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Effects of T3Rα1 and T3Rα2 Gene Deletion on T and B Lymphocyte Development[1]

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Thyroid hormones bind to several nuclear receptors encoded by T3Rα and T3Rβ genes. There is now accumulating evidence that thyroid hormones act on the immune system. Indeed, mice deficient for thyroid hormones show a reduction in lymphocyte production. However, the mechanisms involved and, in particular, the role of the different thyroid hormone receptors in lymphocyte development have not been investigated. To address that question, we have studied lymphocyte development in mice deficient for the T3Rα1 and T3Rα2 gene products. A strong decrease in spleen cell numbers was found compared with wild-type littermates, B lymphocytes being more severely affected than T lymphocytes. A significant decrease in splenic macrophage and granulocyte numbers was also found. In bone marrow, a reduction in CD45$^+$/IgM$^-$ pro/pre-B cell numbers was found in these mice compared with wild-type littermates. This decrease seems to result from a proliferation defect, as CD45$^+$/IgM$^-$ cells incorporate less 5-bromo-2′-deoxyuridine in vivo. To define the origin of the bone marrow development defect, chimeric animals between T3R$\alpha^{-/-}$ and Rag1$^{-/-}$ mice were generated. Results indicate that for B cells the control of the population size by T3Rα1 and T3Rα2 is intrinsic. Altogether, these results show that T3Rα1 or T3Rα2 gene products are implicated in the control of the B cell pool size. ***The Journal of Immunology*****, 2000, 164: 152–160.**

The role of thyroid hormones in the general development of organisms has been known for a long time. There are also increasing data supporting reciprocal interactions between thyroid hormones and the immune system (1). These interactions have been revealed by experimentally induced fluctuations of thyroid hormones in a number of species, including amphibians (2), avians (3), and rodents (4).

The influence of thyroid hormones on the immune system has been extensively studied in mice deficient for hormones of the pituitary/thyroid axis. Snell dwarf (*dw/dw*) mice are deficient in anterior pituitary hormones, i.e., growth hormone (GH),[4] prolactin, and thyroid-stimulating hormone (TSH), due to a point mutation in the gene encoding the *Pit-1* transcription factor (5). Lack of GH and TSH production subsequently causes a drop in seric concentrations of insulin-like growth-factor-I (IGF-I) (6) and thyroid hormones, respectively. The numerous studies of the immune system in Snell dwarf mice have revealed a role for thyroid hormones in B and T cell development (7–13). For example, an increase in thymocyte cellularity is induced by exogenously administered thyroxine (T_4) in Snell dwarf mice, and this effect has been confirmed in the thyroid hormone deficient *hyt*/*hyt* strain of mice (13) and in normal mice (4). For the B cell lineage, there is a drop in cell numbers at the pro/pre-B cell stage (10, 12, 13), which could result from a proliferation or differentiation defect.

Deficient thyroid hormone production seems the main factor responsible for impaired B cell development in Snell dwarf mice. Indeed, pre-B cell numbers reach normal levels after thyroxine injections, while they are not restored by prolactin, GH, or IGF-I treatment (12, 14). The latter findings are consistent with the observation that B cell development occurs normally in IGF-I, GH, and prolactin-deficient mice (13, 14). The predominant effect of thyroid hormones on B cell development was confirmed by the recovery of the pre-B cell compartment in the *hyt/hyt* thyroid hormone-deficient mice following T_4 injections (13).

In these studies, the target of thyroid hormones was not identified, i.e., it is still unclear whether they regulate the size of the B cell pool by acting directly on B cell progenitors or by optimizing the cellular environment necessary for their development. In addition, the thyroid hormone receptors that influence lymphoid development have not been identified. The thyroid gland produces T_4 that is metabolized by thyroxine 5′-deiodinases to its active form: triiodo-thyronine (T_3) (15, 16). T_3 binds to several nuclear receptors encoded by the T3Rα and T3Rβ loci (17, 18). T3Rα (19)-, T3Rβ (20)-, and T3Rα1 (21)-deficient mice have recently been generated by targeted gene disruption. The T3Rα KO mice are deficient for the T3Rα1 and T3Rα2 isoforms (19). However, they are still able to express the T3R$\Delta\alpha$1 and T3R$\Delta\alpha$2 short transcripts (22), which function is unknown. These mice have a general growth defect and die between 4–6 wk of age. A number of defaults, including bone and small intestine development, have been investigated (19).

We have studied the development of lymphocytes in these T3Rα KO mice. We have found that the size of the mature B cell

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[4] Abbreviations used in this paper: GH, growth hormone; BrdU, 5-bromo-2′-deoxyuridine; IGF, insulin-like growth factor; KO, knockout; T_3, triiodo-thyronine; T_4, thyroxine; TSH, thyroid-stimulating hormone.

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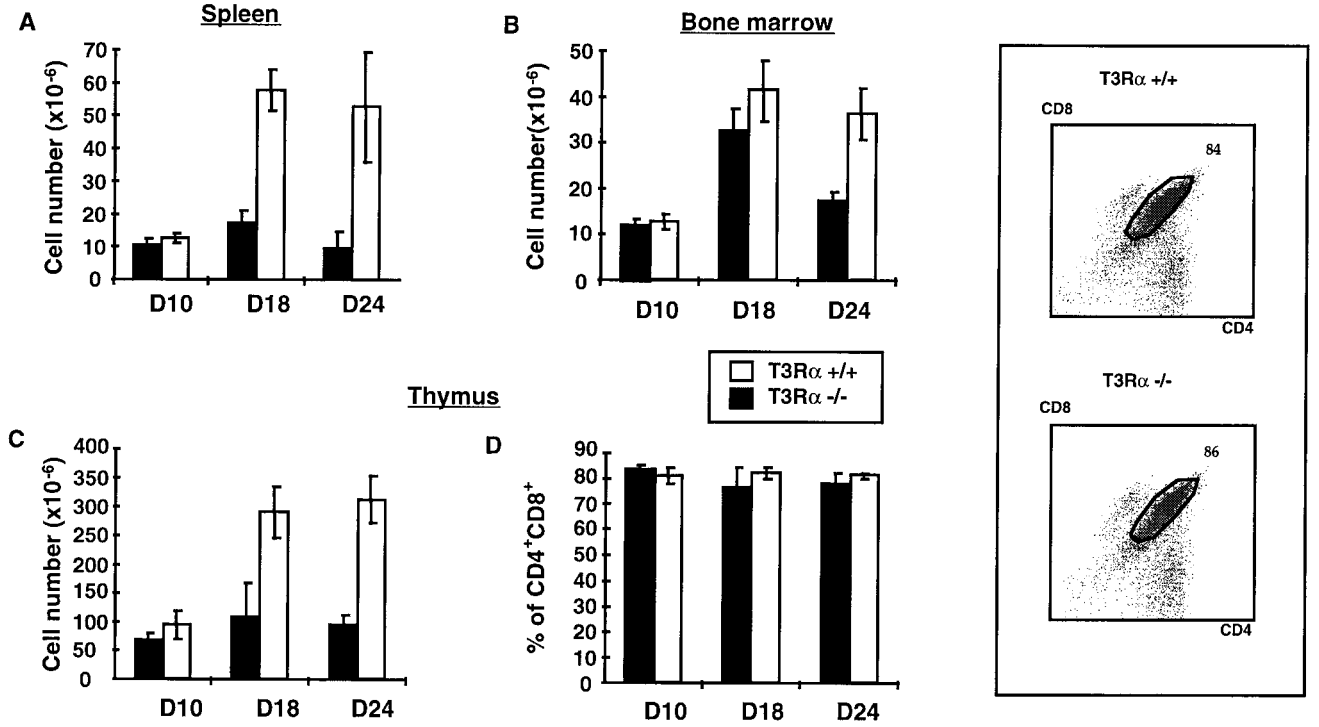

FIGURE 1. Lymphocyte numbers in T3Rα KO mice. The numbers of splenocytes (*A*), bone marrow cells (*B*), and thymocytes (*C*), and the percentages of $CD4^+CD8^+$ double-positive thymocytes (*D*) in 10-, 18-, and 24-day-old T3Rα KO (■) and wild-type (□) mice are shown. *Inset*, An example of double anti-CD4/anti-CD8 staining of thymocytes from 18-day-old T3R$\alpha^{+/+}$ (*top*) and T3R$\alpha^{-/-}$ (*bottom*) mice is shown. The figures represent the percentages of gated double-positive thymocytes.

pool is particularly affected. This default was at least partly due to a lack of B cell progenitor proliferation. Finally, we show that intrinsic expression of T3Rα1 or T3Rα2 gene products is needed for the development of a normal B cell pool.

Materials and Methods

Mice

$Rag1^{-/-}$ mice were purchased from Transgenic Alliance (l'Arbresle, France). T3Rα KO mice, developed on the outbred OF_1 background, were generated in our laboratory (19). All mice were bred in the institute's animal facility and kept under pathogen-free conditions. As homozygous T3Rα KO mice die between 4 and 6 wk of age, heterozygous animals were routinely crossed to generate the T3R$\alpha^{-/-}$ and T3R$\alpha^{+/+}$ littermates used in the experiments. The genotype of the offspring was analyzed by PCR, using a mixture of oligonucleotides specific for T3Rα (A and B) and a lacZ oligonucleotide (lacZ-A5) specific for the targeting vector. A PCR product is amplified with the oligonucleotides A and B when the wild-type allele is present, and with the oligonucleotides A and lacZ-A5 when the gene is disrupted (A, 5′-GGACAAGTCTCTGAAGACTTCC-3′; B, 5′-GTCT GACCCACACTCCACCTTG-3′; lacZ A5, 5′-CCTCTTCGCTATTACGC CAGCTGG-3′). The amplification conditions used were 20 s at each of the following temperatures: 94°C, 58°C, and 72°C, for 32 cycles.

Fluorescence staining and flow cytometry sorting

For surface marker analysis, spleen cells were stained as previously described (23). The following Abs were used: 2B8-biotin (anti-CD117 (c-*kit*)), anti-CD4-PE, and anti-CD8α-Tricolor from Caltag Laboratories (Burlingame, CA); R6-60.2-biotin (anti-IgM), M1/70-biotin (anti-CD11b/Mac-1), S7-biotin (anti-CD43), 7D4-FITC (anti-CD25), and RB6-8C5-biotin (anti-Ly-6G/Gr-1) from Becton Dickinson (Le Pont de Claix, France); RA3 6B2-PE (anti-B220) from Sigma Immunochemicals (St. Quentin Fallavier, France); 145.2C11-FITC (anti-CD3) and M1.93-biotin (anti-CD45) were prepared in house. Avidin-Tricolor (Caltag Laboratories) was used to reveal biotin-conjugated Abs.

BrdU labeling and staining were performed as previously reported (23). Briefly, mice were injected twice i.p. with 1 mg BrdU (Sigma-Aldrich Chimie, St. Quentin Fallavier, France) at a 30-min interval. Six hours later, animals were sacrificed and the lymphocytes were surface stained. Cells were then fixed and permeabilized, and after partial DNA digestion, BrdU incorporation was revealed by a FITC-conjugated anti-BrdU Ab from Becton Dickinson (Le Pont de Claix, France; clone B44).

Cells were analyzed using the FACScan device (Becton Dickinson, Mountain View, CA) and the CellQuest software (Becton Dickinson Immunocytometry Systems, San Jose, CA). For cell cycle analysis, cells were surface stained and then fixed in 70% cold ethanol. Before analysis, cells were washed in PBS and incubated for 30 min to 2 h in 0.5 μg/ml Hoechst 33342 (Molecular Probes, Leiden, The Netherlands). Cells were analyzed in the presence of the die on a FACStar^{Plus} (Becton Dickinson) equipped with an UV argon laser.

Hormone assays

Cortisol levels in sera were assayed by a private laboratory (Laboratoire d'Analyses Médicales Collombel-Orfeuvre, France) using an enzyme-linked fluorescent assay (ELFA).

Generation of chimeric animals by morulae aggregation

$Rag1^{-/-}$ and T3R$\alpha^{+/-}$ animals were crossed independently. On 2.5 days postcoitum, mice were sacrificed, oviducts were dissected out, and morulae were isolated. The zona pellucida was removed from morulae by acid Tyrode's solution. Morulae from the $Rag1^{-/-}$ cross were aggregated to the morulae from the T3R$\alpha^{+/-}$, as previously described (24). After overnight in vitro expansion, the resulting blastocysts were transferred to the uteruses of 2.5-day postcoitum pseudo-pregnant females. For each offspring, the T3Rα genotype of the morulae derived from the T3R$\alpha^{+/-}$ cross was determined on mature lymphocytes. Indeed, as mature lymphocytes cannot be derived from the $Rag1^{-/-}$ morulae, this cellular subset is not chimeric. The degree of chimerism was estimated by measuring the ratio between PCR products corresponding to the wild-type and targeted T3Rα gene locus, amplified from hepatocytes.

Results

T3Rα KO mice show a defect in lymphoid organ cell numbers

To assess the effect of T3Rα1 and T3Rα2 deletion on lymphocyte development, we first determined cell numbers in primary (thymus and bone marrow) and secondary (spleen) lymphoid organs of T3Rα KO mice, at different times following birth. As shown in

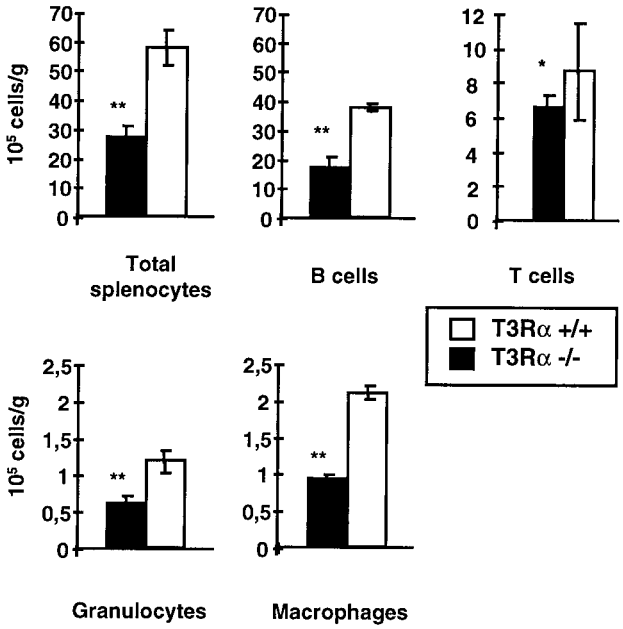

FIGURE 2. Splenocyte subpopulations in T3Rα KO mice. Splenocytes from 17- to 19-day-old mice were counted and stained with anti-B220 and anti-CD3 Abs to identify B and T lymphocytes, respectively. Granulocytes and macrophages were identified by anti-Ly-6G and anti-CD11b Abs, respectively. The cellularity index was calculated as the number of cells per organ divided by the weight of T3Rα$^{-/-}$ (■) or wild-type control (□) animals. The cellularity index values are expressed as 10^5 cells/g. *, $p < 0.1$; **, $p < 0.01$ (Student's *t* test).

Fig. 1, at 10 days of age, cell numbers of T3Rα KO mice are not significantly different when compared with wild-type littermates. In contrast, by day 18 there is a sharp difference in thymocyte and splenic lymphocyte numbers in T3Rα KO compared with wild-type animals, while bone marrow cell numbers remain equivalent in both types of mice. By day 24, cell numbers in all three lymphoid organs are drastically reduced in KO mice. This defect is further increased at later time points (data not shown).

However, T3Rα KO mice also suffer from stress-inducing defects, including decreased nutritional functions due to a delayed maturation of the small intestine (19). The stress response influences the immune system, notably through glucocorticoid production, which down-regulates immune processes and induces apoptosis of lymphoid cells (25). In particular, the percentage of double-positive thymocytes is a good indicator of stress, as these cells are the most sensitive to glucocorticoid-induced depletion (26). Hence, to assess stress levels, we have measured cortisol levels in the blood of mice aged 18 or 32 days. At 18 days of age, there is no significant difference in the levels of cortisol measured in T3Rα KO and wild-type mice (28 ± 8 nM vs 21 ± 1 nM). However, by day 32, T3Rα KO mice show a sharp rise in their levels of circulating cortisol compared with wild-type mice (76 ± 25 nM vs 28 ± 12 nM). Moreover, we have found that the CD4$^+$CD8$^+$ double-positive subset of thymocytes in T3Rα KO mice is not significantly affected up to day 24 of age (Fig. 1), confirming the absence of major stress-induced modifications of the immune system during early life in these mice.

Therefore, to study the impact of T3Rα1 and T3Rα2 deletion on the immune system without potential interference of the stress response, we have performed all further analyses on 17- to 19-day-old animals. Moreover, in all experiments, the percentage of double-positive thymocytes was measured, and the few mice (less than 3%) showing a preferential deletion of double-positive thymocytes, i.e., mice in which the double-positive subset represented less than 80% of total thymocytes, were not included in the analysis.

Finally, as T3Rα KO mice are smaller than wild-type littermates (19), cellularity in lymphoid organs was normalized by calculating a cellularity index, which corresponds to the total cell number divided by the weight of the animal (27).

Splenic B lymphocytes are severely affected by T3Rα1 and T3Rα2 deletion

To further characterize splenic subpopulations affected in T3Rα KO mice, splenocytes were stained with anti-CD45R (B220), CD3, CD4, and CD8 Abs. As shown in Fig. 2, both B and T lymphocyte numbers were decreased in spleens from T3Rα KO animals. However, if T lymphocyte cellularity was reproducibly decreased by about 30% as compared with the control littermates (Table I), the small number of animals analyzed in each group and the wide variability among them led to a poorly significant difference between deficient and wild-type animals in each experiment ($0.05 < p < 0.1$; Student's *t* test, Fig. 2). It is noteworthy that the numbers of CD4$^+$ and CD8$^+$ T lymphocytes were reproducibly reduced to the same extent (data not shown).

In contrast, in all experiments, splenic B (i.e., B220$^+$) lymphocyte cellularity was significantly ($p < 0.005$; Student's *t* test) and reproducibly (Table I) decreased by about 60% in T3Rα-deficient mice compared with wild-type littermates (Fig. 2). Similar results were obtained when IgM$^+$ splenic cells were measured, indicating that all B cell subsets were affected in the periphery (data not shown). Thus, T3Rα1 and T3Rα2 gene deletion affects splenic B cells more profoundly than splenic T lymphocytes.

As shown in Fig. 2, the numbers of splenic granulocytes and macrophages, identified by anti-Ly-6G (Gr-1$^+$) and anti-CD11b

Table I. *In spleen T3Rα1 and T3Rα2 deletion affects more profoundly B than T lymphocytes*[a]

		Total Splenocytes		B Lymphocytes		T Lymphocytes	
Expt.	*n* (KO; wt)	Cell index (KO; wt)[b]	Ratio, KO/wt (%)[c]	Cell index (KO; wt)[b]	*Ratio, KO/wt (%)[c]	Cell index (KO; wt)[b]	Ratio, KO/wt (%)[c]
1	(4; 1)	(33; 84)	39	(18; 58)	31	(11; 17)	68
2	(3; 3)	(27; 58)	47	(17; 38)	45	(7; 9)	75
3	(2; 1)	(30; 60)	49	(18; 42)	43	(9; 13)	66
4	(2; 2)	(20; 43)	48	(12; 33)	37	(6; 7)	81

[a] Seventeen- to 19-day-old T3Rα KO (KO) and wild-type (wt) mice were sacrificed. Splenocytes were counted and stained with anti-B220 and anti-CD3 to detect B and T lymphocytes, respectively.

[b] Cellularity index was calculated as the number of cells per organ divided by the weight of the animals. The values are expressed as 10^5 cells/g.

[c] For each experiment the percentage shown corresponds to: [(mean of cellular indices in KO mice/mean of cellular indices in wild-type mice)] × 100.

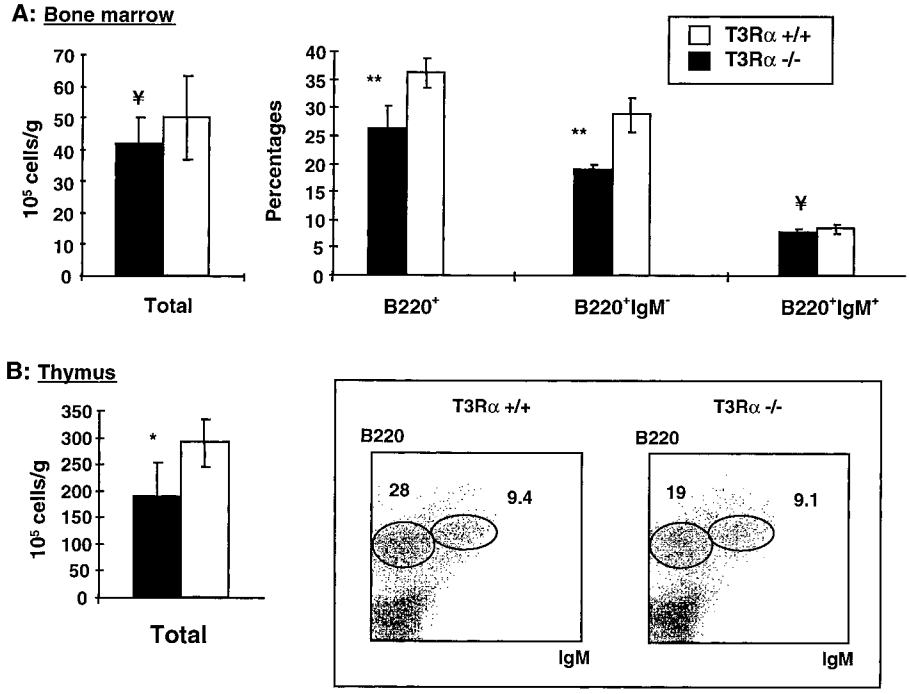

FIGURE 3. Lymphoid development in T3Rα KO mice. Bone marrow nucleated cells (*A*) and thymocytes (*B*) obtained from 17- to 19-day-old mice were counted and stained. *Inset*, An example of double anti-B220/anti-IgM staining of bone marrow nucleated cells from T3R$\alpha^{+/+}$ (*left*) and T3R$\alpha^{-/-}$ (*right*) mice is shown. The figures in the *upper left* and *right corners* represent the percentages of $B220^+IgM^-$ and $B220^+IgM^+$ bone marrow cells, respectively. The cellularity index was calculated as the number of cells per organ divided by the weight of the animals. The cellularity index values are expressed as 10^5 cells/g. Results for T3R$\alpha^{-/-}$ (■) or wild-type control (□) mice are shown. *A*, Bone marrow lymphocytes were double stained for B220 and IgM expression and analyzed on FACS. As T3Rα KO and wild-type animals show no significant differences in bone marrow cellularity indices, the size of the different bone marrow subsets is expressed as a percentage of total cell numbers. ¥, $p > 0.2$; ∗, $p < 0.1$; ∗∗, $p < 0.01$ (Student's *t* test).

(Mac-1^+) Abs, respectively, were also significantly reduced by about 50% in T3Rα-deficient mice compared with wild-type aged-matched control animals.

T3Rα1 and T3Rα2 deletion affects lymphocytes in primary lymphoid organs

As T3Rα1 and T3Rα2 gene deletion affects both B and T lymphocyte numbers in the periphery, we questioned whether they developed normally in primary lymphoid organs. As shown in Fig. 3*A*, the total number of cells in bone marrow, when corrected for the weights of mice, was not significantly different in wild-type and T3Rα KO mice. However, the numbers and percentages of B lineage ($B220^+$) bone marrow cells were significantly (Fig. 3*A*) and reproducibly (Table II) reduced by about 20% in T3Rα-deficient animals. This decrease in the number of B lymphocyte precursors preferentially affects the pro/pre-B cell ($B220^+IgM^-$) stage (Fig. 3*A*, Table II). Immature/mature ($B220^+IgM^+$) B cell numbers were not reproducibly reduced in bone marrow of T3Rα KO mice (Table II). To further pinpoint the B cell progenitor defect, we have used anti-CD43, CD25, and CD117 (c-*kit*) Abs to look at the different progenitor B cell subsets, as defined by A. Rolink and colleagues (28). Results in Table III show that only the $B220^+CD25^+$ pre-BII cells were significantly decreased in T3Rα KO animals. When cells were analyzed according to their size, we found that only the small $B220^+CD25^+$ pre-BII cells were affected (Table III).

As observed for splenic T cells, T3Rα1 and T3Rα2 gene deletion reproducibly (Table II) induces a slightly significant ($0.025 < p < 0.05$; Student's *t* test, Fig. 3*B*) decrease of about 30% in thymocyte numbers. Interestingly, thymic subpopulations were equally affected as the percentages of $CD4^-CD8^-$, $CD4^+CD8^+$, $CD4^+CD8^-$, and $CD4^-CD8^+$ thymocytes were not modified in 17- to 19-day-old T3Rα KO animals (data not shown).

Lymphocyte precursors from T3Rα KO mice proliferate less in primary lymphoid organs

To determine whether the reduction in lymphocyte numbers in both primary and secondary lymphoid organs of T3Rα KO mice was due to a decreased proliferative capacity of these cells, we have measured their proliferation in vivo. For that purpose, T3Rα

Table II. *The size of the $B220^+IgM^-$ pre/pro B cells subset is decreased in T3Rα KO mice*[a]

		Thymus		Bone Marrow							
						% $B220^+$		% $B220^+IgM^-$		% $B220^+IgM^+$	
Expt.	*n* (KO; wt)	Cell Index (KO; wt)[a]	Ratio, KO/wt (%)[b]	Cell index (KO; wt)[a]	Ratio, KO/wt (%)[b]	(KO; wt)[c]	Ratio, KO/wt (%)[d]	(KO; wt)[c]	Ratio, KO/wt (%)[d]	(KO; wt)[c]	Ratio, KO/wt (%)[d]
1	(3; 3)	(19; 29)	66	(43; 41)	104	(24; 33)	74	(17; 23)	72	(8; 10)	79
2	(3; 3)	(15; 24)	64	(42; 36)	116	(26; 38)	70	(19; 31)	62	(8; 9)	79
3	(2; 2)	(20; 38)	53	(46; 34)	136	(45; 54)	84	(28; 33)	83	(18; 19)	97
4	(3; 3)	(24; 34)	69	(42; 41)	102	(34; 47)	72	(19; 28)	68	(13; 19)	69
5	(3; 3)	ND	ND	ND	ND	(37; 42)	89	(23; 30)	77	(14; 12)	121
6	(3; 3)	ND	ND	ND	ND	(39; 45)	86	(19; 27)	71	(20; 19)	104

[a] Mononucleated cells were counted and cellularity index was calculated as the number of cells per organ divided by the weight of the animals. Values are expressed as 10^5 cells/g.
[b] The percentage shown corresponds to: [(mean of cellular indices in KO mice/mean of cellular indices in wild-type (wt) mice)] × 100.
[c] Bone marrow cells were stained with anti-B220 and anti-IgM Abs, and the percentages of each subset was determined using flow cytometry.
[d] The percentage shown corresponds to: [(mean values for KO mice/mean values for wt mice)] × 100.

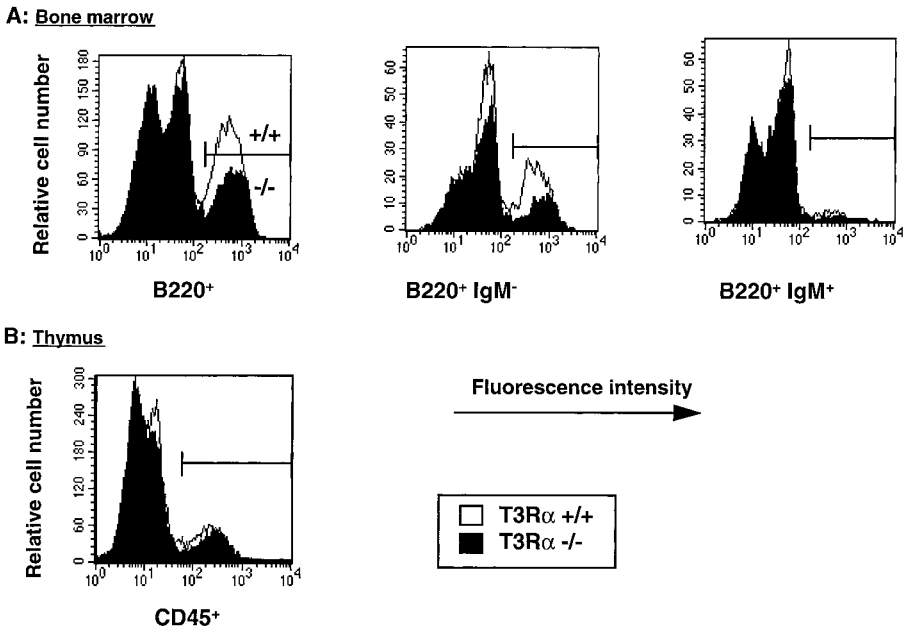

FIGURE 4. In vivo proliferation of bone marrow cells and thymocytes from T3Rα KO mice. T3Rα KO (filled histograms) or wild-type (open histograms) mice (17–19 days old) were injected twice with 1 mg BrdU at a 30-min interval. Six hours later, mice were sacrificed, and bone marrow (*A*) and thymic (*B*) cells were surface stained for B220 and IgM, or CD45 expression, respectively. BrdU-positive cells were then revealed, as described in *Materials and Methods*. Results for one representative experiment of three are shown.

KO mice and wild-type littermates were injected twice i.p. at a 30-min interval with 1 mg BrdU. This thymidine analogue is incorporated in the DNA of cycling cells. Incorporation of BrdU in bone marrow and thymic cells was measured 6 h after the last injection. Cells were first stained for surface markers to identify lymphocyte subsets. $BrdU^+$ cells were then revealed by a FITC-conjugated anti-BrdU Ab, as described in *Materials and Methods*. PBS-injected animals were used as a negative control for background staining.

As shown in Fig. 4*A* and Table IV, the proportion of bone marrow $B220^+$ cycling cells is decreased by 40% in T3Rα KO mice. When looking at bone marrow subpopulations, as expected (29, 30), most of the cycling B lymphocyte precursors were contained in the pro/pre-B cell subset (Table IV). However, in T3Rα KO animals, the percentage of $BrdU^+$ cells was decreased in both the pro/pre-B cell ($B220^+IgM^-$) and the immature/mature B cell ($B220^+IgM^+$) subset by 30% and 50%, respectively, compared with wild-type controls.

To determine which subsets among pro/pre-B cells were affected in their cycling capacity, we have performed cell cycle analysis using Hoechst 33342 labeling on cells that were stained for B220, IgM, CD25, CD43, or c-*kit*. In T3Rα KO mice, a small (15%) but significant ($p \sim 0.05$) reduction in the percentage of cycling cells was observed among all B cell progenitor subsets defined by these markers (Fig. 5, data not shown). Thus, T3Rα1 and T3Rα2 gene deletion affects the proliferation of all subsets of progenitor B cells. This effect was particularly obvious in large $B220^+CD25^+$ pre-BII cells, which represent the major subset of cycling cells (28) (Fig. 5*B*). This reduced proliferation might explain the defect in the number of small $B220^+CD25^+$ pre-BII cells observed in T3Rα KO mice.

Thymocytes from T3Rα KO mice also proliferate less in vivo than thymocytes from wild-type animals. Under the experimental conditions, using BrdU, the percentage of cycling thymocytes is decreased by about 20% (Fig. 4*B*).

T3Rα1 or T3Rα2 gene products control the size of the B cell pool by intrinsic mechanisms

As T3Rα1 and T3Rα2 are widely expressed among murine tissues (19, 31, 32), we questioned whether the deficient lymphocyte development was intrinsic to these cells or whether it was due to a defect in environmental factors. To address this question, we produced $T3R\alpha^{-/-} \times Rag1^{-/-}$ chimeric animals. $T3R\alpha^{+/-}$ mice were crossed and the resulting morulae were aggregated to morulae from Rag1-deficient mice. Rag1 is necessary for TCR and Ig gene recombination (33), and mice deficient for this enzyme are not able to develop mature T or B lymphocytes (34). Thus, in $T3R\alpha \times Rag1^{-/-}$ chimera, all mature B and T lymphocytes can only derive from the T3Rα morulae, while all other cell types are a mix of cells derived from T3Rα or $Rag1^{-/-}$ morulae. The degree of chimerism was analyzed by PCR analysis on liver cells, as described in *Materials and Methods*. For the experiment described, it was estimated that $Rag1^{-/-}$ $T3R\alpha^{-/-}$ chimeric animals contained about 60% to 75% of liver cells from $Rag1^{-/-}$ (i.e., $T3R\alpha^{+/+}$ genotype) origin. This implies that in $T3R\alpha^{-/-} \times Rag1^{-/-}$ chimeric animals, mature lymphocytes that do not ex-

Table III. *Analysis of bone marrow progenitor B cell subsets of T3Rα KO and wild-type mice*[a]

Subpopulations	$B220^+$c-*kit*$^+$	$B220^+$c-*kit*$^-$	$B220^+CD43^+$	$B220^+CD43^-$	$B220^+CD25^+$	$B220^+CD25^-$	Larges, $B220^+CD25^+$	Petites, $B220^+CD25^+$
% (KO; wt)[b]	(1.2; 1.0)	(39.8; 46.6)	(3.2; 3.2)	(43.8; 50.0)	(12,1; 17.0)	(35.9; 37.2)	(2.1; 2.0)	(10.0; 15.0)
Ratio KO/wt[c]	118¥	85*	102¥	88*	71**	96¥	107¥	67**

[a] Results are representative of one experiment out of two, including seven mutant and seven wild-type animals.
[b] Bone marrow cells were stained with anti-B220, CD117 (c-kit), CD25 and CD43 antibodies and the percentages of each subset was determined using flow cytometry.
[c] The percentage shown corresponds to: [(mean values for KO mice/mean values for wild-type mice)] × 100.
¥, $p > 0.2$; *, $p < 0.05$; **, $p < 0.005$ Student's *t* test).

Table IV. *Lymphocyte precursors in primary lymphoid organs from T3Rα KO mice proliferate less*[a]

Cell Types	% of BrdU$^+$ T3Rα KO	% of BrdU$^+$ Wild Type
Bone marrow		
B220$^+$	10.9	18.9
B220$^+$IgM$^-$	20.9	29
B220$^+$IgM$^+$	2.3	4.9
Thymus		
CD45$^+$	12.8	15.3

[a] T3Rα KO and wild-type mice were injected twice with 1 mg BrdU. Six hours later, they were sacrificed and bone marrow and thymic cells were surface stained for B220 and IgM or CD45 expression. After permeabilization and DNA digestion, BrdU$^+$ cells were revealed with an anti-BrdU FITC-conjugated Ab using flow cytometry. Results for one representative experiment out of three are shown.

press T3Rα1 and T3Rα2 have developed in a cellular environment in which a significant proportion of cells express these proteins. Hence, any decrease in lymphocyte cell numbers generated in these chimeric mice would indicate an intrinsic involvement of T3Rα1 or T3Rα2 gene products in the control of the development of these cells.

As shown in Fig. 6*B*, there is no significant difference in thymocyte numbers between T3Rα$^{-/-}$ × Rag1$^{-/-}$ and T3Rα$^{+/+}$ × Rag1$^{-/-}$ mice. In contrast, splenic cellular indices are significantly reduced by about 55% in T3Rα$^{-/-}$ × Rag1$^{-/-}$ mice compared with the T3Rα$^{+/+}$ × Rag1$^{-/-}$ control chimera. This decrease results mainly from a strong reduction in splenic B cell numbers (Fig. 6*A*). Indeed, splenic T cells, granulocytes, and macrophages are found in equivalent numbers in T3Rα$^{-/-}$ × Rag1$^{-/-}$ and T3Rα$^{+/+}$ × Rag1$^{-/-}$ mice. This indicates that the defect in T cell numbers observed in T3Rα KO mice was not intrinsic to T3Rα$^{-/-}$ T cells and, more importantly, that the cellular environment provided in chimeric animals was able to support T lymphocyte, granulocyte, and macrophage development. Thus, these results indicate that B cells, in contrast to T cells, are direct targets for thyroid hormone regulation through the T3Rα receptor.

Discussion

Defective lymphocyte development in T3Rα KO mice

The aim of this study was to determine the role of T3Rα receptor isoforms in the immune system. Mice deficient for T3Rα1 and T3Rα2 isoforms show a reduced T and B lymphocyte development that results in a decreased cellularity. However, lymphocyte development seems qualitatively normal, as no difference was observed in terms of surface phenotype and function. Indeed, in vitro, spleen lymphocytes from T3Rα KO mice show a normal proliferative response following LPS or anti-CD3 activation (data not shown). Furthermore, we show that T3Rα1 and T3Rα2 gene products are involved in the proliferation control of progenitor cells in primary lymphoid organs. This does not exclude a role for these proteins in the differentiation of the T or B lymphocyte precursor cells. The reduced lymphocyte cellularity observed in T3Rα KO mice could also result from an increased sensibility to programmed cell death (35). This does not seem to be the case, at least for bone marrow cells, as we did not find sub-G_1 apoptotic cells following Hoechst cell cycle analyses on freshly isolated bone marrow cells. Similarly, no detectable increase in apoptosis after in vitro bone marrow cell culture was found (data not shown).

Multiple levels of T_3 actions on the immune system development

Previous observations in Snell dwarf mice strongly supported a direct effect of T_3 on B cell development, but they could not completely exclude a possible indirect effect on GH/IGF-I production by the cellular environment. Indeed, thyroid hormones regulate GH/IGF-I production by enhancing GH gene transcription in a pituitary cell line (36). Furthermore, IGF-I has been shown to promote B lymphopoiesis (37, 38). Our results show that T_3 seems to act directly on B lymphocytes. This could be explained by the direct control by T3Rα1 or T3Rα2 of genes involved in B cell progenitor proliferation and/or differentiation. Indeed, it has been shown that bone marrow-derived B lineage cell lines express these isoform of the T_3 nuclear receptors (39). Still, one cannot exclude that T_3 acts only to up-regulate GH/IGF-1 secretion by B lineage cells (40, 41). However, this seems unlikely, as it has been shown

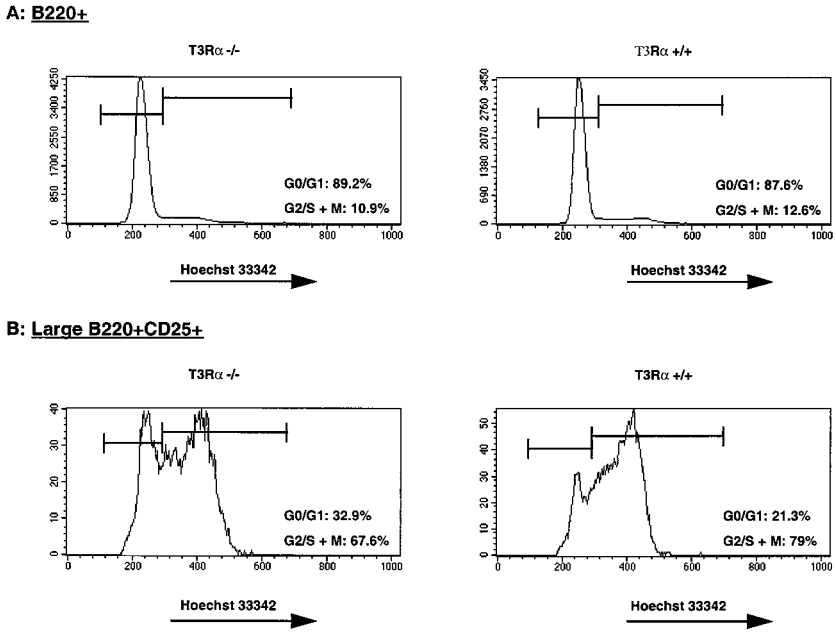

FIGURE 5. Cell cycle analysis of freshly isolated bone marrow nucleated cells from T3Rα KO mice. Bone marrow nucleated cells were first stained for B220 and CD25 expression. After ethanol fixation, cells were washed in PBS, incubated with Hoechst 33342 die, and analyzed on FACs. Hoechst stainings of B220$^+$ (*A*) and large B220$^+$CD25$^+$ (*B*) cells from T3Rα$^{-/-}$ (*left*) and T3Rα$^{+/+}$ (*right*) mice are shown. Representative of two experiments.

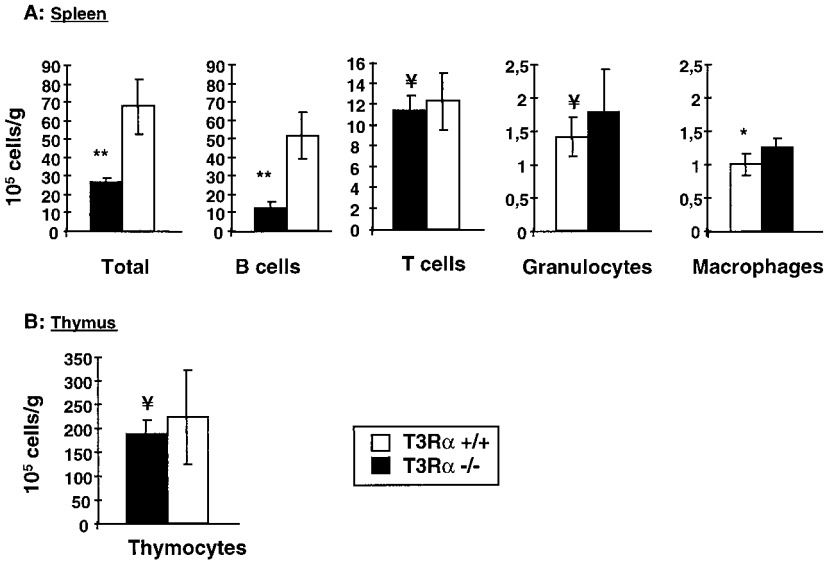

FIGURE 6. Lymphocyte numbers in T3Rα KO × Rag1 KO chimeric animals. T3Rα$^{-/-}$ and T3Rα$^{+/+}$ morulae were fused to Rag1$^{-/-}$ morulae (see text for more details). The splenocytes (*A*) and thymocytes (*B*) from 17- to 19-day-old T3Rα$^{-/-}$ × Rag1$^{-/-}$ (■) and T3Rα$^{+/+}$ × Rag1$^{-/-}$ (□) chimeric offsprings were counted. *A*, Splenocytes stained with anti-B220 and anti-CD3 to identify B and T lymphocytes, respectively. Granulocytes and macrophages were identified by anti-Ly-6G anti-CD11b Abs, respectively. Cellularity indices were calculated as the number of cells per organ divided by the weight of the animals. The cellularity index values are expressed as 10^5 cells/g. ¥, $p > 0.2$; ∗, $p < 0.1$; ∗∗, $p < 0.01$ (Student's *t* test).

that GH is unable to fully restore the pre-B cell compartment in Snell dwarf mice (12).

Conversely, defective T cell development in T3Rα KO mice was shown to depend only on cellular environment, as it is fully restored in T3Rα$^{-/-}$ × Rag1$^{-/-}$ chimeric animals. This is in accordance with previous reports showing that thymic cellularity is enhanced by treatment with IGF-I (42) or GH (11, 43). Furthermore, these hormones are locally produced in the thymus (25, 44). T_3 could also influence thymocyte development through the regulation of other thymic-origin hormonal factors, such as thymulin (Zn-FTS), which induces thymocyte proliferation and differentiation (45). Indeed, the production of thymulin is correlated to seric concentrations of T_3 and T_4 (46, 47). However, T3Rα1 or T3Rα2 expression seems to influence the immune system development at multiple levels, as it acts directly on B lymphocytes and through the cellular environment on T lymphocytes.

In the case of myeloid cell development, we have found a deficit in macrophage (Mac-1$^+$) and granulocyte (Gr-1$^+$) splenic cell numbers in T3Rα KO mice. However, T_3 again seems to act at another level, as it is unlikely that a defective myeloid central development accounts for this decreased macrophage and granulocyte splenic cellularity. Indeed, we did not observe any significant differences in the number of Gr-1$^+$ and Mac-1$^+$ bone marrow myeloid precursors (data not shown), as previously reported in Snell dwarf mice (12). As myeloid cells do not need Rag1 enzyme expression during their development, our experiments with chimeric animals did not allow us to identify the thyroid hormone target. However, a direct effect of T_3 on macrophages and granulocytes has been reported in a number of systems. Indeed, thyroid hormones modulate the metabolism and the functions of purified rat macrophages (48, 49) and stimulate phagocytosis by purified human polymorphonuclear granulocytes (50). Moreover, the production of TSH by cultured monocytes following thyrotropin-releasing factor stimulation is inhibited by T_3 (51).

The role of the different isoforms in the immune system development

The immune deficiencies observed in T3Rα KO mice are directly attributable to the absence of the T3Rα1 and T3Rα2 gene products. Gene transcriptional regulation by T_3 nuclear receptor isoforms is complex. The first degree of complexity is given by the numerous isoforms of T_3 nuclear receptors, some of which do not bind T_3 nor act as transcription factors. Furthermore, these isoforms act as homodimers or heterodimers together with other members of the nuclear receptor family (52, 53) and, depending on the targeted genes and the presence of their ligand, coactivators or corepressors either activate or repress gene transcription (17, 53–55). T_3 nuclear receptor isoforms can also inhibit one another's transcriptional activities (22, 56). Finally, T3R isoforms specifically retro-control thyroid hormone production in a complex fashion. Indeed, although thyroid hormones negatively retro-control pituitary TSH production, T3Rβ-deficient mice have elevated seric levels of T_3 and T_4, together with high TSH serum concentrations (20, 57), while the level of all three hormones is reduced after 3–4 wk of age in T3Rα KO mice compared with wild-type littermates. (19). In T3Rα1-deficient mice (21), seric T_3 is unaffected, while T_4 seric concentrations are reduced only in male animals. It is important to note that in our experiments using 17- to 19-day-old animals, T_3 and T_4 seric concentrations were always found in the normal range, definitely establishing a specific role for T3Rα1 and T3Rα2 gene deletion in the observed phenotype.

Given the complexity of the regulation of gene transcription by nuclear hormone receptors, it would be of particular interest to correlate the hormonal status and immune deficiencies in different mice deficient for T3R isoforms and other members of the nuclear receptor family. We have preliminary data showing a decreased granulocyte, macrophage, and T and B lymphocyte cellularity in spleens from T3Rβ KO mice (data not shown). Our preliminary experiments also show drastically reduced numbers of these cells in the spleens of mice deficient for all T3R isoforms, indicating an additive effect of T3Rα and T3Rβ gene deletions (data not shown). This further supports a role for T_3 in B lymphocyte development and suggests a partial redundancy between thyroid hormone α and β receptors. It has also been shown that T3Rα plays a major role in the control of the proliferation of the crypt cells in the intestine, and that partial redundancy between T3Rα and T3Rβ also occurs in this system (58). Moreover, additive effect of T3Rα and T3Rβ gene deletions excludes the possibility that in absence of T3Rα1, T_3 would act through T3Rβ to reduce cell cycle progression. Indeed, it has been shown that thyroid hormones regulate proliferation arrest and differentiation of oligodendrocyte precursor cells and that the level of T3Rβ1 receptor expression in these cells is involved in determining the time when they will withdraw from cell cycle and differentiate (59).

In conclusion, this study shows that T3Rα1 and T3Rα2 gene products are involved in immune system development. It also shows for the first time that expression of these receptors in bone marrow B lineage cells is intrinsically needed for a proper B lymphopoiesis. Finally, it supports increasing amounts of data demonstrating the importance of cross-talks between neuro/endocrine and immune systems. In terms of functional consequences of these receptors' defect, we have found that T3Rα-deficient lymphocytes proliferate after polyclonal activation (data not shown), but we have not been able to test Ag-specific responses in T3Rα KO mice that die within a few weeks after birth. However, this needs to be investigated in the different thyroid hormone-deficient mice. Indeed, in humans with thyroid hormone resistance due to mutations in T3Rβ receptors, a cohort study showed a high frequency of nose, ear, and throat infections, which could indicate a partial impairment of their immune system (60).

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References

1. Fabris, N., E. Mocchegiani, and M. Provinciali. 1995. Pituitary-thyroid axis and immune system: a reciprocal neuroendocrine-immune interaction. *Horm. Res. 43:29.*
2. Rollins-Smith, L. A., A. T. Davis, and P. J. Blair. 1993. Effects of thyroid hormone deprivation on immunity in postmetamorphic frogs. *Dev. Comp. Immunol. 17:157.*
3. Marsh, J. A., and C. G. Scanes. 1994. Neuroendocrine-immune interactions. *Poult. Sci. 73:1049.*
4. Villa-Verde, D. M., V. de Mello-Coelho, D. A. Farias-de-Oliveira, M. Dardenne, and W. Savino. 1993. Pleiotropic influence of triiodothyronine on thymus physiology. *Endocrinology 133:867.*
5. Li, S., E. B. d. Crenshaw, E. J. Rawson, D. M. Simmons, L. W. Swanson, and M. G. Rosenfeld. 1990. Dwarf locus mutants lacking three pituitary cell types result from mutations in the POU-domain gene *pit*-1. *Nature 347:528.*
6. Van Buul-Offers, S. C., R. J. Bloemen, M. G. Reijnen-Gresnigt, H. A. van Leiden, C. M. Hoogerbrugge, and J. L. Van den Brande. 1994. Insulin-like growth factors-I and -II and their binding proteins during postnatal development of dwarf Snell mice before and during growth hormone and thyroxine therapy. *J. Endocrinol. 143:191.*
7. Fabris, N., W. Pierpaoli, and E. Sorkin. 1971. Hormones and the immunological capacity. III. The immunodeficiency disease of the hypopituitary Snell-Bagg dwarf mouse. *Clin. Exp. Immunol. 9:209.*
8. Fabris, N., W. Pierpaoli, and E. Sorkin. 1971. Hormones and the immunological capacity. IV. Restorative effects of developmental hormones or of lymphocytes on the immunodeficiency syndrome of the dwarf mouse. *Clin. Exp. Immunol. 9:227.*
9. Duquesnoy, R., and G. Pederson. 1981. Immunologic and hematologic deficiencies of the hypopituitary dwarf mouse. In *Immunological Defects in Laboratory Animals*, Vol. 1. M. Gershwin and B. Merchant, eds. Plenum Press, New York, p. 309.
10. Murphy, W. J., S. K. Durum, M. R. Anver, and D. L. Longo. 1992. Immunologic and hematologic effects of neuroendocrine hormones: studies on DW/J dwarf mice. *J. Immunol. 148:3799.*
11. Murphy, W. J., S. K. Durum, and D. L. Longo. 1993. Differential effects of growth hormone and prolactin on murine T cell development and function. *J. Exp. Med. 178:231.*
12. Montecino-Rodriguez, E., R. Clark, A. Johnson, L. Collins, and K. Dorshkind. 1996. Defective B cell development in Snell dwarf (*dw*/*dw*) mice can be corrected by thyroxine treatment. *J. Immunol. 157:3334.*
13. Montecino-Rodriguez, E., R. G. Clark, L. Powell-Braxton, and K. Dorshkind. 1997. Primary B cell development is impaired in mice with defects of the pituitary/thyroid axis. *J. Immunol. 159:2712.*
14. Horseman, N. D., W. Zhao, E. Montecino-Rodriguez, M. Tanaka, K. Nakashima, S. J. Engle, F. Smith, E. Markoff, and K. Dorshkind. 1997. Defective mammopoiesis, but normal hematopoiesis, in mice with a targeted disruption of the prolactin gene. *EMBO J. 16:6926.*
15. Berry, M. J., and P. R. Larsen. 1992. The role of selenium in thyroid hormone action. *Endocr. Rev. 13:207.*
16. St. Germain, D. L., and V. A. Galton. 1997. The deiodinase family of selenoproteins. *Thyroid 7:655.*
17. Lazar, M. A. 1993. Thyroid hormone receptors: multiple forms, multiple possibilities. *Endocr. Rev. 14:184.*
18. Hsu, J.-H., and G. Brent. 1998. Thyroid hormone receptor gene knockouts. *Trends Endocrinol. Metab. 9:103.*
19. Fraichard, A., O. Chassande, M. Plateroti, J. P. Roux, J. Trouillas, C. Dehay, C. Legrand, K. Gauthier, M. Kedinger, L. Malaval, B. Rousset, and J. Samarut. 1997. The T3R α gene encoding a thyroid hormone receptor is essential for postnatal development and thyroid hormone production. *EMBO J. 16:4412.*
20. Forrest, D., E. Hanebuth, R. J. Smeyne, N. Everds, C. L. Stewart, J. M. Wehner, and T. Curran. 1996. Recessive resistance to thyroid hormone in mice lacking thyroid hormone receptor β: evidence for tissue-specific modulation of receptor function. *EMBO J. 15:3006.*
21. Wikstrom, L., C. Johansson, C. Salto, C. Barlow, A. Campos Barros, F. Baas, D. Forrest, P. Thoren, and B. Vennstrom. 1998. Abnormal heart rate and body temperature in mice lacking thyroid hormone receptor α1. *EMBO J. 17:455.*
22. Chassande, O., A. Fraichard, K. Gauthier, F. Flamant, C. Legrand, P. Savatier, V. Laudet, and J. Samarut. 1997. Identification of transcripts initiated from an internal promoter in the c-*erbA* α locus that encode inhibitors of retinoic acid receptor-α and triiodothyronine receptor activities. *Mol. Endocrinol. 11:1278.*
23. Pihlgren, M., P. M. Dubois, M. Tomkowiak, T. Sjogren, and J. Marvel. 1996. Resting memory $CD8^+$ T cells are hyperreactive to antigenic challenge in vitro. *J. Exp. Med. 184:2141.*
24. Nagy, A., and J. Rossant. 1993. Production of completely ES cell-derived fetuses. In *Gene Targeting: A Practical Approach.* A. Joyner, ed. IRL Press, Oxford University Press, Oxford.
25. Wilder, R. L. 1995. Neuroendocrine-immune system interactions and autoimmunity. *Annu. Rev. Immunol. 13:307.*
26. Gruber, J., R. Sgonc, Y. H. Hu, H. Beug, and G. Wick. 1994. Thymocyte apoptosis induced by elevated endogenous corticosterone levels. *Eur. J. Immunol. 24:1115.*
27. Cross, R. J., J. S. Bryson, and T. L. Roszman. 1992. Immunologic disparity in the hypopituitary dwarf mouse. *J. Immunol. 148:1347.*
28. Rolink, A., U. Grawunder, T. H. Winkler, H. Karasutama, and F. Melchers. 1994. IL-2 receptor α chain (CD25, TAC) expression defines a crucial stage in pre-B cell development. *Int. Immunol. 6:1257.*
29. Osmond, D. G. 1986. Population dynamics of bone marrow B lymphocytes. *Immunol. Rev. 93:103.*
30. Osmond, D. G. 1990. B cell development in the bone marrow. *Semin. Immunol. 2:173.*
31. Forrest, D., M. Sjoberg, and B. Vennstrom. 1990. Contrasting developmental and tissue-specific expression of α and β thyroid hormone receptor genes. *EMBO J. 9:1519.*
32. Schwartz, H. L., K. A. Strait, N. C. Ling, and J. H. Oppenheimer. 1992. Quantitation of rat tissue thyroid hormone binding receptor isoforms by immunoprecipitation of nuclear triiodothyronine binding capacity. *J. Biol. Chem. 267:11794.*
33. Schatz, D. G., M. A. Oettinger, and D. Baltimore. 1989. The V(D)J recombination activating gene, RAG-1. *Cell 59:1035.*
34. Mombaerts, P., J. Iacomini, R. S. Johnson, K. Herrup, S. Tonegawa, and V. E. Papaioannou. 1992. RAG-1-deficient mice have no mature B and T lymphocytes. *Cell 68:869.*
35. Osmond, D. G., S. Rico-Vargas, H. Valenzona, L. Fauteux, L. Liu, R. Janani, L. Lu, and K. Jacobsen. 1994. Apoptosis and macrophage-mediated cell deletion in the regulation of B lymphopoiesis in mouse bone marrow. *Immunol. Rev. 142:209.*
36. Evans, R. M., N. C. Birnberg, and M. G. Rosenfeld. 1982. Glucocorticoid and thyroid hormones transcriptionally regulate growth hormone gene expression. *Proc. Natl. Acad. Sci. USA 79:7659.*
37. Landreth, K. S., R. Narayanan, and K. Dorshkind. 1992. Insulin-like growth factor-I regulates pro-B cell differentiation. *Blood 80:1207.*
38. Gibson, L. F., D. Piktel, and K. S. Landreth. 1993. Insulin-like growth factor-1 potentiates expansion of interleukin-7-dependent pro-B cells. *Blood 82:3005.*
39. Hastings, M. L., C. Milcarek, K. Martincic, M. L. Peterson, and S. H. Munroe. 1997. Expression of the thyroid hormone receptor gene, *erbA*α, in B lymphocytes: alternative mRNA processing is independent of differentiation but correlates with antisense RNA levels. *Nucleic Acids Res. 25:4296.*
40. Weigent, D. A., J. B. Baxter, and J. E. Blalock. 1992. The production of growth hormone and insulin-like growth factor-I by the same subpopulation of rat mononuclear leukocytes. *Brain Behav. Immun. 6:365.*
41. Geffner, M. 1997. Effects of growth hormone and insulin-like growth factor I on T- and B-lymphocytes and immune function. *Acta Paediatr. Suppl. 423:76.*
42. Clark, R., J. Strasser, S. McCabe, K. Robbins, and P. Jardieu. 1993. Insulin-like growth factor-1 stimulation of lymphopoiesis. *J. Clin. Invest. 92:540.*
43. Murphy, W. J., S. K. Durum, and D. L. Longo. 1992. Role of neuroendocrine hormones in murine T cell development: growth hormone exerts thymopoietic effects in vivo. *J. Immunol. 149:3851.*
44. Savino, W., V. de Mello-Coelho, and M. Dardenne. 1995. Control of the thymic microenvironment by growth hormone/insulin-like growth factor-I-mediated circuits. *Neuroimmunomodulation 2:313.*
45. Bach, J. F., M. Dardenne, J. M. Pleau, and M. A. Bach. 1975. Isolation, biochemical characteristics, and biological activity of a circulating thymic hormone in the mouse and in the human. *Ann. NY Acad. Sci. 249:186.*
46. Fabris, N., and E. Mocchegiani. 1985. Endocrine control of thymic serum factor production in young-adult and old mice. *Cell. Immunol. 91:325.*
47. Fabris, N., E. Mocchegiani, S. Mariotti, F. Pacini, and A. Pinchera. 1986. Thyroid function modulates thymic endocrine activity. *J. Clin. Endocrinol. Metab. 62: 474.*
48. Costa Rosa, L. F. B. P., Y. Cury, and R. Curi. 1992. Effects of insulin, glucocorticoids and thyroid hormones on the activities of key enzymes of glycolysis, glutaminolysis, the pentose-phosphate pathway and the Krebs cycle in rat macrophages. *J. Endocrinol. 135:213.*

49. Pereira, B., L. F. Rosa, D. A. Safi, E. J. Bechara, and R. Curi. 1995. Hormonal regulation of superoxide dismutase, catalase, and glutathione peroxidase activities in rat macrophages. *Biochem. Pharmacol. 50:2093.*
50. Balazs, C., A. Leovey, M. Szabo, and G. Bako. 1980. Stimulating effect of triiodothyronine on cell-mediated immunity. *Eur. J. Clin. Pharmacol. 17:19.*
51. Komorowski, J., H. Stepien, and M. Pawlikowski. 1993. The evidence of thyroliberin/triiodothyronine control of TSH secretory response from human peripheral blood monocytes cultured in vitro. *Neuropeptides 25:31.*
52. Mangelsdorf, D. J., C. Thummel, M. Beato, P. Herrlich, G. Schutz, K. Umesono, B. Blumberg, P. Kastner, M. Mark, P. Chambon, et al. 1995. The nuclear receptor superfamily: the second decade. *Cell 83:835.*
53. Mangelsdorf, D. J., and R. M. Evans. 1995. The RXR heterodimers and orphan receptors. *Cell 83:841.*
54. Brent, G. A., D. D. Moore, and P. R. Larsen. 1991. Thyroid hormone regulation of gene expression. *Annu. Rev. Physiol. 53:17.*
55. Glass, C. K. 1994. Differential recognition of target genes by nuclear receptor monomers, dimers, and heterodimers. *Endocr. Rev. 15:391.*
56. Koenig, R. J., M. A. Lazar, R. A. Hodin, G. A. Brent, P. R. Larsen, W. W. Chin, and D. D. Moore. 1989. Inhibition of thyroid hormone action by a non-hormone binding c-*erbA* protein generated by alternative mRNA splicing. *Nature 337:659.*
57. Weiss, R. E., D. Forrest, J. Pohlenz, K. Cua, T. Curran, and S. Refetoff. 1997. Thyrotropin regulation by thyroid hormone in thyroid hormone receptor β-deficient mice. *Endocrinology 138:3624.*
58. Plateroti, M., O. Chassande, A. Fraichard, K. Gauthier, J.-N. Freund, J. Samarut, and M. Kedinger. 1999. Involvement of T3Rα- and β-receptor subtypes in mediating T3 functions during postnatal murine intestinal development. *Gastroenterology 116:1367.*
59. Gao, F. B., J. Apperly, and M. Raff. 1998. Cell-intrinsic timers and thyroid hormone regulate the probability of cell-cycle withdrawal and differentiation of oligodendrocyte precursor cells. *Dev. Biol. 197:54.*
60. Brucker-Davis, F., M. C. Skarulis, M. B. Grace, J. Benichou, P. Hauser, E. Wiggs, and B. D. Weintraub. 1995. Genetic and clinical features of 42 kindreds with resistance to thyroid hormone: The National Institutes of Health Prospective Study. *Ann. Intern. Med. 123:572.*