis or degradation [1]. Thyroid hormone also has immunomodulatory effects because hyperthyroidism increases the size of the thymus [2] and changes in thyroid function modify the level of thymulin [3], which is one of the thymic hormones and plays an important role in T cell ontogeny. Moreover, thyroid hormone is reported to influence lymphocyte subsets [4, 5], natural killer cell activity [6], and macrophage inflammatory activity [7]. But because some studies have found no effect on the immune system [8, 9], the immunomodulatory effect of thyroid hormone is still uncertain.

The interleukin-2 (IL-2)/IL-2 receptor (IL-2R) system plays a central role in immune function by participating in the activation of T cells and by stimulating their proliferation [10, 11]. With regard to the influence of thyroid hormone on the IL-2/IL-2R system, T_3 has been shown to enhance IL-2R alpha chain expression by lectin-activated splenocytes from *Xenopus laevis* and Cornell K chickens [12, 13], but does not alter IL-2R alpha chain expression in rats [5].

The soluble IL-2R (sIL-2R) alpha chain is released from receptors on the surface of activated T cells [14] and is a useful marker of the development of various diseases [15–17]. We and other investigators have found a good correlation between the serum thyroid hormone level and the sIL-2R level in patients with untreated Graves' disease [15, 18, 19], and serum sIL-2R is increased by administration of triiodothyronine to healthy volunteers or to patients with Graves' disease in remission [15]. This suggests that thyroid hormone may have an immunomodulatory

Received: October 14, 1998

Accepted: March 12, 1999

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effect by acting through the IL-2/IL-2R system.

It is known that various substances, such as interleukin-1 (IL-1), IL-2, tumor necrosis factor and forskolin, can induce IL-2R alpha chain expression by YT cells [20, 21], a natural killer-like leukemic cell line that constitutively expresses the IL-2R beta chain [22].

The purpose of the present study was to examine whether thyroid hormone could induce IL-2R alpha chain expression by YT cells and by peripheral blood lymphocytes.

Materials and Methods

Chemicals

L-triiodothyronine (T_3), forskolin, 12-O-tetradecanoyl phorbol 13-acetate (TPA), and bovine serum albumin were purchased from Sigma Chemical Co. (St. Louis, MO). RPMI-1640 medium and fetal bovine serum were obtained from Flow Laboratories (Irvine, Scotland). Concanavalin A (Con A) was obtained from Difco Laboratories (Detroit, MI). Ficoll 400 was purchased from Pharmacia (Uppsala, Sweden). ^3H -thymidine (29 Ci/mmol) was obtained from Amersham International plc. (Buckinghamshire, UK). Fetal bovine serum was treated with AGX-8 resin (200–400 mesh, chloride form; Bio-Rad Laboratories, Richmond, CA) by the method of Samuels *et al.* [23]. T_3 was dissolved in 0.1 N NaOH and diluted with culture medium (RPMI-1640 containing 10% resin-treated fetal bovine serum). Forskolin and TPA were dissolved in dimethyl sulfoxide and ethanol, respectively, and were diluted with the culture medium. Recombinant IL-2 was kindly provided by Takeda Chemical Industries Ltd. (Osaka, Japan).

Cell culture

Human peripheral blood mononuclear cells (PBMC) were obtained from the heparinized venous blood of healthy volunteers by Ficoll-Conray (1.077 kg/l) gradient centrifugation at 400 x G for 30 min at 4°C. The leukocyte-enriched interface was washed three times in Hanks' balanced salt solution (pH 7.2) and the cells were resuspended in culture medium at 1×10^6 /ml in 24-well plates (Sumitomo Bakelite Co.,

Ltd., Tokyo, Japan). Culture was performed at 37°C under a humidified atmosphere of 5% CO_2 in air, and the cells were stimulated by Con A and/or IL-2 with or without various concentrations of T_3 (1–100 nmol/l). YT cells are IL-2-independent natural killer-like cells that were established from a patient with thymic lymphoma, and were a kind gift from Dr. Yodoi (Institute for Virus Research, Kyoto University, Kyoto, Japan) [20]. YT cells were maintained in culture medium at 5×10^4 /ml with IL-2, TPA, or forskolin in the presence or absence of T_3 . The healthy volunteers who donated PBMC gave their informed consent before being enrolled in the study.

Proliferation studies

PBMC and YT cells were suspended at 1×10^6 /ml and 5×10^4 /ml, respectively, in culture medium, and equal volumes (200 μl) of each cell suspension were placed in quadruplicate into 96-well culture plates (Becton-Dickinson Labware, Bridgewater, NJ). To assess the proliferative response, 0.5 μCi of ^3H -thymidine was added to each well and incubation was continued for 6 hours. Then the wells were harvested (Lab Mash; Ltd., Tokyo, Japan) onto glass fiber filters and the incorporation of thymidine was determined as counts per minute (cpm) in a liquid scintillation counter (Aloka Co. Ltd., Tokyo, Japan).

Measurement of soluble IL-2 receptor alpha chain in culture supernatants

Soluble IL-2 receptor alpha chain levels were measured by an enzyme-linked immunosorbent assay with a CELLFREE Interleukin-2 Receptor kit (T cell Science Inc., Cambridge, MA), as described previously [15]. The assay employed a primary anti-IL-2R monoclonal antibody adsorbed onto the wells and a horseradish peroxidase-conjugated secondary anti-IL-2R monoclonal antibody. The absorbance was measured at 490 nm.

IL-2 receptor alpha chain detection by flowcytometry

Cells were washed and resuspended in RPMI-1640 supplemented with 10% fetal bovine serum. Then the cells were incubated for 30 min at 4°C with a

fluorescein isothiocyanate-conjugated anti-Tac monoclonal antibody (Coulter Co., Hialeah, FL) under saturating conditions in the presence of a 100-fold excess of human gamma globulin (Organo Teknika Co., West Chester, PA) to block Fc receptors for IgG. After washing in phosphate-buffered saline containing 0.1% bovine serum albumin and sodium azide, all samples were suspended in 200 μ l of the same buffer and 10⁴ cells were analyzed by laser flowcytometry (EPICS Division; Coulter Co., Hialeah, FL). Irrelevant IgG2a antibodies were used as the negative controls with the window set so that less than 5% control cells were positive. Analysis of IL-2R expression was performed by calculating the percentage of IL-2R positive cells in the case of YT cells or by determining the mean fluorescence intensity (MFI) of stained cells in the case of PBMC, because a large number of PBMC became positive.

Statistical analysis

Statistical analysis of the data was performed by the paired *t* test or Wilcoxon test, and *p* < 0.05 was considered to indicate a significant difference. Results are shown as the mean $\pm$ standard deviation.

Results

Soluble IL-2 receptor alpha chain levels in culture supernatants

The sIL-2R level in culture supernatants from Con A-stimulated PBMC increased over time from day 1 to day 5. Addition of T₃ (10 nmol/l) had no effect for the first 2 days of culture, but there was an increase in sIL-2R in the culture supernatant from the 3rd day onward in preliminary experiments. PBMC were therefore cultured for 3 days with various concentrations of T₃ in the presence or absence of Con A in the subsequent experiments. There was a significant increase in sIL-2R when PBMC were cultured in the presence of Con A (5 μ g/ml) and various concentrations of T₃ (1 nmol/l; *p* < 0.05, and 5, 7.5, 10, 100 nmol/l; *p* < 0.01, respectively), and the peak sIL-2R level was reached at a T₃ concentration of 7.5 nmol/l (Fig. 1). Addition of T₃ had no effect on the increase in sIL-2R in the absence of Con A. In addition, sIL-2R was not detected in the culture

Fig. 1. sIL-2R alpha chain levels in culture supernatant. PBMC from normal volunteers were cultured for 3 days with Con A (5 μ g/ml) and T₃ at a various concentrations. paired *t* test; **p* < 0.05 vs. no T₃ (*n* = 6), ***p* < 0.01 vs. no T₃ (*n* = 6)

supernatant when YT cells were incubated with IL-2 alone.

Cell surface IL-2 receptor alpha chain expression by PBMC

We examined cell surface IL-2R expression by direct flowcytometry to determine the mean fluorescence intensity (MFI). IL-2R expression by PBMC cultured with Con A plus T₃ reached a peak at T₃ concentrations from 7.5 to 10 nmol/l. When PBMC were cultured with Con A (5 μ g/ml) plus T₃ (10 nmol/l) for 3 days, the MFI was higher than when PBMC were cultured without T₃, but the increase was not significant (Fig. 2). In cultures containing both Con A (5 μ g/ml) and IL-2 (1 U/ml), however, the increase of IL-2R was significantly (*p* < 0.05) greater in the presence of T₃ than in the absence of T₃ (Fig. 2).

IL-2 receptor alpha chain expression by YT cells

We used YT cells to investigate the mechanism underlying the induction of IL-2R alpha chain expression by T₃. YT cells showed increased IL-2R expression after addition of IL-2 to cultures, and the level of IL-2R expression reached a plateau at IL-2 concentrations > 0.1 U/ml after 48 hours, and it also

Fig. 2. Cell surface IL-2 receptor alpha chain expression by PBMC. PBMC were cultured for 3 days with Con A (5 µg/ml) alone (left panel) or Con A (5 µg/ml) plus IL-2 (1 U/ml) (right panel) in the presence or absence of T₃ (10 nmol/l). Anti-IL-2R binding is shown as the mean fluorescence intensity (MFI) of the entire histogram. Wilcoxon test; * $p < 0.05$ vs. no T₃ (n=6)

Fig. 3. Cell surface IL-2 receptor alpha chain expression by YT cells. Cells were cultured in the presence (closed columns) or absence (open columns) of T₃ (10 nmol/l) with IL-2 (1 U/ml), forskolin (10 nmol/l), TPA (10 ng/ml), or no stimulating agent. paired *t* test; * $p < 0.01$ vs. no T₃ (n=6)

reached a peak in the presence of 10 nmol/l T₃. IL-2R expression was significantly ($p < 0.01$) greater in the presence of T₃ plus IL-2 (1 U/ml) than in cultures with IL-2 alone (Fig. 3). In cultures with TPA (10 ng/ml), approximately 20% of the cells were IL-2R-positive after 24 hours, but the positivity rate decreased to 9% and 4% after 48 and 72 hours, respectively. IL-2R expression was also increased by culture with forskolin (10 µmol/l), being approximately 66 % and 54% after 24 and 48 hours. We examined whether T₃ had an influence on IL-2R expression when added together with TPA, or forskolin, or without any stimulating agent, but found no change in the presence of T₃ (Fig. 3).

Proliferative response to IL-2 and/or Con A

There was no significant change in thymidine incorporation after the addition of T₃ to PBMC stimulated with Con A alone or with Con A plus IL-2, and YT cells stimulated with IL-2 (data not shown).

Discussion

The present study showed that a thyrotoxic state induced by exposure to T₃ resulted in augmentation of IL-2R alpha chain expression by YT cells and Con A-stimulated PBMC cultured in the presence of IL-2. This enhancement of IL-2R alpha chain expression by T₃ reached a peak at T₃ concentrations from 7.5 to 10 nmol/l, corresponding to the serum T₃ concentration range in patients with untreated Graves' disease [15].

There have been several previous reports regarding the influence of thyroid hormone on the immune system [3-7]. T₃ enhances the production and secretion of thymulin, which is a thymic hormone with an important role in T cell development and function [3]. The IL-2/IL-2R system plays a central role in immune function by participating in the activation of T cells and by stimulating their proliferation [10, 11]. Therefore, if thyroid hormone influences IL-2/IL-2R expression, it may modify immune function. But T₃ does not alter IL-2R alpha chain expression in rats [5], and we have also found that cell surface expression of the IL-2R alpha chain was not changed by administration of T₃ alone to

Graves' disease patients in remission (unpublished observation). Of course, these findings were obtained after administration of T₃ alone without any lectin or IL-2. If both IL-2 and T₃ were administered simultaneously, expression of the IL-2R alpha chain might be increased, as occurred in our in vitro experiments or in studies on lectin-activated splenocytes of *Xenopus laevis* and Cornell K chickens [12, 13].

Since the sIL-2R level and IL-2R alpha chain expression were not influenced by T₃ when PBMC were cultured without Con A, or YT cells were cultured without IL-2, this hormone did not have a direct effect on expression of the IL-2R alpha chain, but T₃ seems to act as a copromoter of IL-2R alpha chain expression in the presence of IL-2, because IL-2R alpha chain expression was superinduced by the simultaneous addition of IL-2 and T₃. Con A-stimulated PBMC also contain monocytes, so some interaction between accessory cells and T cells or the products of accessory cells may be related to the augmentation of IL-2R alpha chain expression by T₃. But YT cells exposed to T₃ also showed superinduction of IL-2R alpha chain expression, suggesting that T₃ may be directly involved in the process of the IL-2R alpha chain expression. Forskolin and TPA also induced IL-2R alpha chain expression by YT cells. Forskolin activates adenylate cyclase and TPA activates protein kinase C [24,25], whereas IL-2 activates tyrosine kinase via the IL-2R [26] to enhance IL-2R alpha chain expression. These differences in the signal transduction pathways of IL-2, forskolin and TPA may explain why the effect of T₃ on IL-2R enhancement was only noted in the presence of IL-2.

The high-affinity IL-2R consists of three subunits (the alpha, beta, and gamma chains). Both the beta and gamma chains are responsible for signal transduction and cell growth [10, 11], and the absolute number of high-affinity receptors per cell is limited by the levels of these two chains. As thymidine incor-

poration did not change in the presence or absence of T₃ in our experiments, T₃ may have no influence on the expression of these two chains. This conclusion is compatible with the report that expression of the transferrin receptor, which is induced on cells in the late G1 phase and is essential for DNA synthesis and cell growth [27], is unchanged by the administration of T₃ to rats with hyperthyroidism [5].

Although the IL-2R alpha chain is only a low-affinity receptor itself, it plays an important role in formation of the high-affinity receptor [11]. IL-2R alpha chain overexpression relative to the level of high-affinity receptors may be beneficial for the autocrine system when cells are cultured at low densities [28]. Even if IL-2R alpha chain expression is superinduced by T₃, the high-affinity receptor level may not change at a cell density of 10⁶/ml, as was used in our experiments, but when the IL-2R alpha chain is superinduced in a setting where the autocrine system is active such as in the thyroid gland, formation of high affinity-receptors may show a marked increase.

The abnormalities of lymphocytes infiltrating the thyroid gland in patients with autoimmune thyroiditis are generally thought to result from the primary autoimmune mechanism involved in the pathogenesis of this disease [29]. Nevertheless, our findings indicate that T₃ may modify the immune system by altering IL-2R alpha chain expression, and this suggests that hyperthyroidism may itself be a self-perpetuating factor in the progression of Graves' disease through activation of the IL-2/IL-2R system, although the precise mechanism involved and its role in immune regulation remain unknown.

Acknowledgments

The technical assistance of Mr. Kameda in (flowcytometry) and Mr. Oka (cell culture) is also gratefully acknowledged.

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