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Hormone regulation of murine T cells: potent tissue-specific immunosuppressive effects of thyroxine targeted to gut T cells

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Abstract

Recent studies in athymic mice indicate that the neuroendocrine hormones thyrotropin-releasing hormone (TRH) and thyroid-stimulating hormone (TSH) can significantly influence the development of lymphoid cells associated with intestinal intraepithelial lymphocytes (IEL). In the present study we have examined the effects of those hormones, as well as of thyroxine (T_4), a thyroid-derived hormone regulated by TSH, on IEL development in euthymic mice. As reported here, whereas IEL in euthymic mice were unaffected by TRH and TSH treatment, T_4 administered to adult euthymic mice at 3 or 6 weeks of age caused a dramatic reduction in the numbers of TCRαβ, CD8αβ IEL, i.e. the same subsets previously shown to be up-regulated by TRH and TSH in athymic mice. When given to euthymic mice >8 weeks of age, after TCRαβ and CD8αβ subsets had reached normal levels, T_4 had minimal effect on IEL, suggesting that the mode of action of T_4 is directed to developing but not mature IEL. That possibility was confirmed in experiments in which T_4 treatment of bone marrow radiation chimeras during an active phase of T cell regeneration temporarily halted all IEL development at a stage characteristic of immature IEL. Most interesting, the immunosuppressive effects of T_4 were selectively targeted to the intestinal immune system since T_4 had no effect on developing thymocytes or on mature peripheral T cells, in either normal euthymic mice or during hematopoietic reconstitution of radiation chimeras. These findings have implications for understanding intestinal immunity and disease, including chronic intestinal inflammation, in ways not previously appreciated.

Introduction

Intestinal intraepithelial lymphocytes (IEL) are a diverse population of $CD8^+$ T cells which have unique developmental properties and extensive phenotypic complexity (reviewed in 1). In mice, both TCRαβ and TCRγδ IEL are routinely present, although the proportion of those subsets may very substantially. Interestingly, for reasons that are not yet clear, most $CD8^+$ IEL use a homodimeric CD8αα complex. In contrast, only a minor population of the total IEL express the more typical CD8αβ configuration; those cells are almost exclusively TCRαβ IEL. The reasons for these phenotypic peculiarities remain to be elucidated; however, they may reflect differences in IEL developmental pathways, i.e. thymus-dependent versus thymus-independent lineages (2), or may be linked to alternative channels of signal transduction (3)

Paracrine and autocrine interactions have been demonstrated between hormones and cells of the lymphoid immune system For example, thyrotropin-releasing hormone (TRH) can induce the synthesis of thyroid-stimulating hormone (TSH) from lymphoid cell lines, whereas thyroxine (T_4) inhibits TSH synthesis (4) TSH, in turn, has been shown to augment antibody production by B cells (5). However, the specific involvement of hormones in immune regulation overall has yet to be fully realized Recently, it was demonstrated that TRH and TSH up-regulate the development of certain subsets of IEL in athymic mice (6,7). Based on those studies, we sought to determine what effects TRH, TSH and T_4 have on gut T cells in normal euthymic mice. As reported here, administration of T_4, but not TRH or TSH, had strong tissue-specific immunosuppressive effects targeted to T cells of the intestinal immune system The significance of these findings to the overall immunobiology of gut T cells, and to mechanisms of intestinal inflammation and autoimmunity, is discussed.

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Methods

Mice

BALB/c mice were raised at the University of Tulsa vivarium from breeding stocks obtained from The Jackson Laboratory (Bar Harbor, ME).

Hormones

TRH (*p*-Glu–His–Pro amide) (Sigma, St Louis, MO) and TSH (Sigma, T3538) were used as previously reported (6, 7) Briefly, this consisted of daily i.p injections of 100 μg TRH or TSH for 5 days followed by six injections of 100 μg TRH given every third day thereafter, or injections of 7 μg T_4 (8) (Sigma, T0397) per 20 g adult mouse for 5 days followed by 2 days of rest as described by others. Hormone treatments were given for a total of 3 weeks

Isolation of lymphocytes and flow cytometry

Techniques for isolating IEL used by our laboratory have been reported (9). IEL were isolated and studied from individual mice. Antibodies used were phycoerythrin (PE)–anti-Thy-1 2 (CalTag, San Francisco, CA), biotin–anti-TCRαβ; biotin–anti-TCRγδ (GL3), biotin-labeled and unlabeled anti-CD3; PE-labeled and unlabeled anti-CD4; PE–anti-CD5; FITC-labeled and unlabeled anti-CD8α; PE–anti-CD8β; FITC-labeled anti-HSA; PE-labeled anti-CD44; PE- and FITC-labeled species-specific control anti-body (PharMingen, San Diego, CA; all reagents). For one-color staining, cells were reacted with a biotin-labeled primary or control antibody, followed by streptavidin–PE (Jackson ImmunoResearch, West Grove, PA). For two-color staining, cells were reacted with a PE- and a FITC-labeled antibody, washed, and fixed in 2% formaldehyde, or were reacted with biotin-labeled anti-TCRαβ or anti-TCRγδ antibody plus PE-labeled anti-CD5, washed, reacted with streptavidin–Red-613 (Gibco/BRL, Gaithersburg, MD), washed, fixed and analyzed on an Epics 751 flow cytometer interfaced to an MDADS II computer (Coulter Electronics, Hialeah, FL) calibrated with Immuno-Check EPICS Alignment Fluorospheres for optical alignment. Reactivities of antibodies were done at the 99 9% confidence level using KSTest (Coulter) after gating onto $CD45^+$ lymphoid cells.

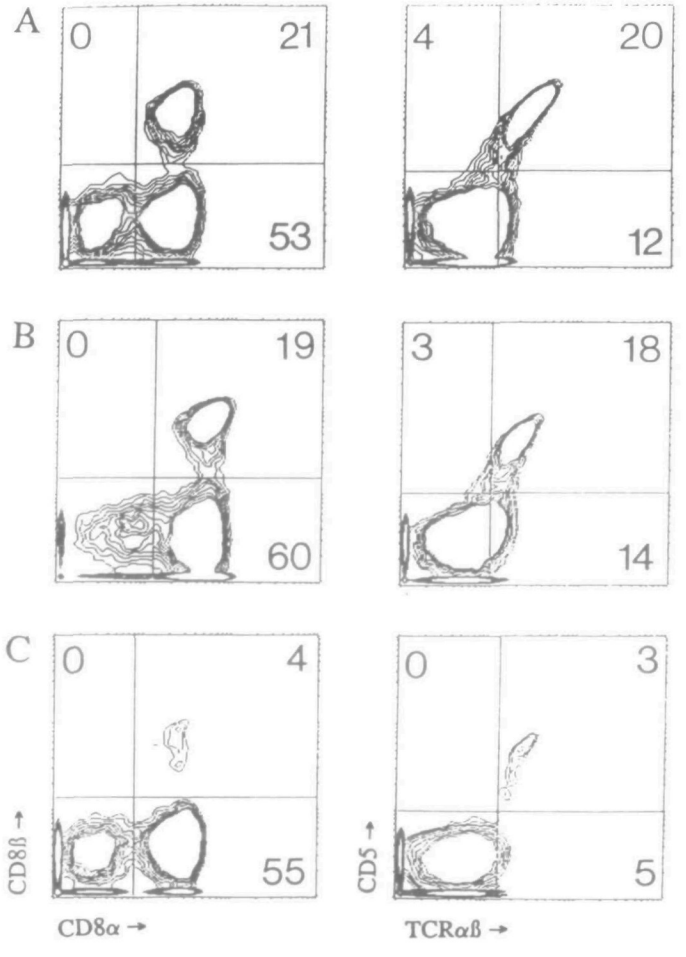

Fig. 1. Expression of CD8α, CD8β, CD5 and TCRαβ on IEL from (A) normal euthymic mice, (B) mice treated with TRH for 3 weeks beginning at 6 weeks of age and (C) mice treated with T_4 for 3 weeks beginning at 6 weeks of age Mice were analyzed at 10 weeks of age.

Radiation chimeras

BALB/c mice, 10–12 weeks old, were irradiated with 9.50 Gy from a ^{137}Cs source and reconstituted with syngeneic bone marrow hematopoietic stem cells depleted of mature T cells by treatment with two rounds of antibody to CD3, CD4 and CD8 plus rabbit complement (Accurate Chemicals, Westbury, NY) At 24 h post-reconstitution, chimeras were started on a regimen of T_4 therapy (described above); control chimeras were left untreated.

Results

In young adult mice, T_4 suppresses the development of $CD8\alpha\beta^+$, $TCR\alpha\beta^+$, $CD5^+$ IEL

Groups of young adult mice (6 weeks old) were given TRH or T_4 for 3 weeks as described (6,8). Lymphocytes from the gut epithelium were isolated from mice at 10 weeks of age, and cells were analyzed for the presence of T cell populations by flow cytometric analyses. Treatment of euthymic mice with TRH (Fig. 1B) had no detectable effect on IEL expressing CD8α, CD8β, TCRαβ or CD5 compared with cells from age-matched control mice (Fig. 1A) or with what has been reported for IEL in mice of that age (1). This is not surprising given that TRH has been shown to up-regulate the development of those IEL subsets; consequently, the effects of TRH on IEL with fully developed T cell populations would be minimal. Most unexpected, however, was the finding that normal euthymic mice treated with T_4 for 3 weeks beginning at 6 weeks of age had greatly reduced numbers of IEL expressing CD8αβ, TCRαβ and CD5 (Fig. 1C). Interestingly, the suppressive effects of T_4 were greatest for the same IEL subsets previously shown to develop in neonatally-thymectomized mice following TRH or TSH treatment (6,7). Other IEL subsets were unchanged in both TRH- and T_4-treated mice (data not shown).

Table 1. T_4, but not TRH or TSH, down-regulates the expression of gut T cell subsets

Marker	Percentage of total IEL[a] by type of treatment (age at start of treatment)						
	Control (6 weeks)	TRH (6 weeks)[b]	TSH (6 weeks)[b]	T_4 (3 weeks)[b]	T_4 (6 weeks)[b]	T_4 (12 weeks)[c]	T_4 (6 weeks)[c]
Thy-1	23 3 ± 4.1	26 1 ± 4.5	28 3 ± 5.2	7 3 ± 0.3[d]	10 5 ± 1.4[d]	31.7 ± 1 8	9 7 ± 1.9[d]
CD5	18.3 ± 3.1	15.6 ± 2 4	16.3 ± 1.9	6.3 ± 1.2[d]	6 6 ± 2.0[d]	23.0 ± 4.0	6 3 ± 0.7[d]
CD8αβ	17 0 ± 2.3	18 5 ± 2 7	16.0 ± 3.8	7.0 ± 0.6[d]	4 8 ± 2.0[d]	21.0 ± 1 4	7.0 ± 0.5[d]
TCRαβ	33.0 ± 3.3	34.0 ± 4 6	29.8 ± 4.8	6.7 ± 1.4[d]	9.4 ± 2.7[d]	38.3 ± 2.8	8.7 ± 0.3[d]
CD8αα	57 3 ± 5.9	57 4 ± 3.7	48 3 ± 4 6	47 4 ± 9.7	55.6 ± 6.1	59.3 ± 6.4	67.0 ± 0.9
TCRγδ	32.0 ± 4.1	33 5 ± 2 9	21.3 ± 3.2	25.6 ± 8.9	23 0 ± 3.8	22.0 ± 4 0	39.3 ± 3.5

[a]Mean values ± SEM of four to seven mice per group analyzed individually
[b]Analyzed at 10 weeks of age
[c]Analyzed at 15 weeks of age
[d]Statistically significant difference ($P < 0.02$) in numbers of IEL compared with control mice, TRH-treated mice, TSH-treated mice or T4-treated mice given hormone at 12 weeks of age using Student's *t*-test for unpaired observations.

T_4-mediated immunosuppression affects developing but not mature IEL and has no effect on T cells outside the intestine

To more thoroughly explore the influence of T_4 on IEL in euthymic mice, groups of mice were treated with T_4, or with TRH or TSH, at various ages. As shown in Table 1, treatment of adult mice with either TRH or TSH had no detectable effect on IEL In contrast, mice treated with T_4 at either 3 or 6 weeks of age had greatly reduced numbers of Thy-1, CD8αβ, TCRαβ and CD5 IEL, though CD8αα and TCRγδ subsets were unaffected. Changes in IEL induced by T_4 treatment at 6 weeks of age persisted several weeks after termination of treatment, as shown by numbers of CD8αβ and TCRαβ IEL that were well below age-matched control mice (Table 1, tested at 15 weeks). Interestingly, unlike mice given T_4 at 3 or 6 weeks of age, changes in IEL subsets were not evident in mice given T_4 after 6 weeks of age. Thus, it appears that the action of T_4 is directed to developing IEL, rather than through the elimination or destruction of mature cells. Note that in mice CD8αβ and TCRαβ IEL do not seed the gut epithelium until ~4–6 weeks of age (10), at the time when the effects of T_4 were most pronounced. In contrast TCRγδ of CD8αα IEL are present the gut epithelium much earlier and would be less affected by T_4 treatment at the times studied in these experiments Unlike the IEL, T_4 treatment did not alter the proportions of thymocytes or lymph node lymphocytes expressing markers common to those tissues compared with non-treated age-matched control mice (Table 2). These findings indicate that the suppressive effects of T_4 are not generalized to all T cells, and are consistent with the overall positive effects reported for thyroid hormones on thymocyte development and peripheral T cell function (8,11–13).

During an active phase of T cell regeneration in bone marrow radiation chimeras, the effects of T_4 are targeted to multiple IEL subsets but not to intrathymic T cells

We sought to determine what effects T_4 would have on the development of IEL subsets other than those described above For these studies, adult radiation chimeras were used since they permit developmental analyses of all IEL subsets, and of lymphocytes in extraintestinal compartments. Adult mice were lethally irradiated and reconstituted with syngeneic bone marrow hematopoietic stem cells. Twenty-four hours later, mice were started on a regimen of T_4 therapy. Three weeks post-reconstitution, at a time of active T cell development in radiation chimeras (14), IEL and thymocytes were collected, stained for expression of T cell markers, and analyzed by flow cytometry. At that time, the numbers of IEL in T_4-treated chimeras were equivalent to non-treated chimeras (Table 3). Most interesting, however, was the finding that the development of IEL in T_4-treated mice was dramatically altered as seen by a lack of markers of mature T cells (Thy-1, CD4, CD5, CD8, TCRαβ and TCRγδ), though nearly all cells expressed heat-stable antigen (Table 3). IEL in age-matched non-treated chimeras, by comparison, expressed markers of mature T cells (Table 3) and were similar to IEL described elsewhere for radiation chimeras of that age (14).

Table 2. Flow cytometric analyses of thymocytes and lymph node cells from T_4-treated and non-treated control mice

Lymphoid cells	Subset	Percentage of total cells[a] by treatment group	
		Control[b]	T_4-treated[c]
Thymocytes	$CD4^+CD8^+$	79.3 ± 1.2	77 2 ± 2.6[d]
	$CD4^+CD8^-$	8.3 ± 1 0	9 0 ± 0 8[d]
	$CD4^-CD8^+$	5.2 ± 0 9	4.8 ± 0 5[d]
	CD3	16 7 ± 0 8	17 0 ± 1.0[d]
	TCRαβ	11 7 ± 0.7	13 2 ± 1 1[d]
Lymph node cells	Thy-1	62.1 ± 2 0	65.2 ± 2 5[d]
	sIg^+	18 3 ± 1.1	16 0 ± 1.4[d]
	$CD4^+CD8^-$	51.1 ± 1.5	50.7 ± 1 7[d]
	$CD4^-CD8^+$	15.3 ± 0.9	16 6 ± 1.4[d]
	CD8αβ	14.7 ± 0.8	15.5 ± 1.3[d]
	CD3	63 4 ± 2.4	65 8 ± 3.1[d]
	TCRαβ	60.2 ± 2.1	63.4 ± 3.0[d]

[a]Mean values ± SEM of four mice per group tested individually
[b]Untreated age-matched BALB/c mice.
[c]BALB/c mice treated with T_4 for 3 weeks beginning at 6 weeks of age
[d]Not statistically significant ($P > 0$ 05) compared with control mice using Student's *t*-test for unpaired observations

Table 3. Analyses of IEL and thymocytes in T_4-treated and non-treated radiation chimeras 3 weeks post-bone marrow reconstitution

Marker	Percentage of total cells[a]			
	IEL		Thymocytes	
	Non-treated (2.1 ± 1.8)[b]	T_4-treated (2.3 ± 1.2)	Non-treated (81 3 ± 13 4)	T_4-treated (76.6 ± 15.3)
Thy-1	40 7 ± 6.5	2.3 ± 1 8[d]	89.4 ± 4.2	91 3 ± 2.9[e]
CD4	18 0 ± 3 8	1 3 ± 1.4[d]	10.2 ± 1.3	9.3 ± 0.8[e]
CD5	30.3 ± 5.6	2.7 ± 0.3[d]	68.7 ± 5 8	70 6 ± 4 4[e]
CD8α[c]	49 6 ± 3 9	3.0 ± 0 5[d]	5.8 ± 2 1	3.3 ± 0.9[e]
TCRαβ	35 3 ± 2 3	2 3 ± 0 5[d]	10.3 ± 0.6	12.0 ± 0.5[e]
TCRγδ	20.0 ± 1.7	1.3 ± 0.4[d]	0.4 ± 0 3	0.6 ± 0 6[e]
HSA	10.3 ± 6.4	95.0 ± 2.1[d]	ND	ND
$CD4^+8^+$	1 0 ± 0 4	0 ± 0	76 3 ± 4.7	79.6 ± 3 0[e]
sIg	0 ± 0	0 ± 0	0 ± 0	0 ± 0

[a]Mean values ± SEM of three mice per group tested individually
[b]Mean values ± SEM of number of cells recovered per animal ($\times 10^6$).
[c]Includes both CD8αα and CD8αβ IEL
[d]Statistically significant difference ($P < 0.001$) compared with IEL from untreated mice using Student's *t*-test for unpaired observations.
[e]Not statistically significant compared with thymocytes from untreated animals

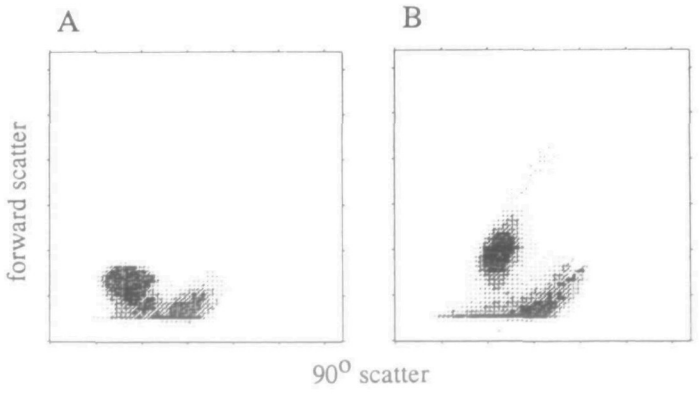

Fig. 2. Characteristics of IEL from (A) T_4-treated bone marrow radiation chimeras or (B) non-treated radiation chimeras according to cell size (forward angle scatter) and granularity (90° scatter).

Moreover, based on properties of cell size (forward scatter) and granularity (90° scatter), IEL in T_4-treated chimeras were smaller and less granular (Fig. 2A) than IEL in non-treated chimeras (Fig. 2B). This is an important distinction since it suggests that IEL in T_4-treated mice are similar to a population of very early IEL precursors, but that they are present much later than normally found in radiation chimeras after hematopoietic reconstitution (15). The tissue-specific effect of T_4 treatment on IEL was evident by the fact that thymocytes in T_4-treated radiation chimeras were phenotypically normal and were present in numbers equivalent to non-T_4-treated chimeras (Table 3). By 4–6 weeks post-reconstitution, differentiated IEL were present despite hormone treatment (data not shown). This may have been due to the increased demand for immune regeneration in newly constructed radiation chimeras or may represent an insufficient dose of T_4 to permanently down-regulate the development of all IEL.

Discussion

The findings reported here, coupled with previous observations linking TRH and TSH to IEL development (6,7), provide strong evidence that the composition and distribution of gut T cells in mice are regulated by neuroendocrine hormones, and indicate that T_4 has potent immunosuppressive effects in that process. Why the immunomodulating effects of T_4 are selectively targeted to the intestinal immune system are not directly evident. However, T_4 has been shown to have broad effects on tissues of the immune system (8,12,13). Within the gut this may reflect variations in the expression of hormone receptors (16) on lymphoid cells (J. Wang *et al.*, unpublished data), differential regulation of lymphocyte-derived hormones associated with the hypothalamus–pituitary–lymphoid axis (4) or be determined by hormone-mediated changes in gut epithelial architecture and/or physiology (17,18). Moreover, it has recently been shown that γδ T cells in the gut epithelium directly influence the growth and development of enterocytes (19), indicating intimate interactions between lymphoid cells and non-hematopoietic cells for the maintenance of intestinal homeostasis. Yet, regardless of the source of T_4, experiments in both euthymic mice and radiation chimeras are consistent with the likelihood that T_4-mediated suppression occurs by halting local maturation of IEL at the precursor cell stage, and that those effects of T_4 are minimal for fully differentiated IEL.

Although T_4 did not measurably alter IEL in older adult mice (see Table 1, T_4-treated mice, 12 weeks), it is possible that suppression of IEL development occurred in those animals as well, but that the effects were masked by the large numbers of mature cells. Clearly, extensive studies will be required to determine the effects of hormones on the development of IEL in those mice. These findings, therefore, raise the novel prospect that hormone control of IEL development occurs locally after precursors have arrived in the gut epithelium, thereby creating a mechanism for differentially regulating IEL throughout the intestine. An obvious advantage of hormone regulation of IEL is the rapid response time for T cell activation and/or suppression. Such expeditious responses would be of particular importance within the gut given the fluctuations in antigen burden compared with most other lymphoid tissues.

Finally, numerous recent studies indicate that the intestinal immune system is a developmentally distinct lymphoid tissue (1,10). The findings reported here, coupled with previous observations (6,7), expand that likelihood by demonstrating that control of the intestinal immune system involves factors which extend beyond classical pathways of immunoregulation. Understanding those complex regulatory processes will need to be addressed in order to gain a comprehensive understanding of intestinal immunobiology. The immunomodulating effects of T_4, and possibly other hormones, now can be explored in experiments aimed at controlling inflammatory responses within the gastrointestinal tract, and for understanding events which govern normal intestinal immunity.

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Abbreviations

IEL	intestinal intraepithelial lymphocytes
PE	phycoerythrin
T_4	thyroxine
TRH	thyrotropin-releasing hormone
TSH	thyroid-stimulating hormone

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