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Growth hormone alters lymphocyte sub-populations and antibody production in dwarf rats in vivo

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Abstract. Female dwarf rats at different ages were treated with recombinant porcine GH or with a potent sheep anti-rat GH serum. Body weight and spleen weight increased with GH and decreased with anti-GH treatment ($p < 0.001$). Neither GH nor anti-GH treatment resulted in a change in circulating WBCs, but GH decreased, while anti-GH increased, RBC counts ($p < 0.001$). Similarly, GH treatment tended to decrease the ratio of $CD4^+$:$CD8^+$ T-cells while anti-GH increased ($p < 0.05$) the ratio. Anti-GH treatment also enhanced the animals' ability to produce specific IgG in response to KLH injection. These results indicate that GH may have a physiological role in suppressing humoral immune function but may enhance cell-mediated immunity.
Key words. Growth hormone; thymus; spleen; lymphocytes; antibodies; immune function.

There is considerable evidence of an interrelationship between growth hormone (GH) and components of the immune system. Hypopituitary and hypophysectomized animals are often immune comprised[1,2], and it has been suggested that immune function may be improved following treatment with exogenous GH[3,4]. Thymus weight is often increased following treatment of animals with exogenous GH[5,6], and leukocytes have been shown to be able to produce GH[7,8] and receptors for GH have been identified on lymphocytes[9]. Furthermore, GH increases proliferation of T-cells both in vitro[10,11] and in vivo[12].

Hypophysectomized animals (and many of the hypopituitary strains of rodents) are not only deficient in GH but also a number of other hormones, and this may confound the conclusions from such studies. In the present studies we have used a line of dwarf rats that have an isolated GH deficiency[13] to investigate more specifically the role of GH on the immune system.

Materials and methods

Female dwarf Lewis rats bred at Ruakura were used in these studies. They were housed in cages at 24 °C with 12:12 h light:dark, and standard laboratory rat food and water were available ad libitum.

Administration of GH. At 30, 60, 90 or 180 days of age dwarf rats were randomly allocated to groups of 10 for each treatment and given daily 100 µl subcutaneous (s.c.) injections of either physiological saline, or recombinant porcine GH (Lucky Ltd., Korea) at 0.3 mg/kg or 3.0 mg/kg, dissolved in saline.

Rats at 30 days of age were injected with 3 mg/kg GH or saline for 10 consecutive days. Rats at 60 and 180 days of age were given either 0.3 mg/kg GH, 3.0 mg/kg GH or saline for 10 days. Rats at 100 and 190 days of age were given saline or 3.0 mg/kg GH for 10 days; other rats (at 90 and 180 days of age) were given saline or 3.0 mg/kg GH for 20 days, such that final ages were similar to those that received treatment for 10 days (table 1). Growth rates were measured over the trial periods.

At the end of the treatment periods, the rats were anaesthetized with CO_2, exsanguinated, killed and dissected. Body, thymus and spleen weights were measured after sacrifice.

GH immuno-neutralisation. Twenty 85-day-old female dwarf rats were given 0.25 mg of keyhole limpet haemocyanin (KLH) in a 1:4 ratio of saline:STM (Span Tween Marcol)[14]. Each rat was injected with a total of 400 µl of the emulsion given s.c. at four different sites. Ten additional rats were left untreated.

Twenty days after KLH treatment, 10 of the KLH-treated rats were given 1 ml of normal sheep serum (NSS) injected s.c. in the back of the neck. The remaining 10 KLH-treated rats, and 5 of the untreated rats, were given 1 ml of sheep anti-rat GH serum (capable of binding 200 µg rat GH in vitro) s.c. Subsequently, 0.75 ml injections of the appropriate serum were given daily for the following 9 days. Five rats remained untreated throughout the trial.

Three days after the start of the serum treatment, the KLH-treated rats were given a second (booster) injection of KLH. Twenty four hours after the last serum injection, the rats were anaesthetized with CO_2, exsanguinated, killed and dissected. Body, thymus and spleen weights were measured after sacrifice.

Lymphocyte sub-population determinations. Total white blood cell (WBC) and red blood cell (RBC) counts were determined and lymphocyte sub-populations were ex-

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Table 1. Average daily growth rate during treatment with GH for female dwarf rats at various ages.

Final age (days)	Treatment	Average daily weight gain (g/day)	Final body weight (grams)
40	Saline	2.59 ± 0.08	75 ± 1
40	10 days GH (3 mg/kg)	3.76 ± 0.08***	85 ± 1***
70	Saline	1.15 ± 0.06	117 ± 2
70	10 days GH (0.3 mg/kg)	1.92 ± 0.13***	126 ± 2**
70	10 days GH (3 mg/kg)	3.28 ± 0.10***	137 ± 3***
110	Saline	0.33 ± 0.05	141 ± 3
110	10 days GH (3 mg/kg)	2.74 ± 0.14***	157 ± 5**
110	20 days GH (3 mg/kg)	2.41 ± 0.09***	184 ± 5***
200	Saline	−0.09 ± 0.10	162 ± 4
200	10 days GH (0.3 mg/kg)	1.70 ± 0.26***	181 ± 5**
200	10 days GH (3 mg/kg)	2.95 ± 0.17***	196 ± 4***
200	20 days GH (3 mg/kg)	2.61 ± 0.17***	214 ± 5***

Values are mean ± SEM, **$p < 0.01$, ***$p < 0.001$ compared with the appropriate saline control.

amined with anti-CD3, anti-CD4, anti-CD8 and anti-CD45 monoclonal antibodies.

Lymphocytes were isolated by centrifugation at 400 × g for 30 min at 20 °C with Histopaque (Sigma). The upper layer was aspirated to within 5 mm of the opaque interface and discarded. The opaque interface was added to 10 ml EPBS (phosphate buffered saline containing 2% fetal calf serum and 0.01% NaN_3), centrifuged at 250 × g for 10 min at 4 °C, and the live cell concentration was determined using trypan blue exclusion. The cells were resuspended in EPBS to a concentration of 3×10^7 cells/ml. The optimum amount of various fluorescently labelled monoclonal antibodies for 10^6 cells was determined by titration and used in 10 µl as follows: anti-CD3 (R-PE anti-rat CD3; Pharmingen, San Diego, USA; 0.2 µg), anti-CD4 (FITC anti-rat CD4; Serotec; 1 µg), anti-CD8 (FITC anti-rat CD8a; Pharmingen; 1 µg), anti-CD45 (FITC anti-rat CD45RC; Pharmingen; 0.5 µg). Ten microlitres of appropriate antibody combinations (anti-CD3 + anti-CD4, anti-CD3 + anti-CD8, anti-CD3 + anti-CD45) were added to 30 µl cell suspension, mixed gently and incubated on ice for 25 min. Ten microlitres of propidium iodine was added to each tube and incubation continued for a further 5 min. The cells were washed with 1 ml EPBS, centrifuged at 250 × g at 4 °C for 5 min, the supernatant discarded, and the cells resuspended in 250 µl EPBS. Cell populations were measured by flow cytometry using an Epics Profile Analyser.

Natural killer (NK) cells were those that were $CD8^+$ but $CD3^-$. B-cell numbers were measured by subtracting the NK cells from the number of cells that were $CD45^+$:$CD3^-$.

Antibody responses. Antibody responses to KLH immunization were determined by ELISA using KLH-coated microtitre plates. Antisera were incubated overnight at 4 °C at 10^{-3} to 10^{-6} dilutions. After washing (×3) in 0.05% PBS-Tween, goat anti-rat IgG/horse radish peroxidase conjugate (ImmunoChemical Products, Auckland, New Zealand) was incubated in the wells for 2 h at 22 °C and after washing the colour reaction was developed with 3,3′,5,5′-tetramethyl benzidine as described elsewhere[15].

Statistical analysis. Analysis of variance and Students' t-test were used to determine the statistical significance of the data.

Results

Administration of GH. Treatment with porcine GH caused a significant ($p < 0.001$), dose-dependent increase in body weight gain in all age groups (table 1) and significantly ($p < 0.001$) increased spleen weight (fig. 1a) and thymus weight ($p < 0.01$) in rats over 40 days of age (fig. 2a). Treatment with higher doses of GH over longer periods of time produced a greater effect than lower concentrations or shorter treatment periods.

Relative spleen weight (as a proportion of body weight) was also generally increased with GH treatment (fig. 1b), with the greatest increase occurring with 3 mg/kg GH per day injected for 20 days. However, with the exception of a slight decrease in 40-day-old animals, and a slight increase in 200-day-old animals, the increase in thymus weight was offset by a change in total body weight and, as a result, thymus weight relative to body weight was unaltered by GH treatment (fig. 2b).

Administration of GH had no significant effect on WBC counts, or on RBC counts after a 10-day treatment period, but 20 days of GH treatment caused a significant ($p < 0.001$) reduction in RBC counts in 110-day-old rats (table 2).

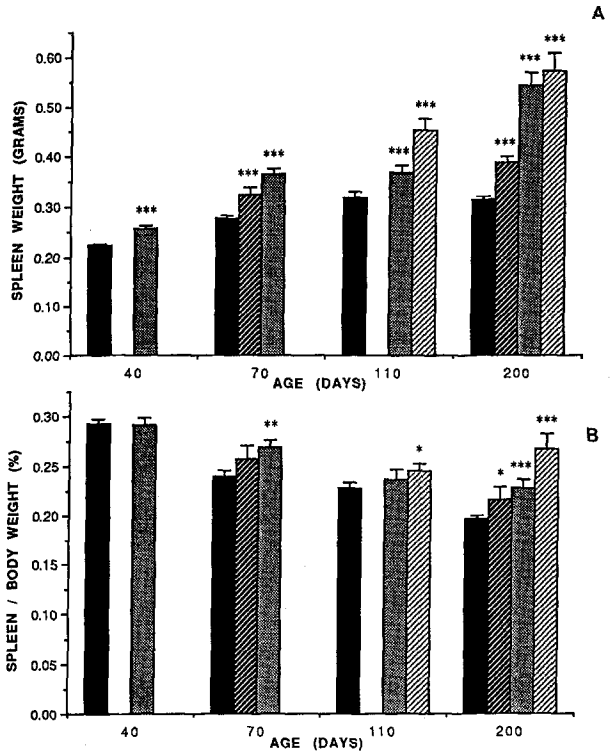

Figure 1. Graph showing the effects of GH treatment on *A* absolute spleen weights, and *B* relative spleen weights of female dwarf rats at different ages. The different treatments include: ■ saline; ▨ 0.3 mg/kg daily GH for 10 days; ■ 3 mg/kg GH daily for 10 days; ▨ 3 mg/kg GH daily for 20 days. Values are mean ± SEM, *p < 0.05, **p < 0.01, ***p < 0.001, compared with the appropriate saline control.

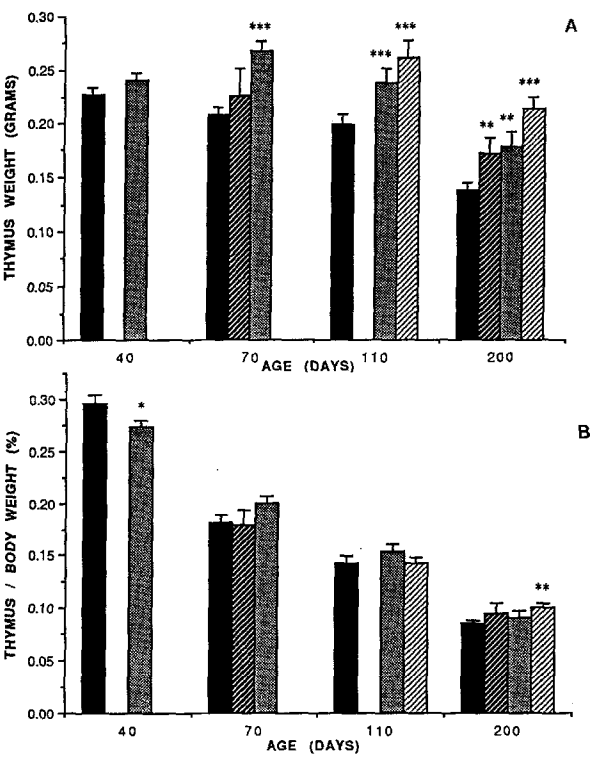

Figure 2. Graph showing the effects of GH treatment on *A* absolute thymus weights, and *B* relative thymus weights of female dwarf rats at various ages. The different treatments include: ■ saline; ▨ 0.3 mg/kg daily GH for 10 days; ■ 3 mg/kg GH daily for 10 days; ▨ 3 mg/kg GH daily for 20 days. Values are mean ± SEM, *p < 0.05, **p < 0.01, ***p < 0.001, compared with the appropriate saline control.

After GH treatment there was no significant effect on the proportion of B-cells, but there was a significant ($p < 0.05$) increase in the proportion of T-cells with a corresponding drop in natural killer cells (fig. 3). Among the T-cell population there was a relatively consistent drop in the ratio of $CD4^+$:$CD8^+$ T-cells with GH treatment for all age groups, but this achieved statistical significance only in the 70-day-old rats (table 3).

GH immuno-neutralisation. There were no growth differences between the KLH- or saline-treated anti-GH rats and the growth data for these groups have been pooled. Treatment with GH antiserum caused an average weight loss of 0.57 ± 0.13 g/day. This was significantly different ($p < 0.001$) from NSS-treated rats which gained 0.18 ± 0.12 g/day. The NSS-treated rats had a significantly lower ($p < 0.05$) growth rate than the untreated rats (0.60 ± 0.16 g/day). It was expected that daily injections of xenogenic proteins would have some effects; for this reason it is important that the anti-GH rats are compared with the appropriate (NSS) controls. Slight decreases in relative and absolute thymus weights were found in anti-GH treated rats, but these decreases were not significantly different from NSS-treated rats (fig. 4). Both relative and absolute spleen weights of anti-GH treated rats were significantly lower ($p < 0.001$) than those of NSS-treated rats (fig. 4).

Table 2. Effect of GH treatment on blood cell counts in female dwarf rats at 110 days.

Treatment	WBC counts 10^6/ml	RBC counts 10^9/ml
Saline	7.09 ± 0.92	8.55 ± 0.10
10 days 3 mg/kg GH	7.13 ± 0.48	8.50 ± 0.72
20 days 3 mg/kg GH	7.94 ± 0.68	7.89 ± 0.08***

Values are mean ± SEM, ***p < 0.001.

Anti-GH treatment had no effect on WBC counts, but resulted in a statistically significant ($p < 0.01$) increase in RBC counts compared with NSS-treatment (table 4). There was no effect of anti-GH treatment on the proportions of T-cells, B-cells or NK-cells (fig. 5), but there was an increase ($p = 0.05$) in the ratio of $CD4^+$:$CD8^+$ cells compared with NSS-treated rats (table 4).

Although there was no significant increase in the proportion of B-cells in the blood of anti-GH treated rats, these animals produced significantly higher ($p < 0.01$) means titres (±SEM) of KLH directed IgG (1:46,800

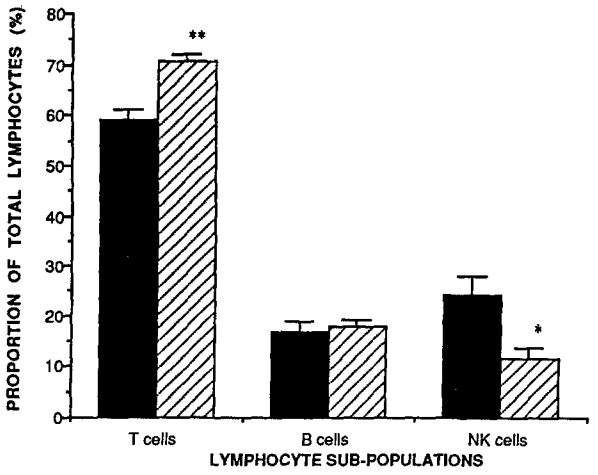

Figure 3. Graph showing the effects of GH treatment on the proportions of lymphocyte sub-populations in 110-day-old dwarf rats. The different treatments include: ■ saline; ▨ 3 mg/kg GH daily for 20 days. Values are mean ± SEM, *p < 0.05, **p < 0.01.

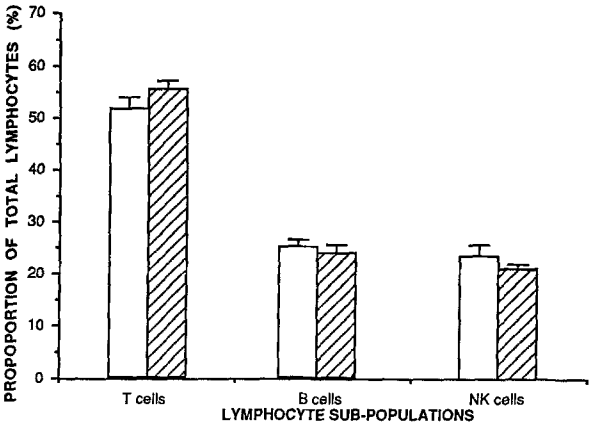

Figure 5. Graph showing effect of anti-GH serum on the proportions of peripheral lymphocyte subpopulations. The different treatments are: □ NSS treatment; ▨ anti-GH serum. Values are mean ± SEM.

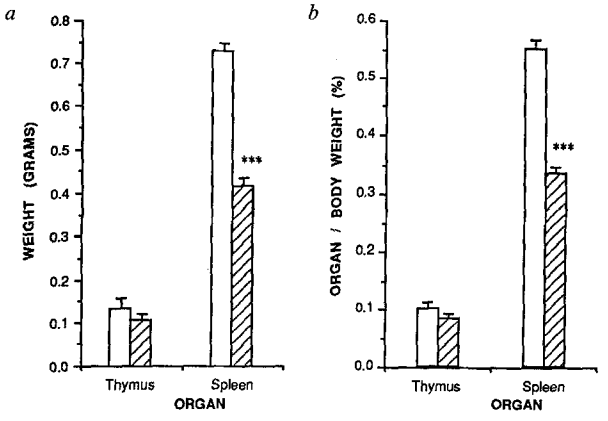

Figure 4. Graph showing *a)* absolute thymus and spleen weights, and *b)* relative thymus and spleen weights, for □ rats treated with NSS; and ▨ anti-GH-treated rats. Values are mean ± SEM, ***p < 0.001.

Table 3. Effect of GH treatment on the ratio of T-helper to T-cytotoxic/suppressor cells in the peripheral blood of female dwarf rats.

Age (days)	Treatment	Ratio of $CD4^+$:$CD8^+$ T-cells
40	Saline	3.10 ± 0.18
40	10 days GH (3 mg/kg)	2.88 ± 0.04
70	Saline	3.06 ± 0.12
70	10 days GH (3 mg/kg)	2.63 ± 0.16*
110	Saline	3.16 ± 0.33
110	10 days GH (3 mg/kg)	3.04 ± 0.15
110	20 days GH (3 mg/kg)	2.99 ± 0.17
200	Saline	3.22 ± 0.13
200	10 days GH (3 mg/kg)	3.09 ± 0.16
200	20 days GH (3 mg/kg)	3.31 ± 0.20

Values are mean ± SEM, *p < 0.05.

Table 4. Comparison of peripheral WBC and RBC counts, $CD4^+$:$CD8^+$ cell ratio and keyhole limpet haemocyanin (KLH) titre for dwarf rats treated with NSS or anti-GH serum.

Treatment	WBC counts 10^6/ml	RBC counts 10^9/ml	$CD4^+$:$CD8^+$	KLH titre ($\times 10^{-3}$)
NSS	8.78 ± 0.93	7.13 ± 0.09	2.74 ± 0.07	1:31.6 ± 2.8
Anti-GH serum	11.05 ± 0.80	7.94 ± 0.09***	2.94 ± 0.07*	1:46.8 ± 4.3**

Values are mean ± SEM, *p = 0.05, **p < 0.01, ***p < 0.001. Comparing NSS treatment with anti-GH serum treatment.

$\pm$ 4300) than did NSS-treated rats (1:31,600 $\pm$ 2800). There was no significant binding of KLH in rats that received anti-rGH alone.

Discussion

The increased body weight with GH treatment confirms that this dose of pGH has biological effects in the rat, while the weight loss with anti-GH treatment is consistent with the antiserum effectively immunoneutralising endogenous GH.

The increased spleen growth with GH, and inhibition of spleen growth by anti-GH serum, concur with similar studies in dwarf mice[5,16,17]. These effects may be partly due to changes in the number of lymphocytes in the spleen[18] as anti-GH serum has been shown to block the proliferative effects of GH on lymphocytes in vitro[19]. These results indicate that GH may play a physiological role in regulating spleen growth, and probably immune status.

The growth of the thymus with GH treatment also agrees with studies in mice. The lack of effect on relative thymus growth at the youngest age was similar to the lack of effect found in 20-day-old mice[5]. This is probably because the thymus is growing at its maximum rate at this age[15]. Pierpaoli and Sorkin[16] reported thymus atrophy with anti-GH treatment in mice, and similarly anti-GH treatment caused a decrease in thymus weight in the present study, although this was not significant compared with NSS-treated control rats.

Although GH is able to stimulate WBC proliferation in vitro[10,11], in vivo studies with pigs[20], dogs[21], cattle[22] and rats (present study) have not been able to detect changes in circulating WBC numbers. Increased WBC counts have been seen following GH treatment of DW/J and Snell-Bagg mice[5,4], but these strains have naturally low levels of WBCs and have undetectable GH levels[23]. Thus proliferation of WBCs may require only trace amounts of GH to maintain normal levels, beyond which increased GH has no effect.

In contrast to its lack of effect on WBCs, GH treatment reduced circulating RBC numbers while anti-GH treatment increased RBCs compared with NSS-treated rats. Decreased RBC counts have been previously reported following GH administration in rapidly growing pigs[20] and rats[24]. Some of this effect may be due to increased retention of RBCs by the spleen (perhaps partly contributing to the increased spleen weight), however some of the effect may be by directly altering RBC numbers. Growth hormone is able to stimulate erythropoiesis in vitro[25], but it has been suggested that the GH-stimulated somatic growth exceeds the rate of erythropoiesis in the rapidly growing animal[24], thus reducing RBC concentration in blood. Furthermore, GH dilutes RBC concentration by increasing plasma volume[26] and can also affect mean corpuscular volume[27]. Similar results were found in the present study (data not shown).

The results from the present study, as discussed so far, are similar to those of previously reported studies. There are, however, few data available on the effects of GH on lymphocyte sub-populations. In contrast to the lack of effects of GH-treatment on B-cells in the present study, growth-deficient children treated with GH have been reported to have decreased B-cell numbers[28]; however, the decrease was only transient.

In all age groups tested, GH-treated rats had a lower ratio of $CD4^+$:$CD8^+$ T-cells, But this decrease only reached statistical significance at 70 days of age. However, strong support for the biological significance of the effect of GH on T-cell ratios is provided by the opposite treatment (anti-GH) having the opposite effect (increased $CD4^+$:$CD8^+$ ratio). These changes in $CD4^+$:$CD8^+$ ratio may have implications for the animals' ability to respond to infection as it has been found that a decrease in the $CD4^+$:$CD8^+$ ratio corresponds with immune suppression[28].

Thus GH produces changes indicative of decrease immune responses. However, the reverse would be expected in the anti-GH treated animals, and indeed the anti-GH treated rats did show an enhanced ability to respond to infection by producing higher antibody titres to KLH than did the control NSS-treated rats. Thus, it appears that while GH may have stimulatory effects on macrophages both in vitro[29] and in vivo[30], the evidence from the present studies suggests that GH suppresses the humoral immune response. The consistency of the opposite treatments having opposite effects strengthens this conclusion. Moreover, GH-deficient dwarf rats show a higher antibody response than normal rats[15]. The specific removal of GH by immunoneutralisation is indicative of a physiological role for GH in regulating immune function.

Using specific GH and prolactin analogues, it has been convincingly shown that GH in vitro works through the prolactin receptor, not the GH receptor, to elicit its effects on lymphocytes in culture[31]. Prolactin levels in the dwarf rat are normal[13]. Thus the differences between the outcomes of these studies and those previously reported may be due to depressed levels of other hormones (particularly prolactin) in the other models.

Further investigations need to be undertaken to determine why lymphocyte sub-populations are sensitive to GH, and to determine how the change in their proportions affects an animal's immune response (both humoral and cell-mediated) to infection.

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