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Evidence for suppression of immune function by insulin-like growth factor-1 in dwarf rats in vivo

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Abstract. These studies were undertaken to investigate the effects of increasing or decreasing IGF-1 levels on aspects of immune function in rats. Female dwarf rats were treated with recombinant human IGF-1 or with a potent sheep anti-IGF-serum. Body weight, thymus weight and spleen weight increased with IGF-1 treatment ($p < 0.001$), while there was no effect of anti-IGF-1 treatment when compared with the appropriate normal sheep serum (NSS) treated controls. IGF-1 treatment significantly decreased WBC and RBC counts, but increased the ratio of $CD4^+$:$CD8^+$ T-cells. Anti-IGF-1 serum had no effect on these parameters compared with NSS. However anti-IGF-1 was associated with increased T-cell numbers, decreased natural killer cells, and enhancement of the animals' ability to produce specific IgG in response to injection of keyhole limpet haemocyanin (KLH). These results indicate that IGF-1 may suppress immune function although increasing the size of immune organs such as spleen.
Key words. Insulin-like growth factor-1; thymus; spleen; lymphocytes; antibodies; immune function.

Various reports have indicated that insulin-like growth factor-1 (IGF-1) may influence certain aspects of the immune system. Sub-populations of white blood cells (WBCs) have been found to synthesise and secrete IGF-1[1, 2] and many reports have shown that WBCs express receptors for IGF-1[3–7]. Several reports have shown that IGF-1 enhances proliferation of lymphocytes in vitro[8–10], although others have also clearly shown that IGF-1 suppressed lymphocyte proliferation[11]. Regardless of its effect on proliferation, IGF-1 has been shown to be a signal for priming neutrophils to secrete superoxide anion[12], suggesting that it may have a role in cell-mediated immune responses. Although there have been some reports on the effects of IGF-1 on lymphoid tissue[13, 14], little work has been carried out to determine the effects of IGF-1 on the immune system in vivo.

The following study examined the effects of IGF-1 administration on lymphoid tissues and peripheral WBC sub-populations in growth hormone (GH)-deficient dwarf rats. Furthermore, since removal of endogenous hormones is generally accepted to be a better indicator of the physiological significance of a hormone than administration of hormones, we have compared the effects of immuno-neutralisation of IGF-1 with administration of the hormone.

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Materials and methods

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

Administration of IGF-1. Fourteen 90-day old female dwarf rats were weighed and allocated to one of two groups of similar average body weight. Seven 2002 (14-day) Alzet mini-osmotic pumps were filled with recombinant human IGF-1 (Ciba-Geigy, Basel) and similarly, seven controls received saline. The minipumps had a nominal pumping time of 14 days, but were found to have an actual life of 17 days, although consistency of the pumping rate was not guaranteed for days 14–17. The pumps were therefore filled with sufficient IGF-1 to allow delivery for 17 days at 1 mg/kg/day. The weighed minipumps were inserted between the scapulae while the rats were under light CO_2 anaesthesia.

Body weight changes were monitored over the trial, and 19 days following minipump insertion the rats were killed with CO_2 gas. Blood was collected by cardiac puncture using EDTA as an anti-coagulant. Spleen and thymus were removed and weighed, and total white blood cell (WBC) and red blood cell (RBC) counts were determined by flow cytometry.

Immuno-neutralisation of IGF-1. To study the possible role of endogenous IGF-1 in regulating immune function, 18 female dwarf rats at 100 days of age were injected with 0.25 mg of keyhole limpet haemocyanin (KLH) to stimulate IgG production. The KLH was

injected in 400 µl of a 1:4 emulsion of saline:STM (Span Tween Marcol)[15] given subcutaneously (SC) at four different sites.

Twenty days after the primary KLH treatment, 9 of the KLH-treated rats were given 1 ml of normal sheep serum (NSS) injected sc in the back of the neck. The remaining 9 KLH-treated rats were given 1 ml of sheep anti-IGF-1 serum[16] subcutaneously. Subsequently, 0.75 ml injections of the appropriate serum were given daily for the following 10 days.

Three days after the start of the serum treatment (i.e. 23 days after the primary immunisation), the KLH-treated rats were given a second injection of KLH. Body weight changes were monitored over the trial, and the day following the final injection of sheep serum (the final age was 130 days) the rats were killed with CO_2 gas. Blood was collected by cardiac puncture using EDTA as an anti-coagulant. Spleen and thymus were removed and weighed, and total WBC and RBC counts were determined by flow cytometry.

Lymphocyte sub-population determinations. Total WBC and RBC counts were determined and lymphocyte sub-populations were examined with anti-CD3, anti-CD4, anti-CD8 and anti-CD45 monoclonal antibodies to determine total T-cell population, class II-restricted T-cells (helper T-cells), class I-restricted cells (cytotoxic/suppressor T-cells) and B-cells, respectively.

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 in the pellet determined using trypan blue exclusion. The cells were re-suspended 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 aliquots as follows: anti-CD3 (R-PE anti-rat CD3; Pharmingen; 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; 0.5µg). 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 iodide were added to each tube to define dead cells on flow cytometry, 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 re-suspended in 250 µl EPBS. Cell populations were measured using an Epics Profile Analyser.

Hormone analysis. Plasma IGF-1 concentrations were measured in final plasma samples by radioimmunoassay following extraction by acid-ethanol cryo-precipitation as described elsewhere[17].

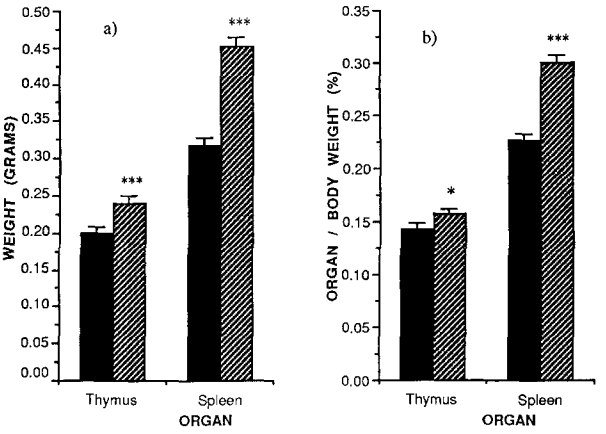

Figure 1. *a*) Absolute and *b*) relative weights of thymus and spleen for ■ saline-treated rats and ▨ rats treated with IGF-1. Values are mean ± s.e.m., *$p < 0.05$, ***$p < 0.001$.

Antibody responses. Antibody responses to KLH immunisation were determined by ELISA using KLH-coated microtitre plates. Antisera at 10^{-3} to 10^{-6} dilutions were incubated overnight at 4 °C. After washing (×3) in 0.05% PBS-Tween, goat anti-rat IgG/horse radish peroxidase conjugate (ImmunoChemical Products, Auckland, NZ) was incubated in the wells for 2 h at 22 °C, and after washing once again the colour reaction was developed with 3,3′,5,5′-tetramethyl benzidine[17a].

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

Results

Administration of IGF-1. The body weight gain of the IGF-1-treated rats (0.91 ± 0.10 g/day) was significantly greater ($p < 0.001$) than that of the control rats (0.33 ± 0.05 g/day). Nineteen days after insertion of the minipumps the plasma IGF-1 concentrations in the IGF-1-treated rats (378 ± 44 ng/ml) were also significantly higher ($p < 0.001$) than in the controls (105 ± 4 ng/ml).

Administration of IGF-1 resulted in a significantly heavier ($p < 0.001$) thymus and spleen, both in absolute

Table 1. Effect of IGF-1 treatment on peripheral WBC and RBC counts and the ratio of $CD4^+$ to $CD8^+$ of female dwarf rats. Values are mean ± s.e.m., *$p < 0.05$, **$p < 0.01$.

Treatment	WBC counts 10^6/ml	RBC counts 10^9/ml	$CD4^+$:$CD8^+$
Saline	8.25 ± 0.40	8.58 ± 0.10	2.66 ± 0.02
IGF-1	6.24 ± 0.55**	8.29 ± 0.06*	2.90 ± 0.10*

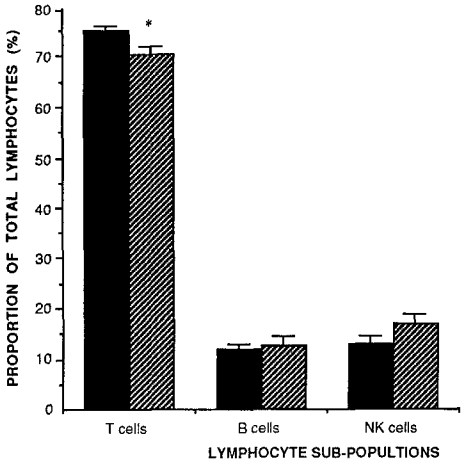

Figure 2. Effect of IGF-1 treatment ▨ compared with saline controls ■ on the proportion of lymphocyte sub-populations in the blood. Values are mean ± s.e.m., *$p < 0.05$.

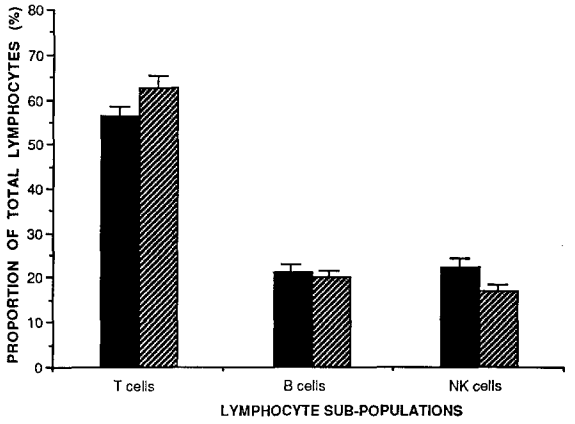

Figure 4. Effects of anti-IGF-1 serum treatment ▨ compared with controls receiving normal sheep plasma ■, on the proportions of peripheral lymphocyte sub-populations. Values are mean ± s.e.m.

terms (figure 1a) and when expressed relative to the body weight of the animals (figure 1b). Treatment with IGF-1 was also associated with a decline ($p < 0.01$) in circulating WBC counts and a reduction ($p < 0.05$) in peripheral RBC counts (table 1).

There was a significant ($p < 0.05$) reduction in the proportion of T-cells with IGF-1 administration (figure 2), and a corresponding increase in the proportion of natural killer (NK) cells, but this did not reach statistical significance ($p = 0.07$). The ratio of $CD4^+$:$CD8^+$ T-cells increased significantly with IGF-1 treatment (table 1).

IGF-1 Immuno-neutralisation. There was no significant difference between anti-IGF-1 and NSS-treated rats with respect to average daily weight gain (0.10 ± 0.16 g/day compared with -0.27 ± 0.12 g/day, respectively). There was also no significant difference between anti-IGF-1 and NSS-treated rats in absolute or relative thymus or spleen weight (figure 3a, 3b) nor in WBC and RBC counts (table 2). Compared with lymphocyte populations in NSS-treated rats, anti-IGF-1 treatment increased $CD3^+$ T-cell number ($p = 0.07$) and slightly decreased NK cells (figure 4), but there was no effect on the CD_4^+:$CD8^+$ ratio (table 2).

The mean titre (±SEM) for antibodies produced against KLH in the anti-IGF-1 treated rats ($1:72{,}800 \pm 8000$) was significantly higher ($p < 0.001$) than titres found in the NSS-treated rats ($1:33{,}000 \pm 5600$).

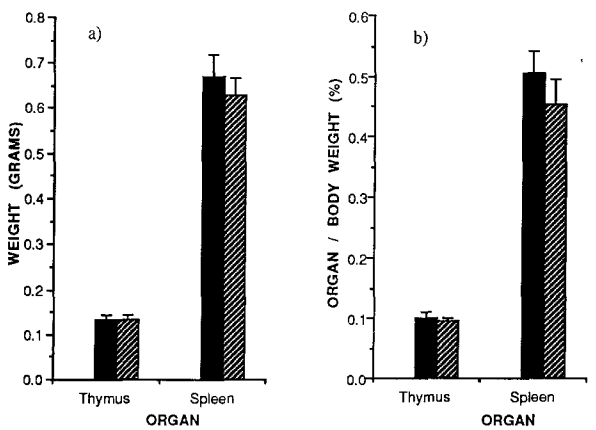

Figure 3. *a*) Absolute and *b*) relative weights of thymus and spleen in rats treated with NSS ■, and anti-IGF-1 ▨. Values are mean ± s.e.m.

Discussion

The increased body weight with IGF-1 administration to GH-deficient rats agrees with other studies[18–20]. This shows that IGF-1 is partially able to substitute for GH in GH-deficient animals, but the stimulation of growth is much less than can be achieved by GH therapy [20, 21]. The lack of effect of anti-IGF-1 on growth is consistent with other reports in rats[16] and guinea pigs[22], and provides further evidence that circulating endogenous IGF-1 is of minimal importance in regulating growth.

Administration of IGF-1 stimulated both absolute and relative weight of the thymus and spleen. The specific stimulation of weight of these tissues confirms the findings of others[19, 23, 24]. Although cell populations in these organs were not measured in the present studies, others[13, 14] have suggested that the weight gain of these organs may be due to increased numbers of T- and B-cells. Administration of IGF-1 antiserum had no effect on thymus weight, suggesting that plasma IGF-1 is not of physiological importance in maintaining thymus weight, and may indicate that the age-related decline in IGF-1 may not be responsible for thymus regression in adult rats. In the case of spleen weight, anti-IGF-1 serum did not have the opposite effect to

Table 2. Effect of anti-IGF-1 serum treatment on the peripheral WBC and RBC counts, $CD4^+$:$CD8^+$ and keyhole limpet haemacyanin (KLH) titre in female dwarf rats. Values are mean ± s.e.m. ***$p < 0.001$.

Treatment	WBC counts 10^6 cells/ml	RBC counts 10^9 cells/ml	$CD4^+$:$CD8^+$	KLH titre ($\times 10^{-3}$)
NSS	10.28 ± 1.47	7.41 ± 0.10	2.55 ± 0.10	1:33.5 ± 8.0
Anti-IGF-1 serum	11.04 ± 1.46	7.56 ± 0.16	2.70 ± 0.10	1:72.8 ± 5.6***

IGF-1. This may be due to a non-specific immune reaction in response to the large amounts of xenogenic antigens (sheep immunoglobulins) being injected each day obscuring the treatment differences.

The decrease in peripheral WBC counts and in the proportion of peripheral T-cells with IGF-1 treatment in vivo, contrasts with reports that IGF-1 stimulates WBC[7, 9, 10] and T-cell[6, 7] proliferation in vitro, but they do agree with the findings of Hunt and Eardley[11]. However, as this treatment increased the spleen weight, it is quite possible that IGF-1 administration induced the sequestration of lymphocytes in the spleen, resulting in a reduced number in the peripheral circulation. In mice, IGF-1 treatment causes WBC and T-cell proliferation in the spleen[14], but it seems that they are not released into the blood.

The slight decline in RBCs also apparently contrasts with in vitro studies, indicating IGF-1 stimulates erythropoiesis[23, 25]. However, GH also stimulates erythropoiesis in vitro[26] but does not lead to increased RBCs in vivo, and it has been suggested that this is because the GH-stimulated somatic growth exceeds the rate of erythropoiesis in the rapidly growing animal[27]. This may also be an explanation for the blood cell results with IGF-1 treatment in the present study. The lack of significant effect of IGF-1 antiserum on these parameters does little to resolve this conundrum, although the trend with anti-IGF-1 treatment compared with NSS-treated animals (increased WBC and RBC) is the opposite of the decrease with IGF-1 treatment.

The relatively high number of IGF-1 receptors present on NK cells (Kooijman et al., 1992b) may be related to the slight increase in NK cell numbers with IGF-1 treatment. Although the increase was not statistically significant, the opposite effect (a decrease in NK cells) with anti-IGF-1 treatment would support the speculation. Similarly, the lack of effect of either IGF-1 or anti-IGF-1 treatment on B-cells is consistent with the low numbers of IGF receptors on their surface[8].

Kooijman et al.[8] also demonstrated a higher number of IGF receptors on $CD4^+$ than on $CD8^+$ cells. The treatment with IGF-1 may therefore cause a specific proliferation of $CD4^+$ cells (as demonstrated in mouse spleen[14]) thus causing an increase in the $CD4^+$:$CD8^+$ ratio, as in the present studies.

Appropriate models for experiments with IGF-1 administration in vivo are scarce. Often, hypophysectomized animals are used but, unlike the GH-deficient dwarf used in these studies which has a discrete GH deficiency[28], hypophysectomized and hypopituitary rodents are also deficient in other hormones. In view of the likely role of prolactin in regulating immune function[29], the GH-deficient dwarf may be a better model for normal animals in hormone replacement studies[30]. The usefulness of this model can be seen with the growth response to the dose of IGF-1 used. While the doses used in the present study are higher than those usually used in humans, they are less than those used in most rodent studies[14, 18, 20, 31].

Although alteration of IGF-1 levels did not have a major influence on circulating lymphocytes, anti-IGF-1 treatment was associated with an increased specific IgG antibody production. This finding is similar to that reported for anti-GH treatment in rats[30]. Since most antibody production occurs in lymphatic tissue, it is possible that changes in cell populations in the spleen (which was considerably increased in weight with this treatment), had an influence in producing the high IgG response. Further work needs to be carried out to determine the effects of anti-IGF-1 on cells within lymphatic tissue, and whether this influences secondary antibody response.

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