Influence of insulin-like growth factor-1 (IGF-1) on components of the haematopoietic and lymphatic systems

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ABSTRACT

Increased thymic weights have been reported in sheep selected for resistance to internal parasites and treatment with IGF-1 increases thymic and splenic weights in sheep, suggesting that IGF-1 may have a role in regulating immune function. This hypothesis was examined in four experiments using mice as a model. *Expt 1* Unselected Swiss mice (n=24) were injected 2x daily with 30 µg/d rhIGF-1 or saline for 26 days from 28 days of age. IGF-1 treatment increased spleen weight at a common body weight (118.2 vs 95.9, pooled standard error (PSE) = 6.2 mg, P<0.05), but did not affect thymic regression (thymic weights at the end of treatment: 85.5 vs 81.9, PSE = 4.0 mg). *Expt 2* Unselected female Swiss mice (n=22, age=84.8±0.8 days) were injected 3x daily with 30 µg/d rhIGF-1 or saline for 17 days. IGF-1 treatment increased thymic weight at a common body weight (78.4 vs 58.0, PSE = 3.0 mg, P<0.01), but had little effect on splenic weight (180.1 vs 163.0, PSE = 7.5 mg, P>0.10). *Expt 3* Histological sections of spleens from High (H), Control (C), and Low (L) IGF-1 selected mice at 30 (n=30) and 120 (n=35) days of age were analyzed for the density of megakaryocytes (precursor cells for thrombocytes), and the percentage of the area covered by white pulp (lymphatic tissue). L mice had, compared to C and H mice, lighter spleens (62.6 vs 76.8 vs 89.6, PSE = 5.3 mg, at 30d; 60.2 vs 109.5 vs 114.5, PSE = 7.4 mg, at 120d; P<0.05), and a lower density of megakaryocytes (9.2 vs 13.4 vs 14.7, PSE = 1.6 n/mm^2, at 30d; 2.1 vs 13.9 vs 14.3, PSE = 2.0 n/mm^2, at 120d; P<0.05), but were not different in the percentage of the area covered by white pulp (16.1 vs 14.1 vs 13.4, PSE = 1.3%, at 30d; 28.3 vs 23.1 vs 23.8, PSE = 2.4%, at 120d). *Expt 4* Mice from the three IGF-1 selection lines (n=13 vs n=13 vs n=14 for L vs C vs H, age=86.8±2.3 days) showed no difference in spleen weights (110.8 vs 137.4 vs 139.0, PSE = 11.8 mg; P>0.10). In response to an antigenic challenge with sheep red blood cells (SRBC), there was a small effect (P=0.10) of selection line on the number of plaque-forming (immunoglobulin M (IgM)-producing) cells (PFC) per spleen (880x10^3 vs 954x10^3 vs 1187x10^3, PSE = 130x10^3), but the differences in anti-SRBC antibody titres (IgM) in the plasma (10.8 vs 11.1 vs 11.7, PSE = 0.6) were not significant. It is concluded that IGF-1 affects splenic and thymic weights independently, that it influences the density of megakaryocytes in the spleen, and that it has an effect on the number of PFC per spleen in response to a SRBC challenge. However, the biological significance of these effects is still to be established.

Key words: insulin-like growth factor-1; immune system; spleen; megakaryocytes; thymic regression; humoral immune response.

INTRODUCTION

There is increasing evidence that insulin-like growth factor-1 (IGF-1) influences the growth and function of the thymus and spleen, and possibly influences immune status. Thus accelerated splenic growth has been observed in: mice transgenic for human IGF-1 (Matthews *et al.*, 1988); Snell dwarf mice, hypophysectomized rats, and mutant GH-deficient rats infused or injected with IGF-1 (van Buul-Offers *et al.*, 1986; Guler *et al.*, 1988; Skottner *et al.*, 1989); and mice selected for high circulating IGF-1 concentrations (Siddiqui *et al.*, 1992). The situation is less clear with respect to the effects of IGF-1 on thymic growth and regression of this organ with aging. Van Buul-Offers *et al.* (1986) found that IGF-1 treatment of 6-8 week old Snell dwarf mice produced only a small and non-significant increase in thymic weight after 4 weeks of treatment, whereas the effect of human GH (hGH) was significant. Conversely, in another study (Guler *et al.*, 1988) human IGF-1 normalised the thymic weights of 9-10 week old hypophysectomized rats (*ie.* returned them to weights similar to those of intact rats), whereas hGH did not. However, we have shown that mice selected for high circulating IGF-1 concentrations have greater peak thymic weights at 28 days of age than mice selected for low IGF-1 concentrations, although the rate of decline in thymic weight with aging was similar in the two selection lines (Siddiqui *et al.*, 1992).

Specific receptors for IGF-1 have been found on both resting and activated human T-lymphocytes (Kozak *et al.*, 1987; Tapson *et al.*, 1988), on human peripheral blood monocytes and B-lymphocytes (Stuart *et al.*, 1991), and on circulating bovine mononuclear cells and neutrophils (Zhao *et al.*, 1992). This suggests that IGF-1 may have a generalized proliferating effect on cells of both the lymphatic and haematopoietic systems.

Clark *et al.* (1993) infused adult male mice with rhIGF-1 (4 mg/kg/d) for 7 or 14 days, and found prominent increases in the weight of spleen and thymus, and in the number of lymphocytes in both organs. In addition to an increase in T cells, an increase in

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splenic B cells was observed which was accompanied by an enhanced responsiveness to dinitrophenyl ovalbumin resulting in increased immunoglobulin G (IgG) production *in vitro*. Hence, there is some evidence that IGF-1 may have a role in regulating immune function *in vivo*. Increased thymic weights have also been reported in sheep genetically resistant to internal parasites (Presson *et al.*, 1988). The possible role of IGF-1 in regulating components of the haematopoietic and lymphatic systems was therefore addressed in the present study using mice as a model for farm animals.

MATERIALS AND METHODS

Experiment 1: Effect of IGF-1 on thymic regression.

Unselected Swiss mice were assigned to one of two treatment groups (n=12). As a pilot study had shown that thymic weights in Swiss mice peaked at 21 to 28 days of age, treatments commenced when the mice were 27.6 ± 0.1 (mean ± SEM) days old. One (control) group (3 females, 9 males) was injected subcutaneously (neck) for 26 days with saline 2x daily (0800h and 2000h), while the second group (3 females, 9 males) was injected with recombinant human IGF-1 (rhIGF-1, 30μg/day) 2x daily in the same way. On the day of slaughter (day 27 of the study) the mice were weighed, euthenased, and the thymus and spleen recovered, blotted dry, and weighed.

Experiment 2: IGF-1 and splenic/thymic growth in aged mice.

Unselected female Swiss mice were assigned to one of two treatment groups (n=11) commencing at 84.8 ± 0.8 days of age. One (control) group was injected subcutaneously (neck) for 17 days with saline 3x times daily (0800h, 1500h and 2200h), while the second group was injected with rhIGF-1 (30μg/day) in the same way. On the day of slaughter (day 18 of the study) the mice were weighed, euthenased, and the spleen and thymus recovered, blotted dry, and weighed.

Experiment 3: Splenic cell types in IGF-1 selected lines.

This study utilised mice from the Massey University lines divergently selected for plasma IGF-1 concentrations at 42 days of age (Blair *et al.*, 1989). They were derived from the 8th generation of random breeding after 7 generations of divergent selection. At 30 and 120 days of age, males of the Low (L) IGF-1 line (n=11 and n=12, respectively), Control (C) line (n=9 and n=12) and High (H) IGF-1 line (n=10 and n=11) were weighed and euthenased. The spleens were excised, blotted dry, weighed, fixed in 10% neutral formal saline, and embedded in paraffin wax. Longitudinal sections were cut (6μm thick), mounted on glass slides, and stained with haematoxylin and eosin. The total area of the spleen and the area covered by the white pulp were measured, and the density of megakaryocytes counted on one slide per animal.

Experiment 4: Response to immune challenge in IGF-1 selected lines

This study utilised 13 L, 13 C and 14 H mice from the Massey University lines. They were derived from the 17th generation of random breeding after 7 generations of divergent selection, and were 86.8 ± 2.3 days old. On day 0 the mice were injected intraperitoneally with 0.3 ml of 5% packed sheep red blood cells (SRBC) in physiological saline. On day 5 the animals were blood sampled from the orbital sinus, euthenased, and the spleens recovered, weighed, and kept on ice in HEPES-buffered balanced salt solution (BSS) containing 5% fetal calf serum (FCS). A haemolytic plaque assay based on the slide method using microchambers (Marbrook, 1980) was performed, and the number of plaque-forming cells (PFC) per spleen calculated. Haemolysin titres were determined by a microhaemolysin technique in standard plates (Vögeli *et al.*, 1984). Titres were expressed as the $\log_2$ of the reciprocal of the highest dilution giving complete haemolysis.

Animal housing and feeding

The mice were housed in cages (3 to 5 per cage) in 16h light:8h dark (lights on at 0600h) and ambient temperature of 21°C. They were given *ad libitum* access to fresh water and a pelleted complete diet throughout the study.

Statistical Analysis

Data from Experiments 1 and 2 were subjected to analysis of covariance (with final live weight as the covariate) to test effects of IGF-1 treatment on organ weights, adjusted to a common sex and rearing rank (Expt 1). Data from Experiments 3 and 4 were subjected to analysis of covariance (with age as the covariate) to test effects of IGF-1 selection line adjusted to a common litter size (Expt 3) or sex (Expt 4). Analyses were performed using the statistical package SAS (1988).

RESULTS

Young mice treated twice daily for 26 days with IGF-1 (Expt 1) did not differ from saline-treated mice in thymic weight (Table 1), whereas spleen weights were substantially increased by IGF-1 treatment (+23%, P<0.05). The opposite effect - ie no increase in spleen weight but a 35 % (P<0.01) increase in thymic weight - was observed in Expt 2 when adult mice were treated 3x daily with IGF-1 (Table 1).

TABLE 1: Spleen and thymus weights (adjusted to a common final liveweight) in Swiss mice treated with either saline or rhIGF-1 for 26 days from 28 days of age (Expt 1) or for 17 days from 85 days of age (Expt 2).

Parameter	Saline	IGF-1	Pooled S.E.
Expt 1			
n	12	12	
Spleen (mg)	95.9	118.2 *	6.2
Thymus (mg)	81.9	85.5	4.0
Expt 2			
n	11	11	
Spleen (mg)	163.0	180.1	7.5
Thymus (mg)	58.0	78.4**	3.0

* P<0.05, ** P<0.01

The selection line effect was highly significant for liveweight and spleen weight at 30d and 120d of age in Expt 3 (Table 2). Mice from the H line were heavier (+20% and +33%, P<0.05) and had heavier spleens (+43% and +90%, P<0.05) than L mice, with the C mice midway between the two selection lines. Although H mice from Expt 4 (Table 3) had 25% heavier spleens than L mice, the difference was not significant. At both 30d and 120d of age, H and C animals had significantly greater megakaryocyte densities than L animals (+60% and +681%, P<0.05; Table 2). The percentage of the area covered by white pulp was consistently smaller in H and

C mice than in L mice, but none of the differences was significant (Table 2). Differences between the three selection lines in anti-SRBC titres were not significant but there was a greater number (P=0.10) of PFC in H animals than in L animals, with the C group being intermediate (Table 3).

TABLE 2: Liveweight, spleen weight, percentage of area covered by white pulp, and density of megakaryocytes (adjusted to a common birth rank and age) at the age of 30d and 120d in male mice from lines selected for High, Control, or Low circulating IGF-1 concentrations (Expt 3).

Parameter	High	Control	Low	Pooled S.E.
30 days of age				
n	10	9	11	
Liveweight (g)	17.0[a]	15.8[a]	14.2[b]	0.6
Spleen (mg)	89.6[a]	76.8[ab]	62.6[b]	5.3
White pulp (%)	13.4	14.1	16.1	1.3
Megakaryocyte (n/mm^2)	14.7[a]	13.4[ab]	9.2[b]	1.6
120 days of age				
n	11	12	12	
Liveweight (g)	34.7[a]	30.4[b]	26.1[c]	1.0
Spleen (mg)	114.5[a]	109.5[a]	60.2[b]	7.4
White pulp (%)	23.8	23.1	28.3	2.4
Megakaryocyte (n/mm^2)	14.3[a]	13.9[a]	2.1[b]	2.0

[a, b, c]: Means with different superscripts are significantly different (P<0.05)

TABLE 3: Spleen weight, number of plaque forming cells (PFC), and haemolysin antibody (AB) titre (adjusted to a common sex and age) in mice selected for High, Control, or Low circulating IGF-1 concentrations (Expt 4).

Parameter	High	Control	Low	Pooled S.E.
n	14	13	13	
Spleen (mg)	138.9	137.2	111.3	12.1
PFC (x10^3/spleen)	1186.9	954.2	879.7	130.2
AB titre (log_2)	11.7	11.1	10.8	0.6

DISCUSSION

The positive effect of IGF-1 on spleen growth has been widely reported in the literature for a number of species. Thus, increased spleen weights were observed in mice selected for high circulating IGF-1 concentrations (Siddiqui *et al.*, 1992), and yearling castrate male sheep or adult male mice treated with IGF-1 (Cottam *et al.*, 1992; Clark *et al.*, 1993). In this study, significantly heavier spleens were recorded in young mice treated with IGF-1 vs saline (*Expt 1*, +23%, P<0.05) and in mice selected for High vs Low plasma IGF-1 concentrations (*Expt 3*, +43% at 30d, +90% at 120d, P<0.001) but not in mice greater than 80d of age treated with IGF-1 (*Expt 2*). This suggests that the responsiveness of the spleen to exogenous IGF-1 treatment is influenced by age.

In *Expt 2*, adult mice treated 3x daily with IGF-1 had significantly increased thymus weights (+35%, P<0.01) as compared to animals injected with saline. This is consistent with results from Clark *et al.* (1993) and Guler *et al.* (1988), who reported that exogenous IGF-1 treatment could reverse age-related thymic regression in adult mice and adult hypophysectomized rats. However, no such effect was observed in *Expt 1* when growing mice were treated 2x daily with IGF-1. Thus, IGF-1 administered 3x daily appears to increase thymic weights in animals which have already undergone thymic regression but with 2x daily administration it does not retard the process of thymic regression.

Expt 3 examined the histology of spleens from mice divergently selected for plasma IGF-1 concentrations. The main finding was a higher density of megakaryocytes (precursor cells for thrombocytes) found in H line mice as compared to L line mice at 30d and 120d of age, which is consistent with other reports of IGF-1 having effects on cells of the haematopoietic system (Kurtz *et al.*, 1990; Zhao *et al.*, 1992).

Clark *et al.* (1993) observed an enhanced responsiveness to repeated injections of dinitrophenyl ovalbumin resulting in increased immunoglobulin G (IgG) synthesis *in vitro*. In the study presented here the immune responsiveness of the three IGF-1 selection lines was assessed by the *in vivo* immune response to a single injection of SRBC (*Expt 4*). While the number of PFC per spleen (IgM producing cells) was greater (P=0.10) in H animals than in L animals, the haemolytic test was perhaps not sensitive enough to also detect differences in IgM antibody titres in the plasma.

It is concluded that IGF-1 affects splenic and thymic weights independently, that it influences the density of megakaryocytes in the spleen, and that it has an effect on the number of PFC per spleen in response to a SRBC challenge. However, the biological significance of these effects is still to be established, and we are currently investigating the direct effect of differing plasma IGF-1 concentrations on the resistance to internal parasites in sheep.

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