Effect of Thyroxine and Chicken Growth Hormone on Immune Function in Autoimmune Thyroiditis (Obese) Strain Chicks (43338)

JAMES A. MARSH,*[,1] BRIAN E. JOHNSON,* HYUN S. LILLEHOJ,[†] AND COLIN G. SCANES[‡]

Department of Microbiology, Immunology, and Parasitology, College of Veterinary Medicine, Cornell University, Ithaca, New York 14853; Animal Parasitology Institute,[†] U.S. Department of Agriculture, Beltsville, Maryland 20705; and Department of Animal Sciences,[‡] Cook College, Rutgers University, New Brunswick, New Jersey 08903*

Abstract. **The effect of thyroxine (T_4) and/or recombinant chicken growth hormone (rcGH) supplementation on immune function and on immune cell maturation was examined in Obese strain chickens. Day-old Obese strain chicks received the control treatments or were treated with either T_4 (supplemented in the diet), T_4-rcGH, or rcGH (by daily injection) in a full factorial design. At 4 weeks of age, the proliferative activity of peripheral blood T cells to either mitogenic or allogenic cell (mixed lymphocyte response) challenge was assessed. At the same time, peripheral blood lymphocytes and thymocytes were collected and prepared for flow cytometry analysis. Proliferative responses to both T cell mitogens and allogeneic splenocytes were significantly increased ($P < 0.05$) by rcGH treatment, while the combined T_4-rcGH treatment resulted in a significant increase in allogeneic and in concanavalin A responsiveness, but not in the response to phytohemagglutinin. All supplemented groups showed a significant decrease in the mean fluorescent intensity for CT-1a$^+$ thymocytes, while thymocytes from birds receiving either T_4 or rcGH alone had higher proportions of CD4$^+$ and CD8$^+$ cells. The monoclonal antibody staining of thymocytes from T_4-rcGH-supplemented animals more closely resembled that of the unsupplemented controls. Among the peripheral blood lymphocytes, there were no changes in the numbers of CD4$^+$, CD8$^+$, or sIg$^+$ cells as a result of treatment. The mean fluorescent intensity of sIg$^+$ cells was significantly decreased, however, as a result of T_4 supplementation when given either alone or in combination with rcGH. Finally, the mean fluorescent intensity ratios of CD4$^+$ to CD8$^+$ cells was significantly increased as a result of rcGH supplementation. These results strongly support a role for both the thyroid hormones and growth hormone in regulating and/or enhancing immune function, with changes in functional responses paralleled by concomitant changes in the T cell populations as expressed by shifts in T cell surface marker expression.** [P.S.E.B.M. 1992, Vol 199]

It is clear that the neuroendocrine system is intimately involved in the development and regulation of the immune system (1–3). Conversely, the immune system produces a number of humoral factors or modulators of immune activity that can feed back on the neuroendocrine system through the hypothalamic-pituitary axis (4–6). Many of the same endocrine factors that are basic to growth also appear to be essential to the development of the immune system and thus to an animal's ability to deal with biologically stressful agents.

Of particular interest in these studies are the effects of the thyroid hormones, thyroxine (T_4) and triiodothyronine (T_3), and growth hormone (GH) on immune development and regulation. A positive relationship is known to exist between these hormones and thymic growth and development. This has been demonstrated in both mammalian (7, 8) and avian systems (9–11). Generally, thyroidectomy (7), hypophysectomy (11), severe hypothyroidism (8, 12), hypopituitarism (12, 13), or treatment with anti-growth hormone antibodies (14) results in a atrophy of thymic tissue in a manner that

[1] To whom correspondence and requests for reprints should be addressed at 102 Rice Hall, Cornell University, Ithaca, NY 14853.

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is similar to what is seen in aged individuals. Conversely, supplementation with the appropriate hormone to relieve the deficiency condition reverses these effects and stimulates thymic growth and the mitotic cell index of cortical thymocytes (7, 8). The ability of thyroid hormones to influence functional aspects of immune development has been demonstrated in several systems. A number of apparently contradictory findings relative to the role of the thyroid in immune function have been reported, but many of these apparent conflicts may well be due to the methods used (i.e., hypophysectomy, thyroidectomy, goitrogenic treatments, and high dosages of exogenous T_3 and T_4) to create either hypothyroid or hyperthyroid conditions. All of these manipulations are somewhat drastic and may disturb the balance within the internal milieu too dramatically to allow a determination of the normal role of either the thyroid hormones or GH in immune development and function. Even with these conflicting results, the final conclusion that thyroid hormones do have an influence on immune function appears to be inescapable. The possible role of GH in affecting immune development and function is generally less controversial (reviewed by Ref. 3), but the actual events that are affected and the possible mechanisms of action are still not understood.

The purpose of these studies was to examine the effect of T_4 and/or recombinant chicken growth hormone (rcGH) supplementation on T cell function and development within the Obese strain (OS) of chickens. This strain is known to have a high incidence of spontaneous autoimmune thyroiditis, which appears to be largely due to immunoregulatory deficiencies within this strain (15). Thus, the OS strain becomes an excellent model system to investigate the major hypothesis that T cell-mediated immune function and immunoregulation may be partially controlled by the same endocrine mediators that are known to regulate the growth and development of other tissues and organ systems.

Materials and Methods

Experimental Animals. White Leghorn chickens of the Obese strain that were homozygous for the B^{13} major histocompatibility haplotype were used in these experiments (16, 17). This strain was developed at Cornell University and has been maintained as a closed breeding population for over 20 years. Newly hatched chicks were randomly assigned to treatment groups and housed in temperature-controlled battery brooders with raised wire floors. Feed and water were available *ad libitum* and animals received a 15:9-hr light:dark photoperiod. Animals were weighed every 3–4 days to monitor growth rates.

Experimental Design. Two experiments were performed, each with an identical design and differing only in the number of animals. In the first experiment, day-old chicks were randomly assigned to one of four treatment groups. Treatment Group 1 served as the control and was fed a commercial-type chick starter ration (Cornell D) while receiving daily injections of the vehicle. Treatment Group 2 received the same ration supplemented with 1.0 ppm T_4 and daily injections of the vehicle. Treatment Group 3 was fed the nonsupplemented ration and received daily subcutaneous injections of recombinant chicken growth hormone. Treatment Group 4 received both the T_4-supplemented diet and the daily rcGH injection. Each treatment group consisted of a minimum of 15 male chicks. The second experiment was a smaller one, with six to seven animals per treatment group, and served largely as a replication for main effects and as a source of cells for further flow cytometry analysis. Experiments were terminated after 4 weeks of treatment. Blood samples were collected from which peripheral blood lymphocytes (PBL) and plasma were separated. Thymic tissue was collected for the thymocyte preparations.

Hormone Treatments and Serum Hormone Analyses. The effectiveness of dietary iodothyronine supplementation has been demonstrated previously (18) and is used routinely in our laboratory (9, 10, 19, 20). In this study, the diet was supplemented with 1.0 ppm of 3,3′,5,5′-tetraiodothyronine (T_4; Sigma Chemical Co.) as described previously (19). Recombinant chicken growth hormone was dissolved in 0.01 *M* borate-buffered saline (pH 8.5) and injected daily at a dose of 500 μg/kg body wt. All hormone injections were administered at the same time of day. The rcGH (des Thr_1, Met_1, cGH) was produced by BTG (Israel) and depyrogenated and donated by American Cyanamid (Princeton, NJ). The resulting preparation was determined to be 94.4% monomer and 3.55% dimer by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Thyroxine and triiodothyronine serum levels were measured in duplicate serum samples using commercially available radioimmunoassay kits (Amersham Amerlex). Avian GH serum concentrations were determined in duplicate at two concentrations in a single radioimmunoassay using the specific homologous radioimmunoassay developed by Harvey and Scanes (21).

Cell Proliferation Assays. The culture system, medium, and methods have been described previously in detail (20, 22). Briefly, blood was collected in heparinized syringes and transferred to sterile culture tubes. Following a slow-spin centrifugation (23) of 80*g* at 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 later hormone analysis. The PBL were washed twice in phosphate-buffered saline and resuspended in RPMI me-

dium containing 25 mM Hepes buffer and supplemented with 1 ml of 5×10^{-3} M 2-mercaptoethanol, 1 ml of 200 mM L-glutamine, 2 ml of commercial heat-inactivated chicken serum, 1 ml of Pen-Strep (containing 100 units and 100 μg, respectively), and 1 ml of 0.25 mg/ml of fungizone per 100 ml of RPMI. This supplemented culture medium is hereafter referred to as "complete" medium.

Lymphocyte proliferation in response to either phytohemagglutinin (PHA) or concanavalin A (Con A) was assessed by adding a 0.1-ml PBL suspension containing 1×10^7 viable lymphocytes/ml of complete medium 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 control. On the bases of previous experiments, the concentrations of 25 μg PHA/ml (Sigma) or 100 μg Con A/ml (Sigma) were selected. Each stimulated well received 100 μl of the appropriate mitogen; nonstimulated control wells received 100 μl of complete medium. After 30 hr of culture (5% CO_2, 95% air, and 95% humidity at 40.5°C), 50 μl of [^{3}H]thymidine (30 μCi/ml of complete medium) were added to each well. Cultures were harvested onto glass-fiber filters following an additional 18-hr culture period and the level of label incorporation was measured in a liquid scintillation counter. 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.

One-way mixed lymphocyte response (MLR) activity was assessed as described previously (22). Allogeneic ($B^{15/15}$) and syngeneic ($B^{13/13}$) stimulator splenocyte preparations were irradiated with 1200 rad from a double-^{137}Celsium source and these cells were then aliquoted and frozen until their use in the assay system. After thawing, live cells were separated over Ficoll and adjusted to a concentration of 2×10^7 viable cells/ml. The responder PBL were freshly prepared as described above and adjusted to a concentration of 1×10^7 viable cells/ml; the responder cell preparation (0.1 ml) was mixed with 0.1 ml of either the allogeneic or syngeneic (control) stimulator cell preparation. After 90 hr of culture, the [^{3}H]thymidine label was added, and the cultures were harvested 16 hr later. Data are presented as a stimulation index, where the stimulation index equals proliferation in response to allogeneic stimulators/proliferation in response to syngeneic stimulators.

Flow Cytometric Analysis. 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. The following dilutions of primary antibody were used; CT-1a (as ascites, Southern Biotechnology) 1/200, anti-CD3 (culture supernate, Dr. C. L. Chen) undiluted, anti-CD4 (ascites, H. S. L.) 1/600, anti-CD8 (ascites, H. S. L.) 1/600, anti-sIg (an equal mixture of G-1 anti-τ and M-4 anti-μ, Southern Biotechnology) 1/200. 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). All reagents were kept chilled and procedures were carried out at 4°C. A Coulter Corp. (Hialeah, FL), Epics Profile flow cytometer was used for cytometric evaluation of 10,240 cells from each sample. The argon-ion laser was operated at 12 mW (PBL) or 15 mW (thymocytes). Cells were gated 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.

Statistical Analysis. Data were analyzed using a 2 × 2 factorial analysis of variance model and the SYSTAT statistical package (SYSTAT, Evanston, IL). The main effects within the analyses were diet (T_4 supplemented versus nonsupplemented) and GH injection (vehicle versus GH). Specific planned comparisons among groups were accomplished using the protected LSD t test (24). In all statistical comparisons, the null hypothesis that there was no difference among treatment groups was rejected if $p \leq 0.05$.

Results

Serum growth hormone levels were significantly altered in those groups receiving daily injections of rcGH (Table I). Similarly, both serum T_3 and T_4 levels were significantly increased by T_4 supplementation to the feed. Neither of the hormonal treatments resulted in a significant effect on general body growth. No significant interaction effects were observed between the rcGH and T_4 treatments on serum GH levels, serum thyroid hormone levels, or on body growth rates.

The effect of hormonal supplementation on lymphocyte proliferation due to stimulation either with T cell polyclonal activators (Con A and PHA) or by *in vitro* allogeneic stimulation (MLR) was examined. Stimulation of cultured Obese strain PBL by PHA resulted in a strong proliferative response, as shown in Figure 1. There was, however, no significant effect of either the individual T_4 or rcGH treatments or the combined T_4-rcGH treatment on T cell proliferation as stimulated by PHA. In contrast, the response to Con A was significantly enhanced (Fig. 1) by those treatments involving rcGH supplementation, either alone or in combination with T_4 (i.e., a significant main effect due

Table I. Effect of rcGH and/or T_4 Treatment on Growth and on Plasma GH and T_3/T_4 Levels in Obese Strain Chickens[a]

Treatment	Body Wt (g)	GH (ng/ml)	T_4 (ng/ml)	T_3 (ng/ml)
Control	256.6 ± 4.5*	88.7 ± 10.1*	11.3 ± 0.9*	1.97 ± 0.22*
T_4	269.5 ± 4.9*	90.1 ± 3.8*	74.0 ± 7.5†	3.89 ± 0.61†
rcGH	261.6 ± 4.7*	109.1 ± 10.6†	11.0 ± 1.2*	1.56 ± 0.13†
T_4/rcGH	262.0 ± 5.2*	117.5 ± 11.4†	46.1 ± 2.4‡	2.43 ± 0.15‡

[a] T_4 was administered in the feed (1.0 ppm) and rcGH was administered by daily subcutaneous injections (500 μg/kg body wt), as described in Materials and Methods. Values are the mean ± SE of 15 animals per treatment group. The different symbols (*, †, ‡) within a column indicate significant differences within that column ($P \leq 0.05$).

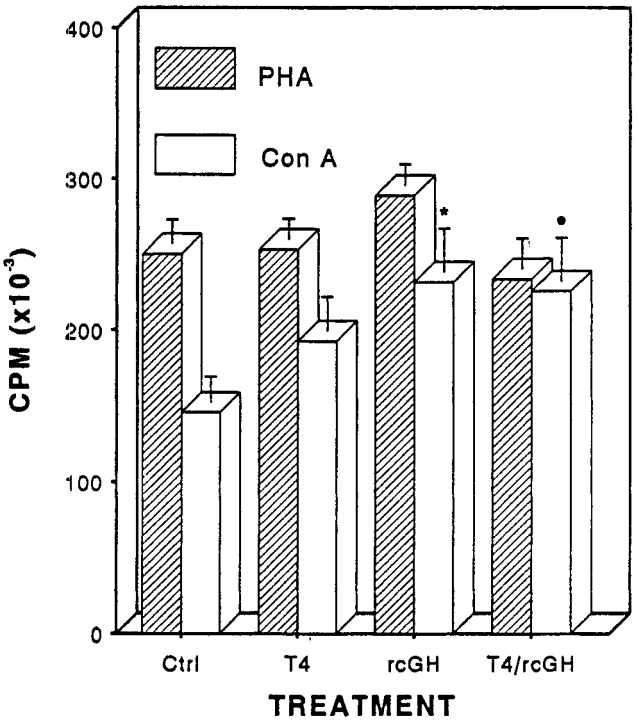

Figure 1. Effect of *in vivo* rcGH, T_4-rcGH, and T_4 supplementation on *in vitro* PBL proliferation (i.e., [^{3}H]TdR incorporation) following 25 μg/ml of PHA or 50 μg/ml of Con A stimulation. Each bar represents the mean ± SE of the 15 animals in each treatment group. Asterisks indicate responses that are significantly different from those of the untreated control ($P < 0.05$).

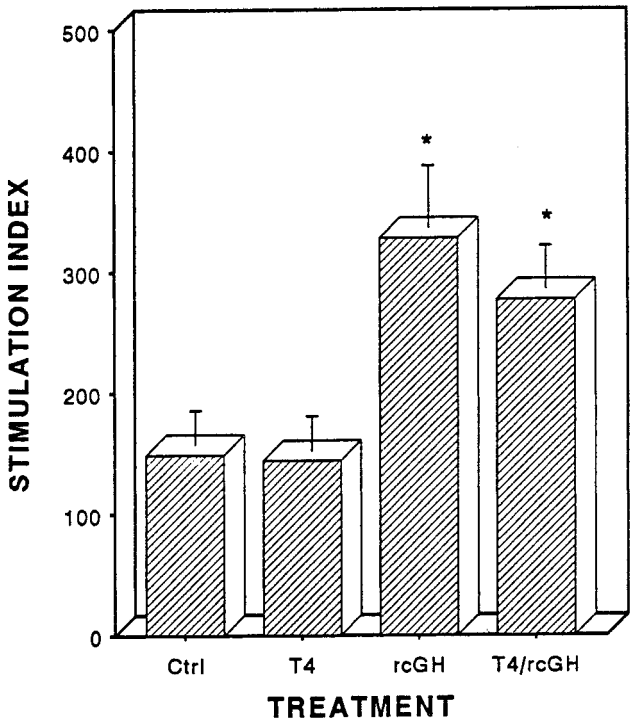

Figure 2. Effect of *in vivo* rcGH, T_4-rcGH, and T_4 supplementation on the *in vitro* allogeneic response (MLR) of $B^{13/13}$ OS responder cells to $B^{15/15}$ stimulator cells. The response is given as the stimulation index, which is the ratio of the $B^{15/15}$ to $B^{13/13}$ response. Each bar represents the mean ± SE of the 15 animals in each treatment group. Asterisks indicate responses that are significantly different from the untreated control ($P < 0.05$).

to rcGH treatment). Supplementation with T_4 alone did not significantly elevate the Con A response, nor was there a significant interaction effect observed due to the combined treatments.

The MLR activity of the PBL preparations from these same treatment groups yielded results similar to that seen in response to Con A stimulation (Fig. 2). A highly significant overall effect ($p \leq 0.001$) of daily rcGH injection was observed when assessing one-way MLR activity. The response of the OS strain-cultured PBL to major histocompatibility complex-compatible ($B^{13/13}$) irradiated stimulator cells ranged between 200 and 2,000 cpm, with an average of 460 ± 360 cpm (mean ± SD). In contrast, the response of the OS-cultured PBL to allogeneic stimulation ranged from approximately 14,000 to over 100,000 cpm and showed marked variation on the basis of treatment groups (Fig. 2). The response of the group receiving 1.0 ppm of T_4 was not different from that of the control group, while those receiving daily injections of rcGH produced a response to allogeneic stimulation that was more than double that of controls. The combined T_4-rcGH treatment was less stimulatory than the rcGH treatment alone, but did result in a significant elevation of the MLR activity. The second smaller experiment (which was done primarily to repeat the flow cytometry studies) resulted in the same general effects, although, with the

smaller sample sizes and the variability inherent in these systems, not all treatments produced significant effects in the functional studies.

No differences were observed in the numbers of CT-1a$^+$ thymocytes from any of the treatment groups. There were, however, significant decreases in the intensity of labeling (as mean log fluorescence) associated with all T_4- and T_4-rcGH- or rcGH-supplemented groups (Fig. 3). The effects of T_4, T_4-cGH, or rcGH on the expression of CD3 by thymocytes from Obese strain chickens are also shown in Figure 3. The percentage of thymocytes staining positive for CD3 was significantly decreased in all supplemented groups. The greatest decrease occurred in birds that received both T_4 and rcGH, while those birds receiving either individual treatment had an intermediate response. The effect of these treatments on the intensity of CD3 expression was similar to the effect on the number of CD3$^+$ cells, i.e., thymocytes from T_4-rcGH-treated birds were more dimly labeled than those from either the unsupplemented control or those receiving only T_4 or rcGH.

The proportion of highly fluorescent CD4$^+$ thymocytes was significantly increased by T_4 and rcGH treatment as compared with the unsupplemented controls, while those receiving the T_4-rcGH treatment were intermediate (Fig. 4). No differences were seen in the mean intensities of CD4$^+$ thymocytes. As with CD4, there was a significantly greater proportion of CD8$^+$ thymocytes in those animals receiving either T_4 or rcGH, but not in the T_4-rcGH-treated group. In contrast to alterations in cell number, T_4-rcGH-treated birds showed a highly significant reduction in labeling intensity of CD8$^+$ thymocytes, whereas those groups receiving either T_4 or rcGH were not significantly different from the untreated control.

In peripheral blood lymphocytes, there were no differences among treatments in the numbers of CD4$^+$, CD8$^+$, or sIg$^+$ cells (Table II). The ratios of CD4$^+$ to CD8$^+$ PBL were equivalent among treatment groups. Although the proportion of cells with these markers remained unaltered, differences were seen in the labeling intensity. PBL from birds given T_4 alone or in combination with rcGH showed significant reductions in mean log fluorescence of sIg$^+$ cells compared with unsupplemented controls or birds supplemented with rcGH alone. Supplementation with rcGH produced a significant increase in the ratio of CD4 to CD8 marker expression intensity (Table II).

Discussion

The OS strain is characterized by a hyperimmune responsiveness in the antibody response (25), the mitogen-induced proliferative response (26), and the autoimmune response to the thyroid and thyroid-related products (15). This general lack of immunoregulation makes it an interesting model for the study of the effects of exogenous hormonal effects on T cell function and differentiation. In previous studies, we have demonstrated that low level testosterone (27, 28) or thyroxine (29) treatments can influence the production of autoantibodies and the lymphoid infiltration of the thyroid. Growth hormone is another product of the neuroendocrine system that has been demonstrated to be inextricably linked to immune development and function (3). Recently, several intriguing results about the possible role of GH and immune function have emerged.

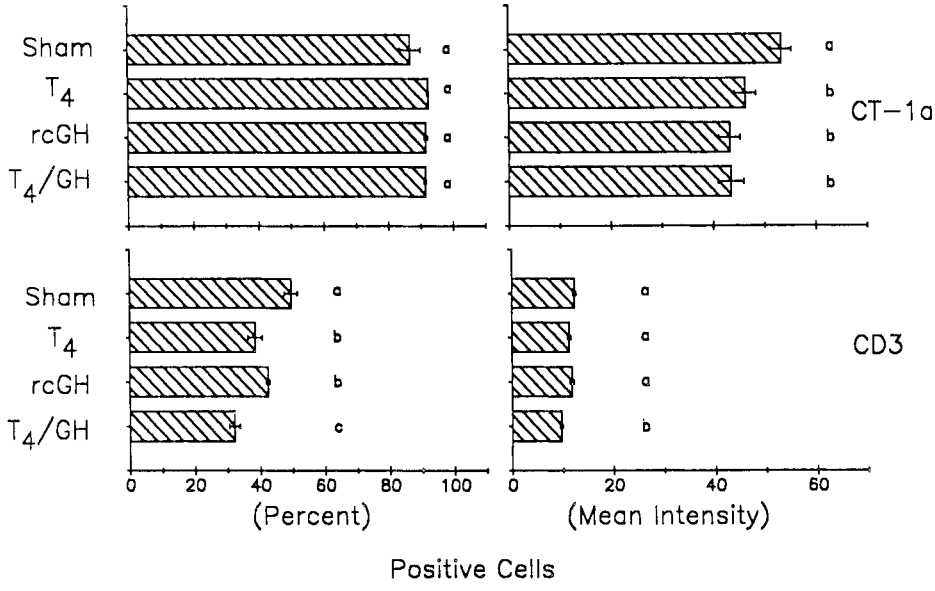

Figure 3. Single-color flow cytometric analysis of thymocytes from the OS strain chickens supplemented *in vivo* with rcGH, T_4-rcGH, and T_4. The relative number and the mean log fluorescence of CT-1a$^+$ and CD3$^+$ thymocytes are shown for six animals per treatment group (mean ± SE). Within a quadrant, treatment groups that share common lowercase letters are not significantly different ($P < 0.05$).

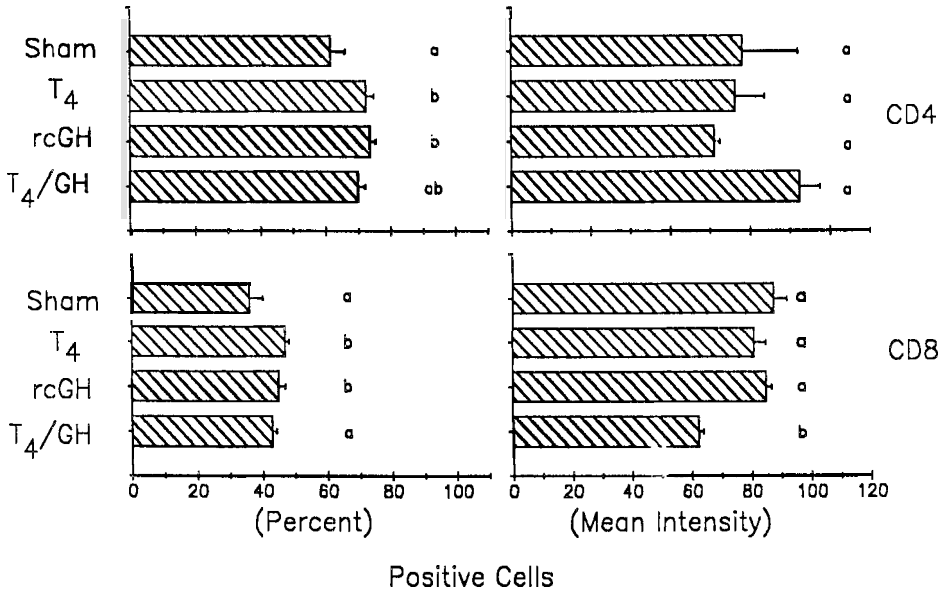

Figure 4. Single-color flow cytometric analysis of $CD4^+$ and $CD8^+$ expression by thymocytes from OS strain chickens supplemented *in vivo* with rcGH, T_4-rcGH, and T_4. The relative number and the mean log fluorescence of $CD4^+$ and $CD8^+$ thymocytes are shown for six animals per treatment group (mean ± SE). Within a quadrant, treatment groups that share common lowercase letters are not significantly different ($P < 0.05$).

Table II. Effect of rcGH and/or T_4 Treatment on Peripheral Blood Lymphocyte Populations Expressing the CD4, CD8, and sIg Surface Markers

Cell surface marker	Number of fluorescent cells[a] Treatment				Mean fluorescent intensity[b] Treatment			
	Control	T_4	rcGH	T_4-rcGH	Control	T_4	rcGH	T_4-rcGH
sIg[c]	1129.7 ± 95.8	1018.8 ± 182.3	1287.7 ± 110.6	957.4 ± 128.6	32.4 ± 1.2	27.8 ± 0.7[d]	31.5 ± 0.6	25.7 ± 1.3[d]
CD4	247.0 ± 58.0	325.7 ± 152.4	401.3 ± 99.9	280.2 ± 162.2	38.8 ± 0.6	37.9 ± 1.2	37.9 ± 1.0	37.3 ± 0.5
CD8	1868.8 ± 631.4	2922.3 ± 659.6	2217.5 ± 462.9	2965.6 ± 445.2	37.6 ± 0.7	37.3 ± 1.3	35.6 ± 0.8	36.1 ± 0.8
CD4/CD8[e]	0.166 ± 0.062	0.112 ± 0.053	0.206 ± 0.063	0.106 ± 0.052	1.218 ± 0.124	1.283 ± 0.074	1.635 ± 0.203[d]	1.131 ± 0.054

[a] Number of cells fluorescent per 10,240 cells counted for the marker indicated when the cytometric delimiters were set to count only those cells staining strongly positive.
[b] Mean fluorescent intensity of the stained cell population for the indicated marker (relative fluorescence units).
[c] Cells stained with a mixture of anti-IgG and anti-IgM monoclonal antibodies.
[d] Significantly different from the control treatment ($P < 0.05$).
[e] Ratio of $CD4^+$ to $CD8^+$ cells and ratio of the mean intensities of $CD4^+$ and $CD8^+$ cells.

Growth hormone has been shown or suggested to: stimulate IL-2 production (30, 31), thymic hormone secretion (30, 32), and tumor necrosis factor-α synthesis (33); affect porcine and macrophage activity *in vitro* and rat macrophages *in vivo* using hypophysectomized rats (34); stimulate the secretion of free radicals by porcine or human neutrophils (35); play a role in the resistance to infection by *Salmonella typhimurium* (36); effect natural killer cell cytolytic activity (37, 38); and influence the maturation of different T cell populations (38–40). Recent reports showing a direct feedback of the cytokines, interleukin 6 (41), TNF-α (42), and interleukin 1 (5) indicate that products of immune effector cells can feed back and act at the level of the pituitary to affect GH synthesis. Of course, the cytokines that are stimulated by GH and the thyroid hormones can exert strong regulatory effects on immune function, as can the production and release of thymic hormones that exert both stimulatory and regulatory actions on the development and function of the T cell populations (43–45). Finally, Weigent *et al.* (46) report that lymphocytes themselves are capable of producing GH. On the basis of these observations, it seems clear that the neuroendocrine system and immune system are interacting at several different levels where one system can exert considerable control over the functioning of the other, and that the thyroid hormones and GH play a central role in these interactions.

The present investigations provide further results to support the above conclusion. As can be seen from the data in Table I, clearly the hormonal treatments were having the desired effect, since the level of the

appropriate hormone was significantly elevated in the serum of the treated animals. It is important to note, however, that these elevations are not such that this would be considered a pharmacologic effect. Roughly 20% elevations in GH were present in rcGH-treated birds (when plasma samples were collected 2–3 hr after injection), while the T_4 supplementation resulted in less than a 2-fold increase in serum T_3 concentrations (the major biologically active form of the thyroid hormones). Given the fact that the OS strain chickens are normally hypothyroid due to the autoimmune thyroiditis, this level of T_4 supplementation actually has the effect of returning serum thyroid hormone levels to near-normal.

The effects of the *in vivo* hormonal treatments on the OS strain's proliferative response leads to several conclusions. First, the high level of responsiveness observed in these experiments to either mitogenic or allogeneic stimulation is consistent with previous reports of OS strain immune function (25, 26). These proliferative responses are approximately double those we routinely see in other strains of chickens (22). Even given the high initial proliferative response of the untreated animals, further increases were seen as a result of hormonal supplementation (Figs. 1 and 2). Clearly, those treatments involving rcGH were most stimulatory to either mitogen-induced proliferation or allogeneic stimulation. It is interesting to note, however, that these treatments did not produce the same effect on the proliferative response to both PHA and Con A. While the Con A response was significantly stimulated by the rcGH treatments, the PHA response was not. This is consistent with the findings in mammals that different subpopulations of T cells respond differently to these mitogens (47). Thus, this result suggests that rcGH is having a differential effect on the T cell subpopulations responding to the mitogenic stimulation and that those cells proliferating in response to Con A are more readily affected by the rcGH treatment. The fact that the proliferative response due to allogeneic stimulation paralleled the Con A response suggests that the same subpopulation may be responsible for much of the proliferation in the MLR.

The conclusion that the hormonal treatments may have a differential effect on specific T cell subpopulations is further supported by the flow cytometry results. In these experiments, we examined four T cell markers associated with maturation and function. An indirect-labeling method was used to assess the expression of the avian homologues of CD3, CD4, and CD8. In addition, a marker for immature T cells that is found on a large proportion of thymocytes (a homologue of mammalian thymic leukemia antigen) defined by the monoclonal antibody CT-1a was examined (48, 49). CD3 is a T cell marker that is coexpressed with the T cell receptor complex of T cells (49, 50). The CD4 marker functions as an accessory receptor in antigen recognition and allows T cells serving an "inducer" function to recognize self-class II major histocompatibility complex antigens on antigen-presenting cells. The CD8 marker serves a similar function on effector T cells in its recognition of self-class I major histocompatibility complex antigens (51). Thymocytes and PBL were evaluated for their expression of these markers on the basis of (i) the number of cells expressing the antigen (i.e., the number of positive cells) and (ii) the intensity of the fluorescence (i.e., the mean log fluorescence) among the positive cells. This means of quantitating fluorescence is used as an indicator of cell-surface antigen density within populations selected on the basis of optical scatter (52).

Clearly, there were shifts in both the number of cells positive for the specific T cell markers and in the intensity of the fluorescence that was observed. This was true when either the thymocyte or PBL preparations were examined. The decrease in $CD3^+$ cells within the thymus that resulted from all the hormonal treatments is perhaps indicative of changes in the rate of T cell maturation and/or trafficking of these cells. This is also consistent with the decrease in the intensity of staining observed when using the CT-1a monoclonal antibody, since this antigen begins to disappear as the thymocytes mature (Fig. 3). At the same time, these treatments resulted in an increase in the number of $CD4^+$ and $CD8^+$ thymoyctes (Fig. 4).

Since all functional assays were performed using PBL, it was of particular interest to determine whether any changes in T cell surface antigen expression accompanied the observed changes in T cell function that resulted from the hormonal treatments. The relative proportion of B cells (sIg^+ cells) and the staining intensity were also examined in the PBL preparation. The hormonal treatments produced no effects on the number of sIg^+ PBL. However, those treatments involving T_4 supplementation, either alone or in conjunction with rcGH, showed a significant reduction in the intensity of staining. Among the peripheral blood T lymphocytes, while there were no changes in the total number of $CD4^+$ or $CD8^+$ cells, shifts in the relative expression of CD4 and CD8 by PBL were apparent when the intensity of labeling was expressed as a ratio (Table II). The *in vivo* administration of rcGH alone resulted in a significant increase in the CD4 to CD8 ratio as compared either with the control group or with those receiving T_4 supplementation. These results suggest that at least qualitative alterations in the peripheral T cells occur as a result of these hormonal treatments. This alteration becomes of particular interest when one compares the profile of T cell marker expression with the results of the functional response assays. The same treatment (rcGH supplementation) that produced the greatest effects in proliferative responsiveness also resulted in

the largest shift in peripheral T cell antigen expression. This observation supports the conclusion that the hormonal status of an animal exerts strong effects on the development of the functional subpopulations of responsive T cells.

Other studies that have examined the effect of *in vivo* GH administration over a period of time have been done primarily in humans receiving GH replacement therapy. Bozzola *et al.* (53) examined the effect of GH treatments on T cell populations over a period of a year and noted some fluctuations, including a decrease in the CD4 to CD8 ratios after 6 months of treatment. By the end of the study, all populations had returned to this near original levels. In a similar study, Petersen *et al.* (54) monitored several immune cell populations over an 18-month period of GH supplementation to GH-deficient children. During this time, the only significant change in cell populations was a decrease in B cells. The treatment appeared to produce a gradual increase with time in the number of $CD4^+$ cells, while the $CD8^+$ population declined. No significant changes in these individual populations were noted and the CD4 to CD8 ratios were not examined. Finally, Kiess *et al.* (38) also examined the effects of GH treatments on immune cell populations in hypopituitary individuals. No differences in T cell subsets were noted and the primary effect of the GH deficiency observed was a depressed natural killer cell activity that was alleviated by GH supplementation.

The present studies differ in several respects from those cited above, including the following: (i) the OS are not GH deficient and thus the administration of GH to these animals represents a GH supplement, not a GH replacement; (ii) the OS exhibit a high frequency of a specific immune-mediated pathology, as opposed to more minor or subtle changes in immune function observed in GH-deficient individuals; (iii) the GH treatments used in these studies began immediately after hatching, when immune development is still underway, as opposed to GH therapy in humans, which often is not begun until the mid- to late prepubertal stages; (iv) none of the cited studies used combined thyroid hormone and GH treatments; and (v) there is the obvious possibility of species differences in the response to these treatments. Regardless of these differences, however, the major conclusions appear to be clear; modifications of circulating levels of GH do result in changes both in functional immune capabilities and in the generation and balance of various immunocyte populations.

The results of the studies reported here strongly support a role for both the thyroid hormones and GH in regulating or enhancing immune function. These changes in functional responses are paralleled by concomitant changes in the T cell populations as expressed by shifts in T cell surface marker expression. The mechanism by which these effects are mediated, however, remains uncertain. As discussed above, both GH and the thyroid hormones have numerous interactions with other mediators of immune development and function (i.e., thymic hormones and various cytokines). Whether effects of the nature observed in these studies are due to a direct action of the hormones on the immunoresponsive cells or whether the effect is an indirect one mediated by other products that regulate immune cell development and function remains to be determined.

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