Influence of Prolactin and Growth Hormone on the Activation of Dwarf Mouse Lymphocytes *In Vivo* (43657)

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Abstract. **The influence of recombinant bovine prolactin (PRL) and recombinant bovine growth hormone (GH) was examined on the popliteal lymph node (PLN) expression of interleukin-2 receptors (IL-2R) in female Snell dwarf mice and normal litter mates after concanavalin A footpad injection. The absolute number of PLN CD4+, CD8+, or B+ cells of dwarf mice was less than that observed for normal litter mates, but when adjusted for the difference in body weight, only the absolute number of B cells was lower in dwarf animals when compared with normal litter mates. The injection of PRL or GH did not alter the observation. The administration of recombinant bovine PRL to normal animals, but not recombinant bovine GH, increased the expression of IL-2R on unstimulated PLN CD4+ and CD8+ subsets. Hormone administration to dwarf animals, however, did not alter the expression of IL-2R on unstimulated PLN T cell subsets. PLN cells from dwarf animals were poorly activated *in vivo* after injection of concanavalin A and the level of IL-2R expression induced was only 50% of that seen in the PLN of normal animals. The administration of PRL and GH completely corrected the defective induction of IL-2R expression on PLN from dwarf animals after concanavalin A stimulation. These findings strongly suggest that PRL and/or GH play an important role at some stage of the T cell activation process *in vivo*. Further studies are needed to precisely identify the defect in the dwarf mice.** [P.S.E.B.M. 1993, Vol 204]

Numerous studies have implicated prolactin (PRL) and growth hormone (GH) in the regulation of the immune system (1–4). Hypophysectomized rats are immunocompromised for both cellular and humoral immunoreactions (5) and the administration of either PRL or GH will prevent the loss of immune function (6, 7). Dwarf mice are not only deficient in GH, but also lack PRL and thyroid-stimulating hormone (8–11). A number of early studies indicated that dwarf mice were immunocompromised (12, 13), but others have not found this to be the case if the animals are raised in a clean environment and kept with the litter beyond the normal weaning period (14, 15). However, even if the animals are raised properly, dwarf mice show defects in immune function (16–19). Thus, the dwarf mouse would be a good model to examine the influence of PRL and GH on immune function.

Recent studies have reported that PRL administration to ovariectomized rats induced interleukin-2 receptors (IL-2R) on splenic lymphocytes (20) and that anti-PRL sera block the induction of IL-2-induced proliferation of L2 cells, a T helper cell line (21). The purpose of this study was to examine the influence of PRL and GH on the activation of dwarf mouse lymphocytes *in vivo* as measured by induction of IL-2R expression. To accomplish this, dwarf mice were injected with either recombinant bovine PRL or GH followed by footpad injection of concanavalin A (ConA) and popliteal lymphocytes were examined for the induction of IL-2R expression.

Materials and Methods

Animals. Snell dwarf mice were purchased as tested breeding pairs from the Jackson Laboratory, Bar Har-

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bor, ME, where the original stock was bred with C3H mice. This line is designated C3H/HeJ-dw^J/$^+$, and homozygous dwarf animals are designated dw/dw and heterozygous normal animals are designated dw/+. Heterozygous animals were paired for breeding at our NIH animal facility, Bethesda, MD, housed in sterile mouse 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 to prevent contamination. Dwarf animals remained with the parents through the second lactation with the new litter. Normal animals were weaned at 21 days. The dwarf animals used in this study were all females 3–5 months of age and thus were sexually mature. Normal litter mates of the same age were used as controls.

Injection Procedure. In a single experiment, 21 female dwarfs (seven per group) and 15 normal female litter mates (five per group) were randomly allocated equally to three groups: control, PRL, and GH. Dwarf animals were injected subcutaneously with 10 μg/0.1 ml saline of recombinant bovine PRL (rbPRL; Monsanto Co., St. Louis, MO) or 10 μg/0.1 ml of recombinant bovine GH (rbGH; American Cyanamide Co., Princeton, NJ) twice daily, 12 hr apart for 3 days. Normal litter mates received 30 μg of each hormone/injection to provide a comparable amount of hormone on the basis of body weight. Control animals were injected with 0.1 ml of saline. On the evening of the third day after the last hormone injection, the right hind footpad was injected with 50 μg of ConA (Cat. No. 17–0450–01; Pharmacia, Piscataway, NJ) in 25 μl of saline; the left hind footpad was injected with a similar volume of saline. The following morning the animals were decapitated 10–12 hr after ConA injection and the blood and popliteal lymph nodes (PLN) were collected. The blood was allowed to clot on ice and the serum was recovered after centrifugation. The serum from two to three animals was pooled for each group and two serum pools were obtained for normal mice and three serum pools were obtained for dwarf mice. The right and left PLN from each group were allocated to a single pool so that enough cells could be obtained for flow cytometry.

Popliteal Lymphocytes. Popliteal lymphocytes were obtained by crushing the tissue on fine-meshed stainless steel screens in 5 ml of cold Hanks' balanced salt solution, washed twice with Hanks' balanced salt solution containing 5 m*M* methyl-α-D-mannopyranoside to remove adherent ConA and again washed with Hanks' balanced salt solution alone. An aliquot of cells was mixed with an equal volume of 0.1% trypan blue and counted with a hemocytometer. The viability of the cells was greater than 95%. The number of lymphocytes/PLN was calculated from the pooled values. A total of 0.5×10^6 cells were added to each tube on ice and two-color flow cytometry was performed using 0.4 μg of the following monoclonal antibodies: anti-CD4-PE (clone RM4–5; Pharmingen, San Diego, CA), anti-CD8-PE (clone 53–5.8; Pharmingen), anti-B220-PE (clone RA3–6B2; Pharmingen), and anti-IL-2R-FITC (clone 7D4; Pharmingen). Staining was accomplished in 50 μl for 30 min on ice. Unstained cells were used as controls for background staining. The cells were then washed twice with 3 ml of sort buffer (phosphate-buffered saline-1% fetal calf serum) and evaluated by flow cytometry.

Analytical Flow Cytometry. Two-color flow cytometric analysis was performed using a Becton Dickinson (San Jose, CA) FACScan. Dead cells were eliminated from the analysis by adding 10 μl of a 50 μg/ml red fluorescent dye propidium iodide and gating out the dead cells. A total of 10,000 viable cells were counted in each case.

Nb2 Cell PRL Bioassay. The level of PRL in the serum pools was estimated using the Nb2 cell bioassay (22). The cell line was originally obtained from Dr. P. W. Gout (Vancouver, British Columbia, Canada) and propagated in our laboratory using RPMI 1640 medium supplemented with 10% fetal calf serum (Biofluids, Rockville, MD), 10% horse serum (Sigma, St. Louis, MO), 3.0×10^{-5} M 2-mercaptoethanol, 100 units/ml of penicillin, 100 μg/ml of streptomycin, 2.0 m*M* L-glutamine, and 1.0 m*M* HEPES at final concentration. When used to bioassay serum PRL activity, the cells were washed twice with the synthetic medium AIM-V (Cat. No. 320–20055PK; 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 1.0 m*M* HEPES at final concentration. The cells were adjusted to 50,000 cells/ml with the AIM-V medium. Five microliters of each serum pool were added to 200 μl of the AIM-V medium and serially diluted. Four dilutions in triplicate were examined for each sample. One hundred microliters of the Nb2 cells were added to the diluted serum samples. The total volume was 200 μl/well in a 96-well, flat-bottom microtiter plate. A standard PRL curve was constructed using ovine PRL (NIDDK oPRL 19) ranging from 0.32 pg/ml to 5,000 pg/ml and each dose was run in triplicate. The sensitivity of the assay was 5 pg/ml. The cells were cultured for 72 hr at 37°C in a humidified 7% CO_2 gas environment. One microcurie of [^{3}H] thymidine (Cat. No. NET027, 6.7 Ci/m*M*; NEN, Boston, MA)/20 μl was added 16 hr before harvesting. The cells were harvested using a Tomtec Mach II 96-well harvester and counted with a Wallac Betaplate liquid scintillation counter (Gaithersburg, MD). Control, un-

stimulated cultures gave a cpm value of 1,000, while 1 ng/ml of PRL gave a value of 200,000 cpm.

Results

A comparison of the absolute number of PLN cells between dwarfs and normal litter mates is presented in Table I. The number of total CD4+, CD8+, and B220+ cells was lower in dwarf animals when compared with normal animals. However, if the total number of cells was adjusted for the difference in body weight, only the B220+ cells were lower in dwarf animals when compared with normal animals. The administration of rbPRL and rbGH increased the absolute number of total CD4+, CD8+, and B220+ cells in normal animals, but not in dwarf animals. ConA stimulation of the PLN similarly increased the number of total CD4+ and CD8+ cells in both intact and dwarf animals when adjusted for the differences in body weight. However, the number of B cells was still lower in dwarf animals than in normal animals. The administration of either rbPRL or rbGH to normal animals decreased the absolute number of total and subset cells for the PLN stimulated by ConA when compared with the control-stimulated PLN. The injection of rbPRL and rbGH to dwarf animals did not alter the absolute number of total and subset cells for the PLN stimulated by ConA when compared with the unstimulated PLN.

A comparison of the relative percentage of PLN subsets between dwarfs and normal litter mates is presented in Table II. The relative percentage of CD4+ and CD8+ cells was similar in dwarf and normal animals for unstimulated and ConA-stimulated PLN. The administration of either rbPRL or rbGH did not alter the relative percentage of PLN CD4+ or CD8+ cells between dwarf and normal animals and between the ConA-stimulated and unstimulated PLN. The percentage of B220+ cells was less for dwarf animals when compared with normal animals. The injection of rbPRL and rbGH to dwarf animals did not alter the observation, whereas hormone injection of normal animals nearly doubled the percentage of B220+ cells. Neither ConA stimulation nor hormone injection altered the relative percentage of PLN CD4+, CD8+, and B220+

Table I. Influence of rbPRL and rbGH on the Absolute Number of Popliteal Lymph Node Subsets in Snell Dwarf mice

Lymphocyte subset	Animal type	Group[a]	No. of cells ($\times 10^4$)				Fold increase with ConA	
			Lf-unstim		Rt ConA-stim			
			Actual	/g body wt	Actual	/g body wt	Actual	/g body wt
Total	Normal	Control	25	1.1	299	9.7	12.0	8.8
	Normal	PRL	65	2.8	168	7.3	2.6	2.6
	Normal	GH	59	2.5	185	7.7	3.2	3.1
	Dwarf	Control	13	1.8	76	10.7	5.8	5.9
	Dwarf	PRL	14	2.0	64	9.0	4.6	4.5
	Dwarf	GH	10	1.4	47	6.6	4.7	4.7
$CD4^+$	Normal	Control	14	0.6	153	6.5	10.9	10.8
	Normal	PRL	35	1.5	81	3.5	2.3	5.3
	Normal	GH	32	1.5	96	4.0	3.0	2.9
	Dwarf	Control	9	1.3	52	7.3	5.8	5.6
	Dwarf	PRL	9	1.3	42	5.9	4.7	4.5
	Dwarf	GH	6	0.9	30	4.2	5.0	4.7
$CD8^+$	Normal	Control	7	0.3	68	2.9	9.7	9.7
	Normal	PRL	14	0.6	36	1.6	2.6	2.7
	Normal	GH	14	0.6	44	1.8	3.1	3.0
	Dwarf	Control	4	0.6	20	2.8	5.9	4.7
	Dwarf	PRL	5	0.7	18	2.5	3.6	3.6
	Dwarf	GH	3	0.4	13	1.8	4.3	4.5
$B220^+$	Normal	Control	4	0.2	81	3.4	20.2	17.0
	Normal	PRL	17	0.7	50	2.2	2.9	3.1
	Normal	GH	14	0.6	46	1.9	3.2	3.2
	Dwarf	Control	0.8	0.1	4.7	0.7	5.9	7.0
	Dwarf	PRL	0.5	0.1	3.9	0.6	7.8	6.0
	Dwarf	GH	0.8	0.1	4.1	0.6	5.2	6.0

[a] Normal animals were injected subcutaneously with 30 μg of either rbPRL or rbGH twice daily; dwarf animals were injected subcutaneously with 10 μg of either rbPRL or rbGH twice daily.

Table II. Influence of rbPRL and rbGH on the Relative Percentage of Popliteal Lymph Node Subsets in Snell Dwarf Mice

Lymphocyte subset	Animal type	Group[a]	Percentage of cells		Fold increase with ConA
			LF-unstim	Rt ConA-stim	
$CD4^+$	Normal	Control	56.0	51.1	0.91
	Normal	PRL	53.0	48.1	0.91
	Normal	GH	54.6	51.6	0.95
	Dwarf	Control	66.7	68.1	1.02
	Dwarf	PRL	63.8	65.1	1.02
	Dwarf	GH	58.8	62.8	1.07
$CD8^+$	Normal	Control	28.4	22.6	0.80
	Normal	PRL	21.2	21.2	1.00
	Normal	GH	24.0	23.5	0.98
	Dwarf	Control	29.2	26.2	0.89
	Dwarf	PRL	33.9	28.5	0.84
	Dwarf	GH	33.8	28.1	0.83
$B220^+$	Normal	Control	15.8	27.2	1.72
	Normal	PRL	26.1	29.7	1.14
	Normal	GH	23.4	24.7	1.06
	Dwarf	Control	6.4	6.0	0.94
	Dwarf	PRL	3.2	6.1	1.91
	Dwarf	GH	7.7	8.7	1.13

[a] Normal animals were injected subcutaneously twice daily with 30 μg of either rbPRL or rbGH; dwarf animals were injected subcutaneously twice daily with 10 μg of either rbPRL or rbGH.

cells when compared with their appropriate unstimulated controls.

A comparison of the expression of IL-2R for PLN subsets between dwarf and normal litter mates is presented in Table III. Dwarf and normal animals had a similar expression of IL-2R on CD4+ and CD8+ cells. However, the expression on B220+ cells in dwarf animals was over twice that observed for normal animals. The administration of rbPRL to normal animals markedly stimulated IL-2R expression on the CD4+ and CD8+ cells when compared with saline-injected controls, but did not alter the expression in dwarf animals. The administration of rbGH to either normal or dwarf animals had no influence on the expression of IL-2R on CD4+, CD8+, and B220+ cells. ConA stimulation of the PLN from dwarf animals induced an increase in interleukin-2 expression on CD4+ and CD8+ cells that was half that observed for normal animals. However, the defective induction of IL-2R expression after ConA stimulation of dwarf mice was completely corrected by the administration of either rbPRL or rbGH. Hormone injection to dwarf and normal animals did not alter significantly the expression of IL-2R on B220+ cells for ConA-stimulated PLN.

Validation that the dwarf animals were deficient in PRL was provided by measuring serum PRL levels (Table IV). No PRL was detected by the Nb2 cell bioassay in the serum of dwarf animals and the values presented were the lower limit of the assay. The rbPRL injected was bioactive as evidenced by an elevation in the serum PRL levels over that noted for control and GH-injected animals.

Discussion

The major observations of this study are as follows: (i) In the dwarf animal, there is a deficiency in the number of B+ cells in the unstimulated node that is not corrected by the administration of PRL and GH. (ii) There is a defect in the dwarf PLN that results in a lower increase in the absolute number of B cells when stimulated by ConA. It is likely that this is a defect in the ability of cells to enter the node by some chemotactic process rather than a defect in cell division. It is unlikely that cell division occurs in the node during the short course of time (10–12 hr) after the administration of ConA. (iii) The PLN cells of the dwarf animal are not as efficiently activated by ConA to express the IL-2R as those of the normal animal, and this defect can be corrected by the administration of PRL and GH. (iv) In normal animals, but not dwarf animals, PRL administration increases the expression of IL-2R on CD4+ and CD8+ cells from the unstimulated PLN.

Our observation that the percentage of PLN CD4+ cells was slightly elevated in dwarf animals while the CD8+ subset was similar to normal animals is consistent with the observation of others examining another peripheral lymphoid organ, the spleen (17). These investigators, however, did not observe in the spleen a decrease in the percentage of B+ cells as we did for the PLN of dwarf animals, and this may represent a differ-

Table III. Influence of rbPRL and rbGH on the Expression of IL-2R for Popliteal Lymph Node Subsets in Snell Dwarf Mice

Lymphocyte subset	Animal type	Group[a]	Percentage of cells with IL-2R		Fold increase with ConA
			LF-unstim	Rt ConA-stim	
$CD4^+$	Normal	Control	9.0	45.6	5.1
	Normal	PRL	32.1	59.6	1.9
	Normal	GH	9.7	51.6	5.3
	Dwarf	Control	12.0	28.0	2.3
	Dwarf	PRL	11.9	54.7	4.3
	Dwarf	GH	9.6	63.4	6.2
$CD8^+$	Normal	Control	1.9	31.5	16.6
	Normal	PRL	20.6	44.9	2.2
	Normal	GH	2.0	43.2	21.6
	Dwarf	Control	3.2	18.5	5.8
	Dwarf	PRL	2.0	49.1	24.6
	Dwarf	GH	3.0	54.6	18.2
$B220^+$	Normal	Control	4.5	10.4	2.3
	Normal	PRL	6.8	13.6	2.0
	Normal	GH	2.8	13.6	4.9
	Dwarf	Control	11.1	12.6	1.1
	Dwarf	PRL	14.6	18.4	1.2
	Dwarf	GH	7.3	18.5	2.5

[a] Normal animals were injected subcutaneously twice daily with 30 μg of either rbPRL or rbGH; dwarf animals were injected subcutaneously twice daily with 10 μg of either rbPRL or rbGH.

Table IV. Serum Bioactive PRL Levels in Snell Dwarf Mice and Normal Litter Mates

Animal type	Experimental groups	Body wt[a] (g)	No. of serum pools	Serum PRL[a] (ng/ml)
Normal	Control	23.6 ± 0.5	2	11.0; 6.6
Normal	30 μg rbPRL ×2	23.1 ± 0.6	2	22.1; 4.2
Normal	30 μg rbGH ×2	24.1 ± 0.6	2	8.4; 5.3
Dwarf	Control	7.1 ± 0.2	3	<0.3
Dwarf	10 μg rbPRL ×2	7.1 ± 0.3	3	2.2; 2.2; 1.4
Dwarf	10 μg rbGH ×2	7.1 ± 0.3	3	<0.3

[a] Values are for pooled samples.

ence between the two peripheral lymphoid organs. They reported, however, that mesenteric lymph nodes had atrophic cortical lymphoid follicles, suggesting a deficiency in B+ cells (17). The relative percentage of CD4+ and CD8+ cells in the unstimulated PLN was not altered by either PRL or GH and is in agreement with the results of others examining the mesenteric lymph node (18).

The observation that the normal mouse PLN stimulated by footpad injection of ConA had a marked increase in cell numbers (12-fold) when compared with dwarf animals (6-fold) has not been examined previously. Other investigators reported the influence of *in vitro* ConA stimulation of splenic lymphocytes between dwarf and normal litter mates and noted no difference in proliferative activity (16). Differences in animal age, tissue and *in vivo* versus *in vitro* ConA stimulation make it difficult to compare their results with ours.

The expression of IL-2R on PLN lymphocytes induced by ConA footpad injection to normal animals has been examined extensively (23). These investigators have reported that the number of PLN cells and IL-2R expression on lymphocytes is maximum between 9 and 15 hr after ConA footpad injection. They also observed that T cells had a greater expression of IL-2R than did B cells (23). In our study, the percentage of unstimulated normal PLN cells with IL-2R was 9% for CD4+ cells, 2% for CD8+ cells, and 4.5% for B+ cells and was similar to that reported previously (23). The expression of IL-2R on lymphocytes of dwarf animals was 12.0% for CD4+, 3.2% for CD8+, and 11.1% for B+ cells. ConA stimulation of the PLN from normal animals resulted in 46% of the CD4+ cells, 32% of the CD8+ cells, and 10% of the B+ cells expressing IL-2R, while dwarf animals had 28% of CD4+ cells, 18.5% of CD8+ cells, and 11.1% of B+ cells with IL-2R. Thus,

the expression of IL-2R in dwarf animals for T cells was approximately half that observed for normal animals.

Our observation that PRL injection to normal animals increased the percentage of PLN T cells with IL-2R supports the *in vitro* observation of others who reported an increase in IL-2R for splenic T cells from ovariectomized rats cultured with PRL (20). It was surprising to us that PRL did not stimulate the expression of IL-2R receptors on unstimulated lymphocytes in dwarf animals as it did in normal animals. Here perhaps additional hormones, such as GH and thyroid-stimulating hormone, which are absent in the dwarf animals but present in normal animals, may play a significant role. Upon ConA stimulation, however, either PRL or GH induced IL-2R expression in the dwarf animal comparable to that of the normal animal.

The observation that PLN lymphocytes from dwarf animals were able to express IL-2R to ConA stimulation, albeit at a reduced level, suggests that pituitary PRL and GH are not absolute requirements for the expression. A number of reports have indicated that lymphocytes themselves are capable of producing PRL- and GH-like molecules (24–30) and these cells may contribute to the proliferative response observed in the dwarf mouse. Since we only examined a single time period for the expression of IL-2R, it is possible that PLN lymphocytes may take longer for a full expression of IL-2R, and examination of later time periods after ConA injection may result in a similar expression in normal and dwarf animals. Regardless of the temporal relationships in IL-2R expression, the administration of PRL or GH to the dwarf animal returns the expression of IL-2R to that observed for normal animals.

It should be noted that the present observations are based on a single pool of cells from five to seven animals. In future studies elucidating the involvement of PRL and GH in the regulation of the immune system, the dwarf mouse model should play an increasingly important role.

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