Effect of Triiodothyronine on the Expression of T Cell Markers and Immune Function in Thyroidectomized White Leghorn Chickens

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Abstract. **Hypothyroid K-strain chickens were produced by neonatal thyroidectomy and treatment with 6-propyl-2-thiouracil. Thyroidectomized birds were given 0, 1.5, 4.5, 15, or 45 μg/kg body wt of triiodothyronine (T_3) by daily injection. At 5 weeks of age, thymocytes were prepared for flow cytometric analysis of CT-1a, CD3, CD4, and CD8 expression. Sham-operated birds had the smallest proportion of CT-1a$^+$ cells and the brightest CT-1a$^+$ cells. Unsupplemented thyroidectomized birds presented an inverse picture, while T_3-treated thyroidectomized birds were intermediate. Fewer and less brightly labeled CD3$^+$, CD4$^+$, and CD8$^+$ cells were associated with sham-operated birds or with higher levels of T_3 replacement. Low levels (1.5 μg/kg body wt) or no T_3 treatment produced a greater proportion of positive, highly fluorescent cells. The ratios of CD4$^+$ to CD8$^+$ thymocytes were increased ($P \leq 0.05$) by T_3 supplementation. Functionally, thyroidectomy produced a decrease in mitogen-stimulated proliferation of peripheral blood lymphocytes. This effect was ameliorated by T_3 supplementation. Further, thyroidectomy produced an elevation of plasma growth hormone concentrations. These results suggest that thyroid factors and alterations of thymic status significantly affect the generation of specific thymus-derived lymphocyte populations and their functional capabilities, perhaps due to changes in the thymic microenvironment. These alterations may have important consequences for the development of immunocompetence and disease resistance in chickens.** [P.S.E.B.M. 1992, Vol 199]

It is becoming generally accepted that endocrine factors requisite for normal growth and homeostasis are intimately tied to immunocompetence (1, 2). This relationship underscores the numerous interactions between the neuorendocrine and immune systems. The thyroid hormones (triiodothyronine (T_3) and thyroxine or (T_4)) have been shown to have a modulatory effect on immune responses in a variety of species (3–6). In mammals, hypothyroidism produces a decreased humoral and cellular immune responsiveness (7, 8). In birds, hypothyroidism induced by treatment with the goitrogen 6-propyl-2-thiouracil (PTU) depresses antibody responses to sheep erythrocytes (4). The T_3-deficient SLD strain of chickens (9) has several demonstrable immune deficiencies. These include a depressed mixed lymphocyte reactivity (10), a decreased proliferative response to T cell mitogens (11), a decreased graft-vs-host reactivity (12), and depressed humoral immune capabilities (13). Most of these immune activities are modified or enhanced when the SLD is supplemented with T_3 (14, 15) and some of these enhancements have been associated with shifts in T lymphocyte subpopulations (16).

The thyroid hormones have a pronounced thymotrophic effect in birds (17) and mammals, including humans (8, 18). Thyroidectomy or severe hypothyroidism produces a reduction in peripheral blood lymphocytes and a hypoplasia of the thymus similar to that associated with aging (8, 19, 20). Conversely, supple-

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mentation with T_3 or T_4 can slow or even reverse this process (19, 21, 22). Circulating levels of the thymic hormone, thymulin, have been shown to be strongly correlated to concentrations of T_3 and T_4 (18). In addition, the thymic epithelium is known to produce a number of hormones that induce the proliferation and maturation of T lymphocytes or T cells. These hormones include thymosin (23), thymopoietin (24), and thymulin (25).

Under the influence of the thymic hormones, immature or pre-T cells derived in the bone marrow enter the thymus and undergo proliferation and maturation (26). From the thymus, mature T cells seed the other lymphoid organs and the periphery. It is the postthymic or mature T cells that are responsible for cell-mediated immunity and are intimately involved in the regulation of immune responses. Thus, endocrine factors that influence thymic development or function have the potential to alter immunocompetence. This may result in increased susceptibility to disease or the development of autoimmune disorders.

The present experiments further examine the influence of thyroid hormones on the development and function of the immune system in an avian model. Surgical thyroidectomy, followed by treatment with PTU, was used to produce an abrupt and dramatic reduction of circulating thyroid hormones. Groups of thyroidectomized chicks, placed on T_3 replacement therapy, were compared with unsupplemented and sham-operated birds. Specific aims were (i) to examine the effect of these treatments on specific cell populations or developmental states using flow cytometry, (ii) to examine the immunocompetence of immunocytes *in vitro*, using assays of immune function, and (iii) to monitor treatment effects on plasma concentrations of T_3, T_4, and growth hormone.

Materials and Methods

Animals and Treatments. These studies used the K strain of Single Comb White Leghorn chickens developed in the Department of Poultry and Avian Sciences at Cornell University. These birds are homozygous for the B^{15} haplotype at the major histocompatibility complex (MHC) and have been shown to have normal levels of pituitary (9), thyroid (9), and immune (13) function. This strain has been maintained at Cornell as a closed breeding population for over 20 years.

Birds were housed in temperature-controlled battery brooders with raised wire floors. The photoperiod was set at 15:9-hr light:dark. Commercial chick starter diet (Agway, Inc., Syracuse, NY) and water were available *ad libitum*.

Experimental Design. Two experiments were conducted that differed in two respects: (i) the doses of T_3 administered and (ii) whether single-label or dual-label flow cytometry was used to assess the expression of T cell markers. Experiments were designed to have a minimum of six observations per group; however, due to low cell yields, this number was reduced to no fewer than four. In both experiments, day-old chicks were randomly divided into two groups. Birds were anesthetized with methoxyflurane (Metofane; Pitman-Moore, Inc., Washington Crossing, NJ) and either surgically thyroidectomized or sham-operated (sham-operated controls). Immediately after surgery, thyroidectomized birds were randomly assigned to the various T_3 treatment groups. Beginning on the third day after surgery, all thyroidectomized birds were given two daily injections of PTU (120 mg/kg body wt, suspended in 10% gum arabic). Sham-operated birds received an equivalent volume of 10% gum arabic. The hormone dosages used in the first study were 0, 1.5, 4.5, 15, or 45 μg T_3/kg body wt. In the second experiment, dosages were 0 and 4.5 μg T_3/kg body wt.

At 5 weeks of age, body weights were noted and blood was collected for the isolation of peripheral blood lymphocytes (PBL) and to obtain plasma for hormone analysis. Following this, the birds were sacrificed by decapitation. Thymus and bursa weights were recorded and single-cell suspensions of thymic tissue were prepared for flow cytometry, as described previously (27).

Hormone Treatments and Analyses of Serum Concentrations. In these studies, thyroidectomized birds were supplemented with triiodothyronine on the basis of body weight. Treatments were administered by daily injection (between 0730 and 1000 hr) beginning on Day 1. T_3 was dissolved in 0.01 *N* NaOH and diluted so that approximately 1 ml/kg body wt was injected. At this dilution, the concentration of NaOH was 0.001 *N*.

Concentrations of T_4 and T_3 were measured in duplicate plasma samples, collected at the termination of the experiment, using commercially available radioimmunoassay kits (Amerlex-M; Amersham, Co.). Plasma concentrations of avian growth hormone (cGH) were determined in duplicate at two concentrations in a single radioimmunoassay using the specific homologous radioimmunoassay developed by Harvey and Scanes (28).

Flow Cytometric Analyses. Two-million thymocytes or Ficoll-enriched PBL were dispensed into conical-bottomed 96-well polystyrene plates (Corning) and pelleted. Cells were washed once with phosphate-buffered saline with 1% goat serum and 0.05% NaN_3 (GPBS), pelleted, and resuspended in 40 μl of primary antibody. For single-label analyses, the appropriate dilutions of the following primary antibody were used: CT-1a (as ascites; Southern Biotechnology), anti-CD3 (culture supernate; Dr. C-L. Chen), anti-CD4 (ascites; H. S. L.), anti-CD8 (ascites; H. S. L.), anti-sIg (an equal mixture of G-1 anti-γ and M-4 anti-μ; Southern Biotechnology). After a 20-min incubation period, the cells

were washed twice in GPBS. The cell pellets were resuspended in 40 μl of the secondary, fluorescein isothiocyanate-conjugated, goat anti-mouse antibody (Southern Biotechnology) at a dilution of 1/40 and incubated for 20 min. The cells were washed twice more in GPBS and resuspended in 1 ml of fixative (GPBS with 1% formaldehyde). For dual-label analyses of CD4 and CD8 expression, biotinylated anti-CD4 and fluorescein isothiocyanate-conjugated anti-CD8 were prepared as described (29, 30). This was followed by two washes, a 20-min incubation period with 4 units of streptavidin-conjugated R-phycoerythrin (Molecular Probes, Eugene, OR), and two more washes. All reagents were kept chilled and procedures were carried out at 4°C. An Epics Profile flow cytometer (Coulter Corp., Hialeah, FL) was used for cytometric evaluation of 10,240 cells from each sample. In single-color analyses, delimiters were set that excluded approximately 10–15% of the "dimmest" cells, whereas 2–5% were excluded in dual-color analyses. The argon-ion laser was operated at 12 mW (for PBL) or 15 mW (for thymocytes). Cells were gaited on the basis of forward, low-angle (2°–12°) versus orthogonal scatter (with a 488-nm dichroic filter). Fluorescence determinations were made through a 457–502-nm laser-blocking interference filter coupled with a 515-nm long-pass and a 550-nm short-pass filter. R-Phycoerythrin fluorescence measurements used a final 590-nm long-pass filter, while fluorescein isothiocyanate fluorescence used a final 525-nm band-pass filter.

Lymphocyte Proliferation Assays. The culture system, medium, and methods have been described previously in detail (10, 16). Briefly, blood was collected into heparinized syringes and transferred to sterile culture tubes. Following a slow-spin centrifugation (31) at 80g and 4°C for 7 min, the plasma containing the PBL was carefully removed from each sample. The lymphocytes were then pelleted from the plasma by a second centrifugation and the plasma was removed and frozen for subsequent hormone analysis. The PBL were washed twice in Dulbecco's phosphate-buffered saline and resuspended in a cell culture medium (CCM) based on RPMI 1640 medium containing 25 m*M* Hepes buffer, 50 μ*M* 2-mercaptoethanol, 2 m*M* L-glutamine, 2% commercial heat-inactivated chicken serum, 0.25 mg/ml fungizone, and 1 ml Pen-Strep/100 ml (Gibco, Inc.). Lymphocyte proliferation in response to either phytohemagglutinin-P (PHA; Sigma Chemical Co.) or concanavalin A (Con A; Sigma) was assessed by distributing 0.1 ml of a suspension containing 1×10^7 viable PBL/ml of CCM to each well of a 96-well, flat-bottomed culture plate. Individual PBL preparations were assayed in triplicate for each mitogen and for the unstimulated (medium or background) control. On the basis of previous experiments (10, 16), the concentrations of 25 μg PHA/ml or 100 μg Con A/ml were selected. Each stimulated well received 100 μl of the appropriate mitogen; nonstimulated control wells received 100 μl of CCM. After 24 hr of culture (5% CO_2, 95% air, and 95% humidity at 40°C), 50 μl of [^{3}H-methyl]thymidine (30 μCi/ml of CCM) was added to each well. Following an additional 18-hr incubation period, cultures were harvested onto glass-fiber filters and the level of [^{3}H]thymidine incorporation was measured by scintillation counting. Data are presented as the response of the stimulated cultures minus that of the unstimulated cultures (i.e., background). The average background within the unstimulated cultures was <330 cpm.

Statistics

Data from these experiments were analyzed by one-way analysis of variance, where the main effect being analyzed was thyroid status (sham-operated controls versus thyroidectomized birds). Planned comparisons between groups were made using the protected Fisher's LSD or Tukey's HSD test. Statistically significant differences were defined as those with a probability of 5% or less of rejecting the null hypothesis when true. These data were analyzed with the aid of the Number Cruncher Statistical System (NCSS, Kaysville, UT) and the Systat (Systat, Evanston, IL) statistical packages.

Results

The results of single-label flow cytometric analysis of CT-1a and CD3 expression by thymocytes from vehicle-injected thyroidectomized, T_3-supplemented thyroidectomized, and sham-operated control birds are shown in Figure 1. In these studies, only cells from the vehicle-injected thyroidectomized birds, sham-operated controls, and groups given 1.5 and 4.5 μg T_3/kg were examined by flow cytometry. The proportion of CT-1a$^+$ thymocytes from vehicle-injected thyroidectomized birds was increased ($P \leq 0.05$) compared with sham-operated controls or those from any of the T_3-supplemented thyroidectomized groups. In addition, controls and T_3-supplemented thyroidectomized groups showed an increase in the staining intensity of CT-1a$^+$ thymocytes. Vehicle-injected thyroidectomized groups were "dimmer" in terms of labeling intensity ($P \leq 0.05$), while T_3-supplemented groups were intermediate between the former group and the sham-operated controls.

Proportionately fewer and less brightly labeled CD3$^+$ thymocytes were found in birds treated with 4.5 μg/kg of T_3; however, thyroidectomized birds injected with 1.5 μg/kg were not different from vehicle-injected thyroidectomized birds (Fig. 1). Vehicle-injected thyroidectomized birds were not statistically different from any other group due to a slight reduction in relative brightness and cell number compared with birds given T_3 at 1.5 μg/kg.

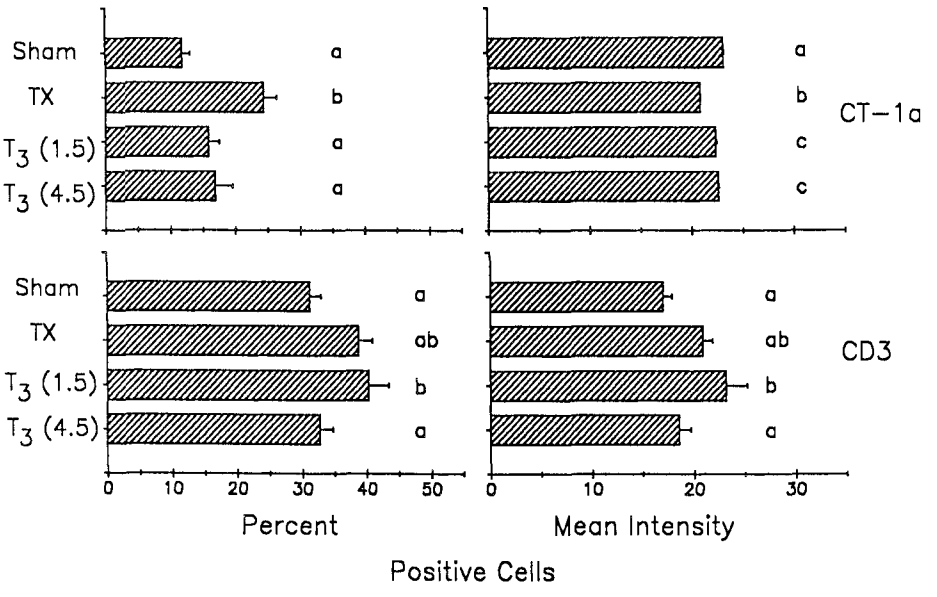

Figure 1. Single-label flow cytometric analysis of CT-1a and CD3 expression by thymocytes. The results are expressed as the mean percentage and mean log fluorescent intensity ± SE of thymocytes from each experimental group. Differences between groups were examined by Fisher's LSD test only when the probability of a type one error was ≤0.05 (protected LSD test). Values within a panel sharing a common superscript, are not significantly different ($P \leq 0.05$).

The expression of CD4 (a T cell phenotype associated with helper/inducer functions) and CD8 (associated with cytotoxic/suppressor functions) by thymic T cells was similar to that of CD3 (Fig. 2). Thyroidectomy resulted in an increase ($P \leq 0.05$) in the number and intensity of labeling of $CD4^+$ and $CD8^+$ thymocytes. In contrast to CD8, CD4 expression showed a dose-dependent decrease, in terms of both relative number and mean intensity of labeling, in response to increased doses of T_3. In dual-labeling experiments (Fig. 3), thyroidectomy resulted in a decrease in $CD4^+$, $CD8^+$ cells (dual-positive cells); however, T_3-supplemented thyroidectomized birds were not different from sham-operated controls. Concomitantly, the relative numbers of $CD4^+$, $CD8^-$ thymocytes from vehicle-injected birds were increased ($P \leq 0.05$), as compared with sham-operated controls. As before, T_3-supplemented thyroidectomized birds were not different from the vehicle-

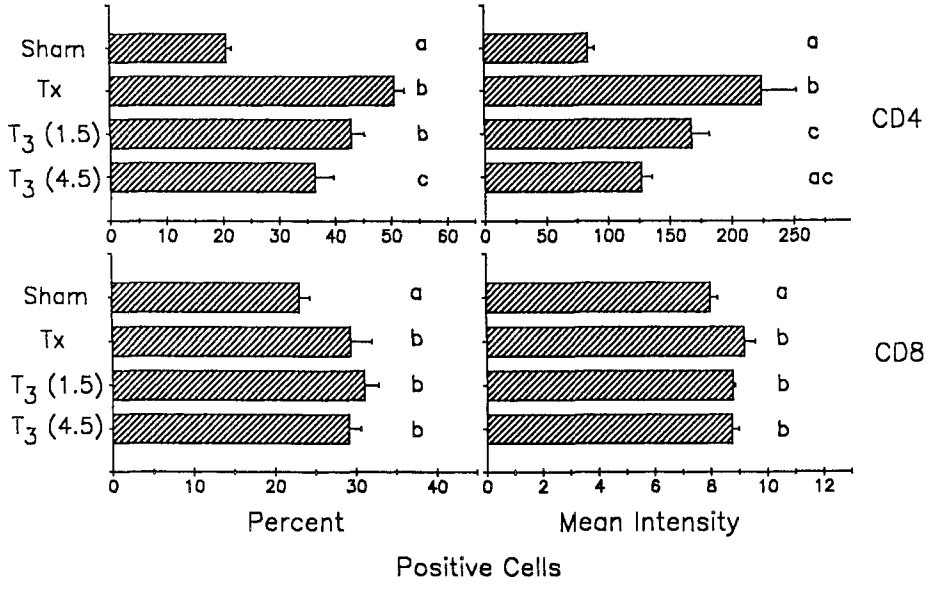

Figure 2. Single-label flow cytometric profiles of CD4 and CD8 expression by thymocytes from sham-operated, thyroidectomized, and T_3-supplemented birds. Bars represent the mean percentage and mean log fluorescent intensity ± SE. Values within a panel sharing a common superscript are not significantly different ($P \leq 0.05$).

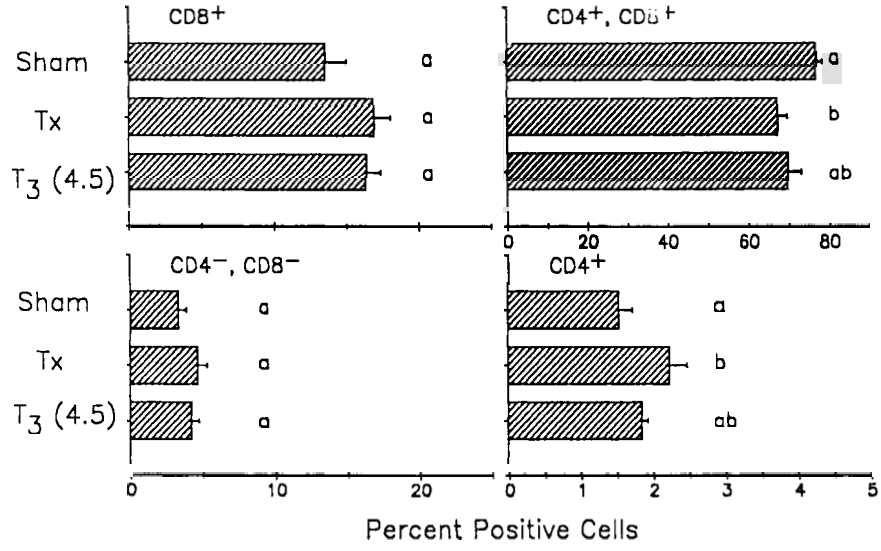

Figure 3. Dual-label flow cytometric analysis of CD4 and CD8 expression by thymocytes. Bars represent the mean percentage of positive cells ± SE. Values within a panel sharing a common superscript are not significantly different ($P \leq 0.05$).

injected controls. In the other single-positive cell population ($CD4^-$, $CD8^+$), no significant changes in relative cell numbers due to treatment were evident, although a similar pattern was observed for both single-positive cell populations. These alterations in CD4 and CD8 expression were also reflected in the ratio of $CD4^+$ to $CD8^+$ cells (Fig. 4). Treating thyroidectomized birds with increasing amounts of T_3 resulted in a dose-related decrease in the ratio of mean intensities of bright $CD4^+$, $CD8^-$ to $CD4^-$, $CD8^+$ thymocytes (Fig. 4, Panel II). Although the same pattern of response is evident in the ratio of relative numbers of bright positive cells (Fig. 4, Panel I) the differences were not significant. In contrast, thyroidectomy resulted in a reduction ($P \leq 0.0001$) in the ratios of dimly fluorescent positive cells. T_3-supplementation of thyroidectomized animals increased the ratio of dimly fluorescent $CD4^+$ to $CD8^+$ cells (Fig. 4, Panel III). No changes due to treatment were observed in the ratios of CD4 to CD8 mean intensities within the dimly fluorescent population (Fig. 4, Panel IV). Differences in the total number of $CD4^+$ or $CD8^+$ cells between single- and dual-color analyses are related to the setting of delimiters on the Epics Profile flow cytometer. Thus, they do not represent biological differences between experiments (see Materials and Methods).

To examine the effect of thyroidectomy on B cells,

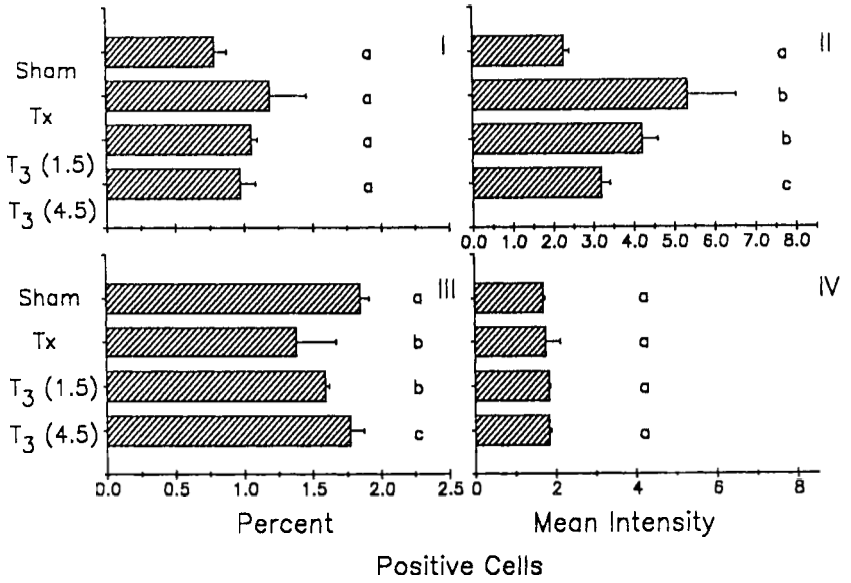

Figure 4. Ratio of CD4 to CD8 expression by thymocytes. Panels labeled I and II represent ratios of highly fluorescent or bright cells, while quadrants labeled III and IV are ratios of dimly labeled cells. Bars represent the mean number and brightness ± SE. Values within a panel sharing a common superscript are not significantly different ($P \leq 0.05$).

PBL expressing surface IgG or IgM immunoglobulins (sIg^+ cells) were evaluated by flow cytometry (Fig. 5). PBL from thyroidectomized birds had the greatest proportion of sIg^+ cells ($P \leq 0.01$). Supplementation of thyroidectomized birds with T_3 abrogated this effect on sIg^+ cells. In addition, sIg^+ PBL from T_3-treated thyroidectomized animals were less intensely labeled than those from untreated birds ($P \leq 0.05$); however, neither group differed significantly from the sham-operated controls.

The effect of thyroidectomy on the proliferative capabilities of T cell subsets to the mitogens Con A and PHA was determined (Table I and Fig. 6). PBL recovered from thyroidectomized animals not receiving T_3 replacement showed reduced proliferative responses to Con A and PHA-P; however, due to the small sample size, this effect was significant ($P \leq 0.05$) only in the second experiment (Fig. 6). The Con A response of thyroidectomized birds treated with 4.5 μg T_3/kg was not different from the proliferative response of cells from sham-operated control animals. This same treatment did not produce an increase in the PHA-induced proliferation of PBL. Additionally, cell yields (Table I) from unsupplemented thyroidectomized birds were substantially lower ($P \leq 0.05$) than those from sham-operated controls. Supplementation with T_3 restored cell yields in a dose-dependent fashion.

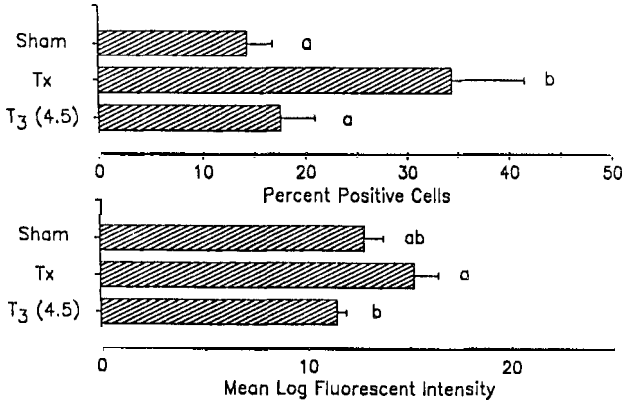

Figure 5. Flow cytometric analysis of surface immunoglobulin (IgM and IgG) expression by peripheral blood lymphocytes. Bars represent the mean percentage of labeled cells (top panel) and the mean fluorescent intensity or brightness (lower panel) ± SE. Values within a panel sharing a common superscript are not significantly different ($P \leq 0.05$).

The plasma levels of T_3, T_4, and growth hormone are shown in Table II. The plasma concentrations of T_3 in all thyroidectomized groups were reduced ($P \leq 0.05$) in both experiments. In Experiment 1, a dose of 4.5 μg T_3/kg produced a maximal elevation ($P \leq 0.05$) in plasma T_3 concentration compared with the other T_3-supplemented groups. This dosage level was chosen for the second experiment, in which it produced the same effect. Thyroidectomized birds showed a reduction ($P \leq 0.05$) in plasma T_4 levels as compared with the sham-operated controls. As expected, T_3 treatments resulted in little or no change in plasma T_4 concentrations. Growth hormone concentrations were increased ($P \leq 0.05$) in vehicle-injected thyroidectomized birds and those given the lowest T_3 dose (1.5 μg/kg). Higher levels of T_3 supplementation of thyroidectomized birds restored plasma GH to concentrations comparable to those of sham-operated controls. These levels were not measured in the second experiment.

The growth of unsupplemented thyroidectomized birds (Table III) was drastically reduced ($P \leq 0.05$), while T_3 supplementation restored body weights to levels comparable to those of sham-operated controls. In a similar fashion, adjusted mean organ weights of thymus and bursa from thyroidectomized birds were reduced ($P \leq 0.05$). Relative thymus and bursa weights were increased with increasing doses of T_3. Adjusted liver weights were increased ($P \leq 0.05$) in thyroidectomized birds. With T_3 supplementation, liver weights

Table I. Effect of T_3 on the Yield and Mitogen-Induced Proliferation of Peripheral Blood Lymphocytes from Thyroidectomized K-Strain Chickens[a]

Experiment 1 treatment (μg T_3/kg)	Sham	Tx	Tx + 1.5	Tx + 4.5	Tx + 15	Tx + 45
PBL/ml ($\times 10^7$)[b]	1.26 ± 0.27*	0.48 ± 0.32†	0.78 ± 0.22*,†	1.08 ± 0.25*,†	1.41 ± 0.21*	1.38 ± 0.29*
n	7	10	10	10	7	10
Con A (CPM $\times 10^{-3}$)	143.21 ± 13.71*	83.59 ± 9.05*	129.79 ± 14.21*	109.73 ± 18.14*	170.49 ± 29.10*	135.93 ± 35.22*
n	7	4	5	7	8	6
PHA-P (CPM $\times 10^{-3}$)	31.32 ± 5.83*	15.90 ± 3.48*	30.61 ± 9.58*	25.94 ± 10.01*	16.09 ± 2.89*	17.87 ± 3.31*
n	7	4	5	7	8	6

[a] Values represent the mean ± SE. Those values within rows sharing common symbols (*,†) are not significantly different ($P > 0.05$) by Tukey's HSD test. Tx, thyroidectomy.
[b] Relative cell yields obtained by slow-spin centrifugation.

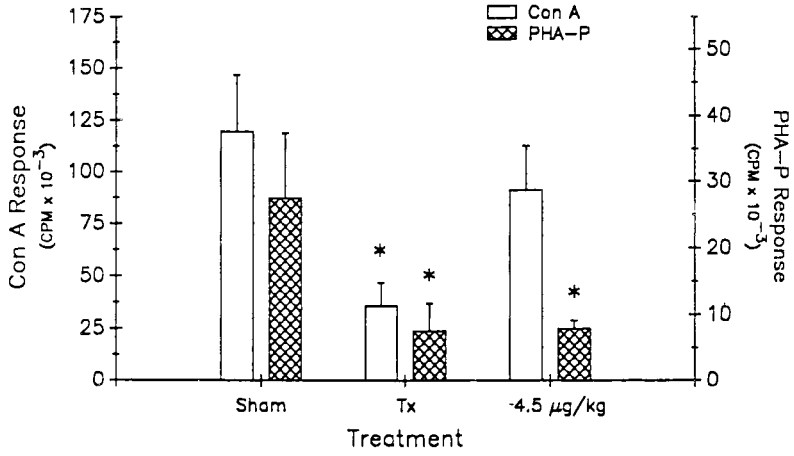

Figure 6. Mitogen response of peripheral blood lymphocytes from the second experiment. Con A and PHA-P-induced proliferation of PBL is expressed as cpm $\times 10^{-3} \pm$ SE. Asterisks indicate treatment values that are significantly different ($P \leq 0.05$) from those of sham-operated controls. Differences between treatment groups were determined as described.

Table II. Effect of T_3 Supplementation on Plasma Levels of cGH, T_4, and T_3 in Thyroidectomized K-Strain Chickens[a]

Treatment (μg T_3/kg)	Sham	Tx	Tx + 1.5	Tx + 4.5	Tx + 15	Tx + 45
Experiment 1						
cGH (ng/ml)	83.6 ± 10.2*	164.5 ± 22.3†,‡	198.2 ± 19.9‡	113.9 ± 14.5*,†	148.6 ± 19.9*,†,‡	106.1 ± 14.2*,†,‡
n	8	10	9	10	7	10
T_4 (ng/ml)	9.64 ± 0.86*	2.99 ± 0.27†,‡	2.94 ± 0.25†,‡	2.85 ± 0.03†,‡	3.50 ± 0.23†	1.95 ± 0.15‡
n	8	10	9	10	7	10
T_3 (ng/ml)	1.93 ± 0.18*	0.64 ± 0.06†	0.68 ± 0.05†	1.02 ± 0.13‡	0.72 ± 0.07†	0.76 ± 0.07†,‡
n	8	10	9	10	7	10
Experiment 2						
T_4 (ng/ml)	8.73 ± 0.97*	1.61 ± 0.14†	ND	2.19 ± 0.24†	ND	ND
n	12	16	ND	10	ND	ND
T_3 (ng/ml)	1.84 ± 0.10*	0.58 ± 0.04†	ND	0.84 ± 0.05‡	ND	ND
n	12	16	ND	10	ND	ND

[a] Values represent the mean ± SE. Those values within rows sharing common symbols (*,†,‡) are not significantly different ($P > 0.05$) by Tukey's HSD test. ND, not determined; Tx, thyroidectomy.
[b] Growth hormone levels were not determined for the second experiment.

Table III. Effect of T_3 Supplementation on Growth and Adjusted Organ Weights in Thyroidectomized K-Strain Chickens[a]

Experiment 1 treatment (μg T_3/kg)	Sham	Tx	Tx + 1.5	Tx + 4.5	Tx + 15	Tx + 45 mg/kg
Body wt (g)	358.1 ± 14.42*	108.0 ± 7.95†	200.6 ± 13.80‡	289.2 ± 7.20§	304.9 ± 15.59§,‖	315.0 ± 10.52*,§,‖
n	8	10	9	10	7	10
Organ weights						
Thymus	0.384 ± 0.026*	0.159 ± 0.017†	0.234 ± 0.021†	0.336 ± 0.028*	ND	ND
n	6	8	6	4		
Bursa	0.408 ± 0.019*	0.073 ± 0.007†	0.155 ± 0.010‡	0.218 ± 0.014‡	0.232 ± 0.020‡	0.331 ± 0.016§
n	8	10	9	9	7	10
Liver	2.197 ± 0.044*	7.007 ± 0.335†	4.309 ± 0.200‡	3.280 ± 0.181§	3.342 ± 0.071§,‖	3.187 ± 0.093§,‖
n	8	10	9	10	7	10
Abdominal fat	0.850 ± 0.096*	0.305 ± 0.033†	1.439 ± 0.229‡	1.620 ± 0.116‡	0.884 ± 0.106*,§	0.654 ± 0.038*,†,‖
n	8	10	9	10	7	10

[a] Values within rows sharing common symbols (*,†,‡,§,‖) are not significantly different ($P > 0.05$) by Tukey's HSD test. ND, not determined; Tx, thyroidectomy.

decreased but did not return to control values. The adjusted mean weight of abdominal fat pads, while reduced in thyroidectomized birds, was increased ($P \leq 0.05$) over sham-operated birds in groups given 1.5 and 4.5 μg T_3/kg. Adjusted fat pad weights from groups supplemented with 15 and 45 μg T_3/kg were comparable to those of sham-operated controls.

Discussion

In these studies, the rationale for surgical thyroidectomy followed by treatment with PTU was to achieve an abrupt and dramatic drop in thyroid hormone concentrations early in the experiment. In addition, PTU treatment results in a complete thyroidectomy and ensures that there is little if any endogenous production of thyroid hormones. King *et al.* (32) showed that PTU alone reduced serum T_3 to 64% of normal concentrations at 7 days of age. The combination of surgical thyroidectomy followed by PTU was shown to reduce T_3 concentrations from nondetectable to 20% of normal concentrations in this same time (33). Although this regimen exposed the animals to the potential side effects associated with PTU treatment, the dosages used were relatively low (34).

It is noteworthy that alterations in lymphocyte surface marker expression, response to mitogens, and body and organ weights were primarily dependent on the level of T_3 supplementation. In addition, these changes occurred in the presence of elevated concentrations of cGH. The retarded growth of thyroidectomized birds, concomitant with elevated concentrations of cGH, is typical. In this model system, supplementation with T_3 resulted in a depression of plasma GH concentrations similar to that described in mammals (35). Elevated levels of GH in thyroidectomized birds was anticipated and has been described previously in association with hypothyroidism (9, 14) and thyroidectomy (36) in chickens. These results imply that the effects of thyroidectomy are not a direct result of changes in circulating GH concentrations. Thus, T_3 may operate to facilitate the effects of growth hormone; however, the influence of GH was not assessed in this study.

Clearly, the hypothyroidism induced in these experiments altered the expression of surface markers on peripheral blood B and T cells and produced shifts in thymic cell populations. Furthermore, these data suggest that T_3 supplementation is capable of reversing the effect of thyroidectomy on the expression of the CT-1a glycoprotein, a marker restricted to thymic T cells (a homologue of a mammalian TL antigen) (37), CD3, which is associated with the T cell receptor complex expressed by both thymic and mature T cells (37, 38), and the accessory receptors, CD4 and CD8. The CD4 and CD8 markers are associated with the antigen-MHC recognition structure of mature T cells (39, 40). In general, thyroidectomy altered both the proportion of positively labeled cells and the intensity of marker expression. The expression of the immature T cell marker, CT-1a, was decreased in thyroidectomized birds, while the expression of CD3, CD4, and CD8, T cell surface glycoproteins associated with cell-cell interactions, was enhanced in these same birds. These findings provide evidence that the modulation of thyroid hormone concentrations can result in qualitative and quantitative changes in the expression of surface markers by lymphoid cells.

Although the majority of thymocytes in the chicken are dual-positive (i.e., $CD4^+$, $CD8^+$) as in humans and other mammals (41), the expression of CD8 appeared to be less sensitive to the effects of thyroidectomy and T_3 supplementation than did CD4. The differences between CD4 and CD8 expression by thymocytes may be related to the temporal expression of these markers. von Boehmer *et al.* (42) and others (43, 44) have suggested that in mammals, thymic T cells first express CD8. After further differentiation, these cells become positive for both CD4 and CD8, finally maturing to T cells expressing either CD4 or CD8. The final maturational step to the single-positive state is thought to involve an interaction between the thymocyte and either an MHC class I- or class II-bearing cell (45, 46). This suggests that T_3 may alter the expression of MHC or other antigens by thymic cells involved in the "education" of thymic lymphocytes. Dardenne and co-workers showed that thyroid and steroid hormones modulate thymic endocrine function *in vitro* and *in vivo* (47). Thus, hypothyroidism may produce changes in the thymic environment, resulting in alterations in T cell maturation and/or trafficking.

The polyclonal proliferative response of lymphocytes exposed to lectins such as Con A and PHA has been regarded as a measure of functional capacity, representing an *in vitro* correlate of the proliferative phase following antigen recognition. In these studies, thyroidectomy resulted in a consistent reduction in mitogen responsiveness. In general, treating thyroidectomized birds with T_3 restored mitogen responsiveness. This is in keeping with observations regarding the differentiation and growth-promoting effects of T_3 and T_4 on lymphocytes *in vitro* (J. A. Marsh and A. M. Khan, unpublished observations). It is likely that these functional differences reflect alterations in thymic T cell populations observed with flow cytometric analysis. Of additional interest, T_3 supplementation produced a response pattern that was lectin-dependent. This is consistent with evidence that various mitogens affect different T cell populations (22). Chan *et al.* (48) reported that peripheral blood lymphocytes, activated for 3 days with Con A, contained approximately 70% $CD8^+$ and 20% $CD4^+$ cells, while PHA-activated PBL contained roughly 20% $CD8^+$ and 40% $CD4^+$ cells. In these studies, the Con A proliferative response was rapidly

restored by T_3 supplementation of thyroidectomy-treated birds. In contrast, the PHA response was not effectively reconstituted in these animals. This may be due to an enhanced susceptibility of $CD4^+$ T cells to the modulation of surface glycoproteins as compared with $CD8^+$ cells.

These results suggest that thyroid hormones play an important role in the maturation and development of immune competence in the chicken. Triiodothyronine showed a marked ability to induce changes in the expression of T cell surface proteins associated with development. In addition, changes in T cell populations were paralleled by changes in functional responses to mitogen. Presently, the mechanism by which these effects are mediated remains unknown; however, they may involve alterations in the thymic environment, cell trafficking through the thymus, or some combination of these and other factors.

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