Influence of Thyroxine and Thyroxine with Growth Hormone and Prolactin on Splenocyte Subsets and on the Expression of Interleukin-2 and Prolactin Receptors on Splenocyte Subsets of Snell Dwarf Mice (43930)

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Abstract. **A number of immune parameters were examined in Snell dwarf mice and compared with normal littermates. The number of splenocytes per gram of body weight were significantly decreased in dwarf animals, and the decrease was distributed throughout the CD4, CD8, B220, and MAC-1 subsets. The percentage of CD4 and CD8 splenocytes was markedly increased, and the percentage of B220 and MAC-1 splenocytes markedly decreased, in dwarf animals. In addition, the percentage of splenocyte T cells constitutively expressing interleukin-2 (IL-2) receptors and prolactin (PRL) receptors was decreased, with the CD4 subset presenting the most dramatic effect.**

The effects of replacing the hormones deficient in the Snell dwarf mouse (i.e., growth hormone [GH], prolactin [PRL], and thyroxine [T_4] on the above immune parameters were also examined. The administration of T_4 alone for 10 days corrected the defect in splenocyte cell numbers per grams body weight for both the CD4 and CD8 subsets, but only partially corrected the defect for the B220 and MAC-1 subsets. The addition of rbGH and rbPRL for the last 3 days of T_4 injection had little additive effect on the number of CD4 and CD8 cells but increased the number of B220 and MAC-1 subsets to values comparable to those of normal animals on the basis of body weight. The decrease in the percentage of CD4 splenocytes in dwarf animals constitutively expressing IL-2R was partially corrected by T_4 injection and completely corrected by the addition of rbGH and rbPRL for the last 3 days. The decrease in CD4 splenocytes constitutively expressing PRLR was partially corrected by T_4 injection alone and the addition of rbGH and rPRL resulted in percentages comparable to that of normal animals. The results indicate that Snell dwarf animals are deficient in immune parameters and that the administration of the hormones lacking in this animal can correct the deficiencies. [P.S.E.B.M. 1995, Vol 210]

Numerous studies have implicated prolactin (PRL) and growth hormone (GH) in the regulation of immune function (1, 2). Recent studies have reported the constitutive expression of cell surface PRL receptors (PRLR) on immunocompetent cells in the rat (3, 4), mouse (5, 6), and human (7) using flow cytometry. There have been no reports identifying specific GH receptors (GHR) on immunocompetent cells. (The use of human GH for this pur-

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pose is not a good choice because the molecule has inherent PRLR binding activity [8].) Although specific GHR have not been demonstrated on immunocompetent cells, the administration of nonprimate GH to animals (9, 10) or its addition to isolated macrophages at high concentrations *in vitro* (11, 12) has been reported to stimulate immune activity.

In classical endocrinology, the effects of hormones on physiologic factors are best observed in animals when the hormone(s) are absent. The Snell dwarf mouse has a defect in the Pit-1 promoter (13, 14) and is lacking thyrotropin (TSH), GH, and PRL (15, 16). This animal has been reported to have defects in immune function (17–20). Recent studies have reported some correction of immune function in the dwarf mouse using GH and PRL (9, 21). We, however, have observed that the administration recombinant bovine GH (rbGH) or recombinant bovine PRL (rbPRL) to the Snell dwarf mouse was not able to alter the expression of interleukin-2 receptors (IL-2R) on lymphocyte subsets under nonstimulated conditions but could do so in normal animals (10). The purpose of this investigation was to extend our previous findings by examining the influence of thyroxine injection alone and in combination with rbGH and rbPRL on the expression of IL-2R and PRLR on immunocompetent cells in the Snell dwarf mouse.

Materials and Methods

Animals. Snell dwarf mice were obtained from our breeding colony at Wayne State University. The original breeding stock was obtained from the Jackson Laboratories (Bar Harbor, ME). The Jackson Laboratories stock was originally bred with C3H mice and the line is designated C3H/HeJ-dw^J/$^+$. The homozygous dwarf animals are designated dw/dw and the heterozygous normal animals are designated dw/+. Both dwarf and normal animals were housed in sterile isolation cages and given sterile mouse chow and water. Cage changing, examination of animals, and injections were performed in a laminar flow hood, and animal handlers and the investigator were gowned, masked, and gloved during all procedures to prevent contamination. Dwarf animals remained with their parents through the second lactation with the new litter. Normal animals were weaned at 21 days. Both dwarf and normal litter mates used in this study were both male and female, divided as equally as possible among the groups. They were approximately 2 months old when the experiment was initiated. The animals were weighed at the initiation of the experiment and upon termination 10 days later.

Hormone Injections. The hormones used were rbGH (American Cyanamide Co., Princeton, NJ), rbPRL (Monsanto Co., St. Louis, MO), and DL thyronine (T_4; cat. no. T-0881; Sigma Chemical Co., St. Louis, MO). The protein hormones were received as a gift from the respective companies. In a single experiment four normal and four dwarf mice were allocated to each group. The groups consisted of control animals injected with 0.1 ml of alkaline saline, and experimental animals injected with either T_4 alone or T_4 + rbGH + rbPRL. All hormones were dissolved in alkaline saline. Thyroxine was injected subcutaneously every other day for a period of 10 days. Pituitary hormones were injected subcutaneously twice daily approximately 12 hr apart for the last 3 days of the 10-day experiment. The animals were sacrificed by decapitation the next morning, 11–13 hr after the last pituitary hormone injection, and the blood was collected into two pools for each group. The serum recovered was frozen at −60°C for future PRL determination using the Nb2 lymphoma cell bioassay. The dose of T_4 administered was 10 μg/0.1 ml for normal animals and 2 μg/0.1 ml for dwarf animals for each injection period. Recombinant bGH and rbPRL were injected at a dose of 50 μg/0.1 ml for normal animals and 10 μg/0.1 ml for dwarf animals twice daily for 3 days. The doses selected for normal and dwarf animals reflected the difference in body weights and were similar to those used previously (10).

Preparation of Splenocytes for Flow Cytometry. Two pools consisting of two spleens from each group were disrupted on fine-messed stainless steel screens in 5 ml of cold Hanks' balanced salt solution (HBSS). Pooled samples were used to obtain enough cells for flow cytometry. The RBC were lysed using ACK buffer. The cells were then washed twice with 5 ml of HBSS, an aliquot of cells was mixed with 0.4% trypan blue, and viable cells were counted on a hemocytometer. The viability was greater than 95%. The number of cells/spleen was calculated from these counts. A total of 0.5 × 10^6 splenocytes was added to each tube on ice and two-color flow cytometry was performed using 0.25 μg/tube of the following monoclonal antibodies: anti-CD4-PE (clone RM4-5 for CD4 cells; Pharmingen, San Diego, CA), anti-CD8-PE (clone 53-5.8 for CD8 cells; Pharmingen), anti-B220-PE (clone 7D4 for B cells; Pharmingen), anti-MAC-1-PE (clone M1/70 for macrophages; Caltag Labs, South San Francisco, CA), and anti-IL-2R-FITC (clone RA3-6B2; Pharmingen). The anti-PRLR monoclonal (U5) was a gift from Dr. Paul A. Kelly (INSERM, Paris, France) and was conjugated to fluorescein in the laboratory as previously reported (6). A total of 5 μg of this preparation was added per tube. Prior to the addition of the above monoclonals, 0.25 μg of the mouse Fc blocking monoclonal (clone 2.4G2; Pharminagen) were added to each tube and reacted with the cells on ice for 20 min. At the end of this time, the monoclonals were added in a total final volume of 100 μl and reacted on ice for 30 min. The cells were then washed twice with 3 ml of sort buffer (PBS with 1% fetal calf serum) and evaluated by flow cytometry after adding 200 μl of sort buffer.

Analytical Flow Cytometry. Two-color analytical flow cytometric analysis was performed using a Becton Dickinson (San Jose, CA) FACScan. Single staining for CD4, CD8, B220, and MAC-1 were performed to set the gates for IL-2R and PRLR staining. Dead cells were eliminated from the analyses by adding 10 μl of 50 μg/ml red fluorescent dye, propidium iodide, and gating out the dead cells. The percentage of cells in each subset with high intensity PRLR-FITC staining was determined by setting the gates one log intensity above that used for the total percentage. A total of 10,000 viable cells were counted for each tube.

Nb2 Cell PRL Bioassay. The level of PRL in the serum was estimated using the rat Nb2 lymphoma cell bioassay. The Nb2 cell line used was originally obtained from Dr. Peter W. Gout (Vancouver, British Columbia, Canada) and its proliferation is specifically induced by PRL (22). The details of the assay have been previously reported (10, 22). In brief, 5 μl of serum were added in triplicate to 96-well, flat-bottom microtiter plates, and a 4-fold serial dilution was performed using AIM-V synthetic medium (cat. no. 12055-018; Gibco, Grand Island, NY) supplemented with 0.1 m*M* minimal essential medium nonessential amino acids, 1 m*M* sodium pyruvate, 3.0×10^{-5} *M* 2-mercaptoethanol, 100 units/ml of penicillin, 100 μg/ml of streptomycin, 2.0 m*M* L-glutamine and 10 m*M* HEPES at final concentration. All supplemental materials were purchased from Gibco. To the diluted serum was added 5000 Nb2 cells in 100 μl of AIM-V medium. Total final volume per well was 200 μl. The cells were cultured at 37°C in a 5% humidified CO_2 environment for 3 days. Sixteen hours prior to harvesting the cells, 1 mCi of [^{3}H]thymidine (cat. no. NET027, 6.7 Ci/m*M*; NEN, Boston, MA)/20 μl was added to each well. To quantitate the level of PRL in the serum a standard curve was constructed between 5 and 5000 pg/ml using natural mouse PRL (Lot #204-136-1; Dr. Y. N. Sinha, Whittier Inst., La Jolla, CA). All serum samples were run in the same assay and the lower limit of sensitivity was 5 pg/ml.

Statistical Analyses. The data was analyzed statistically using either a one- or a two-way analysis of variance (ANOVA) where appropriate. Post hoc statistical comparisons of means was accomplished using Fisher's Protected Least Significant Difference Test. All statistical analysis was performed using the Super ANOVA Computer Program (Abacus, Berkeley, CA) on a Macintosh IIci computer. A value of $P < 0.05$ was considered statistically significant.

Results

Body Weight Gain and Splenocyte Number. The administration of T_4 alone significantly ($P < 0.05$) increased body weight gain of dwarf animals as did the combined injection of T_4 + rbGH + rbPRL ($P < 0.01$) (Table I). The total number of splenocytes in dwarf animals was significantly increased by T_4 injections ($P < 0.05$) and GH and PRL further increased the numbers ($P < 0.01$). The number of splenocytes per gram of body weight was significantly less ($P < 0.01$) in dwarf controls when compared with normal controls. The administration of T_4 alone and T_4 + GH + PRL significantly increased ($P < 0.05$ and 0.01, respectively) the number of splenocytes per gram of body weight for dwarf animals to levels comparable to that of normal control animals. There was no effect of hormone injection on body weight gain and splenocyte numbers in normal animals.

Percentage of Splenocyte Subsets. The percentage of CD4 and CD8 cells was significantly higher ($P < 0.01$) and the percentage of B220 and MAC-1 cells was significantly lower ($P < 0.01$) in dwarf control than those of normal control animals (Table II). The administration of T_4 alone significantly decreased

Table I. Body Weight Gain and Number of Splenocytes of Dwarf and Normal Mice after Hormone Administration

Animal type	Experimental group	Final body wt^{a} (g)	Body wt gain^{a} (g)	Number of splenocytes^{b} (1.0×10^6)	Number of splenocytes/g body wt^{b} (1.0×10^6)
Normal	Control	26.0 ± 2.2	1.8 ± 0.9	30.4 ± 2.6	1.17 ± 0.01
	T_4	29.3 ± 0.2	1.8 ± 1.0	29.2 ± 6.4	1.00 ± 0.21
	T_4 + PRL + GH	30.6 ± 1.9	3.3 ± 0.6	32.9 ± 7.4	1.08 ± 0.29
Dwarf	Control	5.1 ± 0.6	0.2 ± 0.1^{c}	2.3 ± 1.2	0.45 ± 0.18^{c}
	T_4	6.2 ± 0.1	1.0 ± 0.1^{d}	7.0 ± 0.2^{d}	1.13 ± 0.05^{d}
	T_4 + PRL + GH	6.8 ± 0.5	1.5 ± 0.3^{e}	9.9 ± 2.2^{e}	1.46 ± 0.16^{e}

[a] Mean ± SE of four values.
[b] Mean ± SE consisting of two pools each with two spleens.
[c] Significantly different ($P < 0.01$) from normal control.
[d] Significantly different ($P < 0.05$) from respective control.
[e] Significantly different ($P < 0.01$) from respective control.

Table II. Percentage of Splenocyte Subsets in Dwarf and Normal Mice after Hormone Administration

Animal type	Experimental group	Percentage of splenocyte subsets[a]			
		CD4	CD8	B220	MAC-1
Normal	Control	25.1 ± 1.7	9.6 ± 0.1	53.1 ± 0.8	5.2 ± 0.4
	T_4	23.5 ± 2.6	9.6 ± 1.1	52.5 ± 2.4	5.5 ± 0.2
	T_4 + PRL + GH	19.7 ± 1.8^{b}	8.1 ± 0.9	52.8 ± 1.0	4.9 ± 0.4
Dwarf	Control	49.8 ± 0.8^{c}	18.5 ± 1.4^{c}	26.7 ± 1.7^{c}	2.8 ± 0.6^{c}
	T_4	32.0 ± 1.6^{d}	13.2 ± 0.6^{d}	44.7 ± 1.5^{d}	3.5 ± 0.4
	T_4 + PRL + GH	21.8 ± 1.0^{d,e}	7.8 ± 0.6^{d,e}	49.5 ± 0.5^{d,f}	4.0 ± 0.6

[a] Means ± SE (two pools each with two spleens = four values—two from IL-2R experiment and two from PRL experiment).
[b] Significantly different ($P < 0.05$) from respective control.
[c] Significantly different ($P < 0.01$) from normal control.
[d] Significantly different ($P < 0.01$) from respective control.
[e] Significantly different ($P < 0.01$) from dwarf T_4 alone.
[f] Significantly different ($P < 0.05$) from dwarf T_4 alone.

($P < 0.01$) the CD4 and CD8 subset and increased the B220 subset ($P < 0.01$) in dwarf animals when compared with control. The injection of T_4 + GH + PRL further decreased ($P < 0.01$) the percentage of CD4 and CD8 cells, and increased ($P < 0.05$) the percentage of B220 cells in dwarf animals over that of T_4 alone. There was no significant effect ($P > 0.05$) of hormone administration on the percentage of the MAC-1 subset in dwarf animals, although they were somewhat higher. The combination of T_4 + GH + PRL resulted in percentages of splenocyte subsets in dwarf animals comparable to those in normal control animals. There was little effect of hormone administration on the percentage of splenocyte subsets in normal animals except for the CD4 subset where the combined injection of hormones resulted in a significant decrease ($P < 0.05$).

Total Number of Splenocytes Per Subset and the Number of Subsets Relative to Body Weight. Here, statistical comparisons were made only on the basis of numbers of cells per gram of body weight. The total number of all splenocytes per subset per gram body weight were significantly lower ($P < 0.01$) in control dwarf animals than in control normal animals (Table III). T_4 administration significantly increased ($P < 0.01$ or $P < 0.05$) splenocyte subset numbers per gram body weight in dwarf animals when compared with their respective controls. The addition of GH and PRL to T_4 significantly increased ($P < 0.05$) further the number of splenocyte B220 and MAC-1 cells per gram body weight from that observed for T_4 alone but had little to no effect on T-cell subsets. In normal animals, T_4 alone or in combination with GH + PRL significantly decreased ($P < 0.05$) the number per gram body weight of CD4 and CD8 T cells but had no effect on B220 and MAC-1 cells. The combined administration of hormones resulted in comparable splenocyte subset cell numbers/g BW in dwarf animals when compared to normal control animals.

Percentage of Splenocyte Subsets Expressing IL-2R. The constitutive expression of IL-2R for control dwarf splenocytes was significantly lower ($P < 0.05$) only for the CD4 subset when compared with control normal splenocytes although the percentage found in the dwarf CD8 subset was also lower (Table IV). In dwarf animals, only the combined administra-

Table III. Number of Splenocyte Subsets in Dwarf and Normal Mice after Hormone Administration

Animal type	Experimental group	Number of splenocyte subsets[a]							
		CD4		CD8		B220		MAC-1	
		Total (1.0×10^6)	/g body wt (1.0×10^4)	Total (1.0×10^6)	/g body wt (1.0×10^4)	Total (1.0×10^6)	/g body wt (1.0×10^4)	Total (1.0×10^6)	/g body wt (1.0×10^4)
Normal	Control	7.6 ± 0.5	29.4 ± 2.0	2.9 ± 0.2	11.2 ± 0.1	16.1 ± 0.9	62.1 ± 0.9	1.59 ± 0.18	6.1 ± 0.4
	T_4	6.6 ± 0.2	21.6 ± 1.1^{b}	2.7 ± 0.1	8.8 ± 0.4^{b}	15.6 ± 2.6	51.6 ± 9.9	1.62 ± 0.24	5.3 ± 0.9
	T_4 + PRL + GH	6.3 ± 0.3	21.3 ± 1.0^{b}	2.5 ± 0.1	8.6 ± 0.3^{b}	17.5 ± 2.6	59.6 ± 8.7	1.73 ± 0.36	5.9 ± 1.3
Dwarf	Control	1.14 ± 0.34	21.1 ± 4.0^{c}	0.40 ± 0.10	7.5 ± 1.0^{d}	0.65 ± 0.22	11.7 ± 3.0^{d}	0.06 ± 0.01	1.1 ± 0.1^{d}
	T_4	2.23 ± 0.08	33.7 ± 3.2^{e}	0.92 ± 0.03	13.9 ± 1.3^{e}	3.13 ± 0.15	46.6 ± 1.2^{e}	0.24 ± 0.03	3.5 ± 0.3^{b}
	T_4 + PRL + GH	2.10 ± 0.18	34.0 ± 3.3^{e}	0.75 ± 0.05	12.1 ± 0.9^{e}	4.90 ± 0.69	79.3 ± 11.9^{e,f}	0.38 ± 0.03	6.2 ± 0.4^{e,f}

[a] Mean ± SE consisting of two pools each with two spleens.
[b] Significantly different ($P < 0.05$) from respective control.
[c] Significantly different ($P < 0.05$) from normal control.
[d] Significantly different ($P < 0.01$) from normal control.
[e] Significantly different ($P < 0.01$) from respective control.
[f] Significantly different ($P < 0.05$) from dwarf T_4 alone.

Table IV. Percentage of Splenocyte Subsets with Interleukin-2 Receptors in Dwarf and Normal Mice after Hormone Administration

Animal type	Experimental group	Percentage splenocyte subsets with IL-2R[a]			
		CD4	CD8	B220	MAC-1
Normal	Control	8.2 ± 0.3	5.9 ± 3.7	9.0 ± 1.5	7.1 ± 1.2
	T_4	8.5 ± 0.7	1.9 ± 0.3	8.0 ± 1.0	5.6 ± 1.7
	T_4 + PRL + GH	10.1 ± 2.0	2.6 ± 0.7	7.6 ± 1.0	6.8 ± 0.1
Dwarf	Control	3.8 ± 0.5[b]	1.1 ± 0.3	6.8 ± 1.2	6.9 ± 1.0
	T_4	5.7 ± 0.6	1.8 ± 0.2	5.2 ± 1.0	6.3 ± 0.6
	T_4 + PRL + GH	9.2 ± 0.4[c]	2.2 ± 0.1[c]	4.7 ± 1.4	2.9 ± 1.5

[a] Mean ± SE consisting of two pools each with two spleens.
[b] Significantly different ($P < 0.05$) from normal control.
[c] Significantly different ($P < 0.05$) from respective control.

tion of hormones resulted in a significant increase ($P < 0.05$) in IL-2R expression and that was only for the T-cell subsets; the percentage of CD4 cells expressing IL-2R was comparable to that of normal control animals. Hormonal administration had no effect on percentage of B220 and MAC-1 cells expressing IL-2R in dwarf animals and on all splenocyte subsets in normal animals.

Number of Splenocyte Subsets Expressing IL-2R. Statistical comparisons were made only for values relative to body weight. The number of CD4, CD8, B220, and MAC-1 cells expressing IL-2R for control dwarf animals was significantly lower ($P < 0.05$ or $P < 0.001$) than those from control normal animals (Table V). The administration of T_4 alone to dwarf animals increased the number of splenocyte subsets with IL-2R for all subsets but statistical significance ($P < 0.05$) was reached only for the CD8 and B220 subsets. The combined administration of T_4, PRL, and GH significantly increased ($P < 0.05$ or $P < 0.01$) the number of CD4, CD8, and B220 splenic subsets over those of dwarf control animals. Hormone administration to normal animals had no effect on the number of splenic subsets expressing IL-2R.

Percentage of Splenocyte Subsets Expressing PRLR. In control dwarf animals, only the CD4 subset was observed to have a significantly lower ($P < 0.05$) percentage of cells expressing PRLR when compared with control normal animals (Table VI). The combined administration of T_4 + GH + PRL to dwarf animals resulted in a significant increase ($P < 0.05$) in the percentage of CD4 cells expressing PRLR, which was comparable to that observed for control normal animals. Hormone administration did not alter the expression of PRLR on other splenocyte subsets in the dwarf and had no effect in normal animals. In addition, none of the hormonal treatments in either animal type had any effect on splenocyte subsets expressing PRLR with high-intensity fluorescence, except for the B220 subset in dwarf animals where a significant decrease ($P < 0.05$) was observed with the combined injection of hormones.

Number of Splenocyte Subsets Expressing PRLR. Here again, statistical comparisons were made only for values relative to body weight. The number of splenic subsets expressing PRLR in control dwarf animals was significantly lower ($P < 0.05$ or $P < 0.01$) for each of the subsets when compared with normal con-

Table V. Number of Splenocyte Subsets with Interleukin-2 Receptors in Dwarf and Normal Mice after Hormone Administration

Animal type	Experimental group	Number of splenocyte subsets with IL-2R[a]							
		CD4		CD8		B220		MAC-1	
		Total (1.0×10^4)	/g body wt (1.0×10^4)	Total (1.0×10^4)	/g body wt (1.0×10^4)	Total (1.0×10^4)	/g body wt (1.0×10^4)	Total (1.0×10^4)	/g body wt (1.0×10^4)
Normal	Control	61.8 ± 3.3	2.2 ± 0.2	18.0 ± 12.2	0.66 ± 0.42	147.8 ± 37.5	5.6 ± 1.0	11.5 ± 3.8	0.44 ± 0.11
	T_4	56.1 ± 6.6	1.8 ± 0.3	5.2 ± 1.0	0.17 ± 0.04	127.9 ± 50.5	4.2 ± 1.9	10.5 ± 5.7	0.33 ± 0.18
	T_4 + PRL + GH	63.5 ± 16.9	2.2 ± 0.6	6.3 ± 2.4	0.21 ± 0.08	137.3 ± 51.0	4.7 ± 1.7	11.6 ± 3.9	0.40 ± 0.14
Dwarf	Control	4.6 ± 2.8	0.8 ± 0.4[b]	0.5 ± 0.3	0.09 ± 0.03[b]	3.9 ± 1.8	0.7 ± 0.2[c]	0.39 ± 0.12	0.08 ± 0.01[b]
	T_4	12.7 ± 0.7	1.8 ± 0.2	1.7 ± 0.1	0.25 ± 0.01[d]	16.5 ± 4.4	2.4 ± 0.4[d]	1.51 ± 0.09	0.22 ± 0.01
	T_4 + PRL + GH	20.2 ± 3.1	3.2 ± 0.5[d]	1.6 ± 0.2	0.26 ± 0.04[d]	20.7 ± 1.8	3.3 ± 0.2[e]	0.97 ± 0.53	0.16 ± 0.08

[a] Mean ± SE consisting of two pools of two spleens each.
[b] Significantly different ($P < 0.05$) from normal control.
[c] Significantly different ($P < 0.01$) from normal control.
[d] Significantly different ($P < 0.05$) from respective control.
[e] Significantly different ($P < 0.01$) from respective control.

Table VI. Percentage of Splenocyte Subsets with Prolactin Receptors in Dwarf and Normal Mice after Hormone Administration

Animal type	Experimental group	Percentage of splenocyte subsets with PRLR[a]							
		CD4		CD8		B220		MAC-1	
		Total	Hi[b]	Total	Hi[b]	Total	Hi[b]	Total	Hi[b]
Normal	Control	10.0 ± 0.3	1.9 ± 0.1	7.8 ± 0.4	1.7 ± 0.5	78.8 ± 0.6	21.1 ± 1.2	78.2 ± 2.5	8.5 ± 0.2
	T_4	9.9 ± 2.3	1.9 ± 0.4	8.3 ± 1.9	1.4 ± 0.4	76.2 ± 4.8	19.4 ± 2.3	81.1 ± 9.2	8.4 ± 1.6
	T_4 + PRL + GH	12.0 ± 2.6	2.3 ± 0.6	8.5 ± 1.2	2.2 ± 0.4	75.5 ± 2.2	20.2 ± 2.0	71.5 ± 0.5	6.3 ± 1.1
Dwarf	Control	4.2 ± 0.4[c]	0.9 ± 0.1	5.6 ± 1.7	1.3 ± 0.3	75.0 ± 2.5	20.4 ± 0.5	80.7 ± 3.2	5.5 ± 3.3
	T_4	7.1 ± 1.5	1.7 ± 0.5	4.0 ± 0.4	1.1 ± 0.1	78.1 ± 3.1	21.4 ± 1.4	83.3 ± 5.5	7.5 ± 0.9
	T_4 + PRL + GH	8.1 ± 0.5[d]	1.6 ± 0.1	6.5 ± 1.2	1.3 ± 0.1	68.1 ± 2.9	14.2 ± 0.1[d]	82.4 ± 7.0	6.6 ± 0.1

[a] Mean ± SE consisting of two pools each with two spleens.
[b] Hi = percentage of cells with high intensity PRLR.
[c] Significantly different ($P < 0.05$) from normal control.
[d] Significantly different ($P < 0.05$) from respective control.

trols (Table VII). The administration of T_4 to dwarf animals increased the number of splenocytes with PRLR for all subsets, but statistical significance was reached only for the B220 and MAC-1 subsets. The combined administration of T_4 + PRL + GH to dwarf animals significantly increased ($P < 0.05$ or $P < 0.01$) the number of CD4, B220, and MAC-1 subsets. Hormone administration to normal animals had no effect on the number of splenic subsets expressing PRLR.

Serum PRL Levels. In dwarf animals the level of PRL was undetectable (Table VIII). The administration of rbPRL resulted 12–15 hr later in modest levels of PRL in the serum of dwarf animals (approx. 3–4 ng/ml) and elevated serum PRL in one of the pools obtained from normal animals indicating that the PRL injected was absorbed into the circulation and was biologically active.

Discussion

The major findings of this study are as follows: (i) Dwarf animals had a deficit in the number of splenocytes per gram body weight, and this deficit was exhibited throughout the CD4, CD8, B220, and MAC-1 subsets. The administration of T_4 alone was able to correct the defect for T-cell subsets, but it required the addition of GH + PRL to correct the defect in the B220 and MAC-1 subsets. (ii) Dwarf animals had a higher percentage of CD4 and CD8 splenocytes, and a lower percentage of the B220 and MAC-1 subsets. The administration of T_4 partially corrected this defect, but it required the addition of GH and PRL with T_4 to completely correct the defect. (iii) The constitutive expression of IL-2R on dwarf splenocytes was lower only for T cells, and the CD4 subset had the most dramatic effect. The administration of thyroxine partially correct this defect; however, it required the combination of T_4 + GH + PRL to return the defect to normal values. Here, the most dramatic effect was observed for the CD4 subset. (iv) A defect in constitutive expression of PRLR on dwarf splenocytes was most clearly observed for the CD4 subset although the CD8 subset was also lower. Thyroxine alone increased PRLR expression on CD4 and CD8 subsets and the addition of GH and PRL had a modest additive effect only for the CD4 subset.

Although the data represents a single experiment, the results obtained were clear and statistically significant. The ability to replicate this experiment was not possible because the dwarf animal colony had to be sacrificed due to lack of funding. Further, dwarf ani-

Table VII. Number of Splenocyte Subsets with Prolactin Receptors in Dwarf and Normal Mice after Hormone Administration

Animal type	Experimental group	Number of splenocyte subsets with PRLR[a]							
		CD4		CD8		B220		MAC-1	
		Total (1.0×10^4)	/g body wt (1.0×10^4)	Total (1.0×10^4)	/g body wt (1.0×10^4)	Total (1.0×10^4)	/g body wt (1.0×10^4)	Total (1.0×10^4)	/g body wt (1.0×10^4)
Normal	Control	75.4 ± 0.2	2.9 ± 0.3	22.6 ± 3.0	0.87 ± 0.05	1269 ± 103	48.9 ± 0.2	125.2 ± 25.5	4.8 ± 0.6
	T_4	65.3 ± 17.0	2.3 ± 0.5	22.7 ± 5.5	0.75 ± 0.22	1206 ± 417	40.1 ± 15.6	128.2 ± 17.2	4.2 ± 0.8
	T_4 + PRL + GH	75.7 ± 21.6	2.6 ± 0.7	21.5 ± 3.6	0.72 ± 0.11	1319 ± 381	45.3 ± 12.6	123.8 ± 43.2	4.3 ± 1.6
Dwarf	Control	5.2 ± 2.3	0.9 ± 0.3[b]	2.5 ± 1.6	0.45 ± 0.22[c]	47.4 ± 27.3	3.2 ± 1.9[b]	4.5 ± 1.4	0.9 ± 0.1[b]
	T_4	15.7 ± 2.5	2.3 ± 0.1	1.3 ± 0.1	0.54 ± 0.04	245.5 ± 29.5	36.3 ± 0.3[d]	20.4 ± 4.5	3.0 ± 0.3[d]
	T_4 + PRL + GH	17.1 ± 3.7	2.8 ± 0.6[d]	2.3 ± 0.8	0.81 ± 0.25	336.5 ± 95.3	54.6 ± 16.3[e]	31.7 ± 4.2	5.1 ± 0.7[e]

[a] Mean ± SE consisting of two pools of two spleens each.
[b] Significantly different ($P < 0.01$) from normal control.
[c] Significantly different ($P < 0.05$) from normal control.
[d] Significantly different ($P < 0.05$) from respective control.
[e] Significantly different ($P < 0.01$) from normal control.

Table VIII. Serum Prolactin Levels of Dwarf and Normal Mice after Hormone Administration

Animal type	Experimental group	Serum prolactin level[a] (ng/ml)
Normal	Control	25.5 ; 23.1
	T_4	26.4 ; 20.6
	T_4 + PRL + GH	56.9 ; 27.3
Dwarf	Control	<0.3 ; 0.3
	T_4	<0.3 ; 0.3
	T_4 + PRL + GH	3.5 ; 4.1

[a] Individual values of two pools of two animals each.

mals of the number needed for replication are not available from Jackson Laboratories.

Numerous previous studies have indicated that spleen weight (9, 17) and number of splenocytes (19, 21) relative to body weight were dramatically reduced in dwarf mice. The observation in this report that the number of splenocytes per gram body weight was decreased in dwarf mice is in agreement with previous reports. We have observed that T_4 alone corrects the deficit in splenocyte number and similar observations have been made by others (23, 24). The addition of GH and PRL with T_4 results in even a greater number of splenocytes, and this effect can be attributed to an increase in the B220 and MAC-1 cell subsets. The addition of GH and PRL to T_4 does not increase the relative number of splenic T cells over that observed for T_4 alone (Table III). Thus, the relative deficiency of T cells in the dwarf spleen can be corrected by T_4, but the deficiency of B cells and macrophages require all three hormones to return the dwarf animal to normal levels.

Our observation that the percentage of T cells is increased while B cells and macrophages are decreased in dwarf mice splenocytes is in agreement with some investigators (18) though not with others (19, 20). The percentage of CD4, CD8, and B220 cells in the spleen of the normal animals reported here was similar to that reported by others for this strain (19). The partial correction of the T and B cell defect by T_4 alone has not been reported before; however, Dumont *et al.* (18) observed that combined T_4 and bGH injections were capable of returning the values to normal, similar to our observation here using T_4 + bGH + bPRL. While others have shown that bGH alone was capable of restoring total splenocyte cell number (25), no information is available as to its action on specific splenocyte subsets. What is needed are investigations to determine the contribution of each hormone individually and in combination in restoring a normal splenocyte subset pattern. Our intent in this experiment was to determine whether administering all three deficient hormones could return the dwarf spleen lymphocyte subset pattern to that observed for normal animals. Our results indicate that they can. In a number of studies with hypophysectomized rats, GH and PRL have been reported to be equally capable of restoring immune function (26).

This is the first study to examine the constitutive expression of IL-2R on splenocyte subsets in dwarf animals. Our results indicate that dwarf mice have a clear deficit in CD4 IL-2R expression. While administering T_4 alone partially corrects the situation, it required the combination of T_4 + GH + PRL to return the expression to normal levels. Although the CD8 data show a similar trend, the variability among groups did not result in a statistically significant difference. The constitutive expression of IL-2R on B cells and macrophages were not defective and hormone injections had little effect.

This is the first report of the expression of PRLR on immunocompetent cells from dwarf mice. We had previously reported that immunocompetent cells from the popliteal lymph node (PLN) of normal mice constitutively expressed PRLR on 5% of the CD4 cells, 16% of the CD8, and 80% of the B cells using the same U5 PRLR monoclonal antibody used here (6). In addition, we observed that 80% of isolated peritoneal macrophages expressed PRLR (6). In the present report, we have examined the constitutive expression of PRLR on splenocyte subsets and found in normal animals that approximately 10% of the CD4 cells, 8% of the CD8 cells, 75% of the B cells, and 80% of the macrophages express PRLR. Others have also examined mouse splenocyte subsets for PRLR and have reported similar PRLR percentages, for each of the above subsets (5). The deficiency in the expression of PRLR on CD4 cells in dwarf animals was partially corrected by T_4 administration and the addition of GH and PRL to T_4 significantly increased the expression over dwarf control animals to values comparable to that of normal control animals.

The percentage of T cells with high fluorescence intensity for PRLR was not different between animal types and among groups. The percentage of cells with high fluorescence intensity was greatest for the B220 subset, next greatest for the MAC-1 subset, and least for the T-cell subsets. An examination of high-intensity PRLR fluorescence was stimulated by the observation of Gagnerault *et al.* (5), who observed an increase in this cell population for T-cell subsets from lupus autoimmune NZB mice as a function of age and disease progression. It was anticipated that dwarf mice may have a low percentage of these cells, and although a trend existed for the CD4 subset, it did not reach statistical significance.

A recent review article raises the question of whether immunocompetent cells have specific receptors for GH (27). Early studies indicated that the binding of hGH to lymphocytes in culture could be displaced by oPRL and human placental lactogen (28, 29). Recent investigations indicated that the binding of hGH to neutrophils and their activation occurs by way of the PRLR (8). Others have shown that porcine and rat GH could stimulate isolated macrophages to secrete superoxide anion *in vitro* (11, 12). However, to accomplish this 990 ng/ml (45 n*M*) of GH was required, an amount far in excess of normal serum levels (2–6 ng/ml). Numerous *in vivo* investigations (9, 23–25, and others) have administered nonprimate GH and have observed clear effects on immune function. How then can these conflicting observations be resolved? One of the major physiologic effects of GH is to stimulate an increase in circulating insulin-like growth factor-I (IGF-I). The presence of IGF-IR on immunocompetent cells has been clearly demonstrated for both human (30) and mouse cells (31). In addition, administering IGF-I to mice has been reported to increase spleen and thymus weight, secondary to an increase in T- and B-cell numbers (32). Thus, one possible explanation for the action of GH *in vivo* is that it stimulates IGF-I production either systemically or locally, and it is the IGF-I molecule that stimulates immunocompetent cell function. To elucidate this problem, studies are needed to demonstrate the presence of specific GH receptors on isolated immunocompetent cells using nonprimate GH and the expression of the mRNA for GHR. It is only through studies of this nature that we will be able to determine whether GH has a direct action on immunocompetent cells.

The influence of T_4 on immune parameters in the dwarf mouse was extensively examined 20 years ago, however, more recent investigations have lost sight of its significance. We have reported here that for some immune parameters, such as the number of CD4 and CD8 splenocytes relative to body weight, T_4 alone was capable of correcting the defect in dwarf mice (Table III). For other parameters, however, such as the relative number of B cells and macrophages, and the expression of IL-2R on CD4 splenocytes, the addition of GH and PRL are required to return the defect to normal values. These results indicate that hormones play a multifunctional role in immune function and that we are only at the very beginning of understanding where in complicated cascade of immune function specific hormones act to support and/or modulate the system. The dwarf mouse, with its deficiency in GH, PRL, and TSH, will play a critical role in future investigations elucidating the actions of hormones on immune function.

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