

Pregnancy and lactation modulate maternal splenic growth and development of the erythroid lineage in the rat and mouse

Juan J. Bustamante^{A,B,E}, Guoli Dai^C and Michael J. Soares^{A,B,D,F}

^AInstitute of Maternal–Fetal Biology, University of Kansas Medical Center, Kansas City, KA 66160, USA.

^BDivision of Cancer and Developmental Biology, Department of Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas City, KA 66160, USA.

^CDepartment of Pharmacology, Toxicology, and Therapeutics, University of Kansas Medical Center, Kansas City, KA 66160, USA.

^DDepartment of Obstetrics and Gynecology, University of Kansas Medical Center, Kansas City, KA 66160, USA.

^EPresent address: Department of Pharmaceutical Sciences, Texas A&M Health Science Center, Irma Lerma Rangel College of Pharmacy, Kingsville, TX 78363, USA.

^FCorresponding author. Email: msoares@kumc.edu

Abstract. Maternal physiology changes dramatically during the course of gestation and lactation to meet the needs of the developing fetus and newborn. In the present study, we examined the influence of pregnancy and lactation on growth and erythroid gene expression patterns of the maternal spleen. Holtzman Sprague-Dawley rats and CD-1 mice were killed at various stages of gestation and post partum. We observed pregnancy dependent increases in spleen weight and spleen DNA content in both the rat and mouse. In the rat, spleen size was greatest at the end of pregnancy and regressed post partum. In contrast, mouse spleen size peaked by gestational Day 13 and regressed to its non-pregnant weight before parturition. Pregnancy dependent changes in the size of the spleen were primarily due to an increase in red pulp. Maternal spleen expression of erythroid-associated genes (*erythroid Krüppel-like factor*, *erythroid 5-aminolevulinic synthase-2*, *β-major globin*) was influenced by pregnancy and lactation. A pregnancy dependent increase in erythroid progenitors was also observed. In summary, the demands of pregnancy and lactation cause marked adaptations in the maternal spleen. The maternal spleen increases in size and exhibits an expansion of the erythroid lineage.

Additional keywords: erythropoiesis, spleen.

Introduction

Maternal adaptations to pregnancy are essential to reproductive success in mammals, but are poorly understood physiological processes. In the rat, maternal physiology undergoes marked changes during the course of pregnancy (de Rijk *et al.* 2002). These gestational-dependent adjustments permit the development of embryos/fetuses within the reproductive tract and are readily demonstrated by examining the haematology and blood biochemistry of the pregnant animal (de Rijk *et al.* 2002).

The spleen is an organ with multiple responsibilities, including those impacting immunological responses and red blood cell homeostasis, and, under some circumstances, it provides a micro-environment supporting erythropoiesis (Obinata and Yanai 1999; Lenox *et al.* 2005; Mebius and Kraal 2005). The latter task is especially critical during acute erythropoietic stress (Lenox *et al.* 2005). Pregnancy is associated with increased demands for oxygen delivery, which are met by alterations in maternal red blood cell homeostasis. During pregnancy in the mouse, the maternal spleen undergoes marked changes in its structure (Fowler and Nash 1968; Maroni and de Sousa 1973; Mattsson *et al.* 1979;

Sasaki *et al.* 1981). The size of the maternal spleen peaks at midgestation, accompanied by an expansion in splenic red pulp. Thus, the maternal spleen appears to adapt to the demands of pregnancy.

In the present study, we investigated pregnancy dependent adaptations in the maternal spleen of the rat and mouse. Pregnancy associated regulation of splenic gene expression and the development of the erythroid lineage are identified.

Materials and methods

Animals and tissue preparation

Holtzman Sprague-Dawley rats were obtained from Harlan Sprague Dawley (Indianapolis, IN, USA). CD-1 mice were purchased from Charles River Laboratories (Wilmington, MA, USA). Males and females were placed together for mating. The detection of sperm in the vaginal smear of the rat was considered to be gestation Day 0, whereas in mice the presence of a copulatory plug in the vagina was designated as gestation Day 1. Animals were provided food and water *ad libitum*. Animals were placed on a 14 h : 10 h light : dark cycle. Temperature and relative

humidity were maintained at 20–24°C and 40–60%, respectively. Rat maternal spleen and kidney tissues were dissected from non-pregnant and pregnant (gestation Days 4, 8, 11, 13, 15, 18, 20, and 21) rats, as well as on post partum Day 10 from rats without pups (non-lactating) or with pups (lactating). Mouse maternal spleen tissues were dissected from non-pregnant and pregnant (gestation days 4, 8, 10, 11, 13, 15, and 18) mice, as well as on Day 10 post partum from mice without pups (non-lactating) or with pups (lactating). All tissues collected were weighed and then either snap-frozen in liquid nitrogen for biochemical analyses or frozen in dry ice-cooled heptane for histological analysis and stored at –80°C. Litter sizes for rat and mouse pregnancies ranged from 8 to 14 pups. The University of Kansas Medical Center Animal Care and Use Committee approved the animal care and experimental protocols.

DNA content

Splenic tissues were completely digested overnight at 55°C in a buffer containing 100 mM NaCl, 50 mM TRIS-HCl (pH 8.0), 25 mM EDTA, 0.5% sodium dodecyl sulfate (SDS) and 400 µg mL⁻¹ proteinase K. Digested samples were mixed with PicoGreen dsDNA quantitation reagent (Molecular Probes, Eugene, OR, USA). Samples were excited at 485 nm and the fluorescence emission intensity was measured at 538 nm using a spectrofluorometer (Finstuments Fluorokan II; MTX Laboratory System, Vienna, VA, USA). The DNA concentrations were determined using a standard curve of fluorescence emission intensity plotted against DNA concentration.

Histological analysis

Splenic architecture was evaluated by staining 10-µm cryostat-prepared tissue sections with methyl green–pyronin solution (Mattsson *et al.* 1979; Sigma Chemical, St Louis, MO, USA). Sections were stained for 1 h and rinsed thoroughly in water. Stained tissue sections were dehydrated using acetone, 1 : 1 acetone/xylene and xylene and then mounted.

Northern blot analysis

Assessment of mRNA expression was conducted as described previously (Faria *et al.* 1990). RNA was extracted from tissues using TRIzol (Chomczynski and Sacchi 1987). Total RNA (20 µg) was loaded on 1% formaldehyde–agarose gels and transferred to nylon membranes. Northern blot analyses for rat spleen tissues were evaluated using *erythroid Krüppel-like factor* (*EKLF*; GenBank Accession no. AA926284), *β-major globin* (GenBank Accession no. X15009) and *erythroid 5-aminolevulinic synthase-2* (*ALAS-2*; GenBank Accession no. NM_013197) cDNA probes. A glyceraldehyde-3-phosphate dehydrogenase (*G3PDH*; Accession no. NM_017008) cDNA probe was used to monitor loading, RNA integrity and to normalise the *EKLF*, *β-major globin* and *ALAS-2* northern blots. Northern blot analyses for mouse spleen tissues were evaluated using *EKLF* (GenBank Accession no. AI323045), *β-major globin* (GenBank Accession no. J00413) and *ALAS-2* (GenBank Accession no. BI144878) cDNA probes. A *β-actin* cDNA (GenBank Accession no. NM_007393) was used to monitor loading

and RNA integrity. Relative differences in mRNA expression were estimated by densitometry. Autoradiograms were scanned and analysed using the National Institutes of Health (NIH) Image J program (version 1.32; <http://rsb.info.nih.gov/ij/>). Densitometry data for *EKLF*, *β-major globin*, and *ALAS-2* expression in the rat spleen were normalised against *G3PDH*.

Flow cytometry

CD1 mouse spleens were collected and placed immediately on ice. Spleens were dissociated mechanically using a Tenbroeck tissue grinder (Fisher Scientific, Pittsburgh, PA, USA) in phosphate-buffered saline (PBS; pH 7.4) supplemented with 2% fetal bovine serum (FBS). The splenocytes (10⁶ cells per 0.1 mL PBS, 2% FBS) were incubated with rat anti-mouse CD16/CD323 (Mouse BD FC Blocker; BD Biosciences, San Jose, CA, USA) for 15 min. Splenocytes were then incubated for 25 min with both fluorescein-conjugated rat anti-mouse CD71 monoclonal antibody (mAb) and phycoerythrin (PE)-conjugated rat anti-mouse TER119 mAb (BD Biosciences). Controls included the incubation of splenocytes in the absence of antibody, with fluorescein-conjugated rat anti-mouse CD71 mAb only, with PE-conjugated rat anti-mouse TER119 mAb only or with two isotype controls (fluorescein-conjugated rat IgG_{1,k} monoclonal immunoglobulin isotype control and PE-conjugated rat IgG_{2b,k} monoclonal immunoglobulin isotype control; BD Biosciences). Following incubation, the splenocytes were washed twice using cold buffer (PBS, pH 7.4, containing 2% FBS). Splenocytes were resuspended to a final volume of 0.3 mL and analysed by flow cytometry using BD LSRII and FACS Diva (BD Biosciences).

Statistical analysis

Data were analysed by analysis of variance and post hoc comparisons were determined by the Newman–Keuls test. Data of mouse spleen total cell number and percentage CD71/TER119 were analysed with two-tailed Student's *t*-test.

Results

Pregnancy dependent effects on the rat maternal spleen

The rat maternal spleen exhibited a gestational-dependent pattern of growth (Fig. 1a). Splenic growth reached its maximum size by the end of pregnancy, exhibiting an approximate 40% increase in weight. Maternal spleens regressed post partum to their non-pregnant weight independent of lactational state. In comparison, maternal kidneys did not exhibit significant changes in weight during pregnancy but did so during lactation (Fig. 1b). During the post partum period, kidneys were significantly larger in lactating than non-lactating rats (Fig. 1b). The gravimetric changes in the maternal spleen during pregnancy correlated with changes in DNA content (Fig. 2). Maternal splenic DNA content increased significantly as gestation advanced, followed by a marked decrease after parturition to pre-pregnancy levels. The results suggest that the pregnancy dependent increase in weight observed in the maternal spleen is likely due to splenic hyperplasia. Histological analysis of the maternal spleen during pregnancy is shown in Fig. 3. Prominent pregnancy dependent changes in the organisation of the spleen

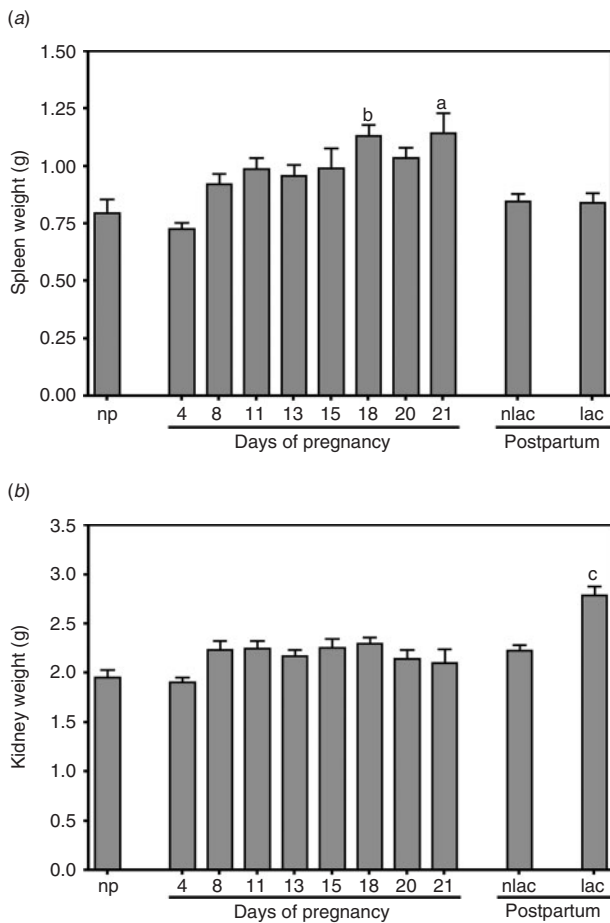


Fig. 1. Maternal weight responses to pregnancy and lactation. (a) Spleen and (b) kidney tissues were collected from non-pregnant (np; $n = 7$), pregnant (gestation Days 4 ($n = 17$), 8 ($n = 9$), 11 ($n = 17$), 13 ($n = 13$), 15 ($n = 6$), 18 ($n = 8$), 20 ($n = 7$) and 21 ($n = 5$)), and Day 10 post partum non-lactating (nlac; $n = 7$) and lactating (lac; $n = 8$) rats and weighed. ^a $P < 0.05$, ^b $P < 0.01$, ^c $P < 0.001$ compared with non-pregnant animals.

were observed. As gestation advanced, the ratio of red pulp to white pulp increased. Accompanying this shift to red pulp was an increase in maternal splenic expression of erythroid-associated genes (*EKLF*, *β -major globin* and *ALAS-2*; Fig. 4). Levels of *EKLF*, *β -major globin* and *ALAS-2* mRNA reached a peak on gestational Day 15, followed by a protracted decline until parturition. Post partum expression of *EKLF*, *β -major globin* and *ALAS-2* genes decreased in non-lactating rats to non-pregnant levels. Lactating rats maintained pregnancy associated up-regulation of splenic erythroid-associated gene expression. The ontogeny of the pregnancy dependent activation of erythroid-associated gene expression was also evaluated. It was found that levels of *β -major globin* and *ALAS-2* mRNA increased by Day 4 of gestation (Fig. 5), suggesting that their expression is independent of embryo implantation. In summary, maternal rat splenic growth and erythroid-associated gene expression are influenced by pregnancy and lactation.

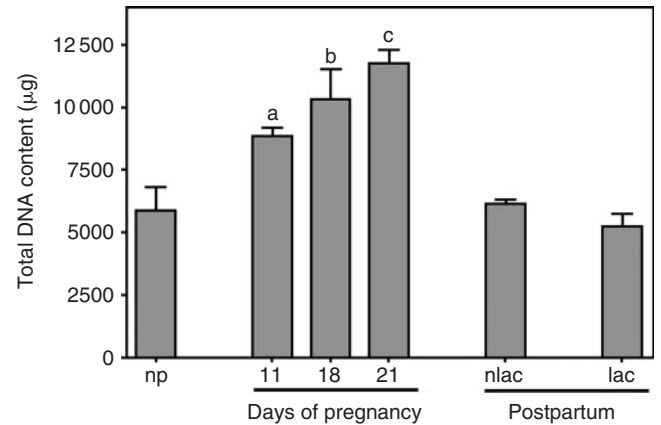


Fig. 2. Measurement of spleen DNA content from non-pregnant, pregnant, non-lactating and lactating rats. Spleen tissues collected from non-pregnant, pregnant (gestation Days 11, 18, and 21) and post partum Day 10 (with and without pups, lac and nlac, respectively) rats were completely digested overnight. The DNA content was measured using a Quant-iT PicoGreen dsDNA Kit (Molecular Probes, Eugene, OR, USA). DNA concentrations were determined using a standard curve of fluorescence emission intensity plotted v. DNA concentration. The analysis was performed in triplicate. ^a $P < 0.05$, ^b $P < 0.01$, ^c $P < 0.001$ compared with non-pregnant animals.

Pregnancy dependent effects on the mouse maternal spleen

A pregnancy dependent increase in splenic size was reported previously in the mouse (Fowler and Nash 1968; Maroni and de Sousa 1973; Mattsson *et al.* 1979; Sasaki *et al.* 1981). We examined the impact of pregnancy on maternal splenic growth and erythroid-associated gene expression in the CD-1 mouse (Fig. 6a). Mouse maternal spleen weights increased 2.34-fold by gestation Day 13 and regressed markedly to non-pregnant weights by gestation Day 18. Splenic DNA content mirrored the pregnancy dependent splenic changes in weight (Fig. 6b). The spleens of post partum lactating mice were significantly larger than the spleens of post partum non-lactating mice (Fig. 6a). Histological analysis of the maternal spleen demonstrated an increase in red pulp associated with increased maternal spleen size at gestation Day 13 (Fig. 6c). Maternal splenic expression of erythroid-associated genes paralleled splenic growth (Fig. 6d). The mRNA levels of *EKLF*, *β -major globin* and *ALAS-2* increased from gestation Days 8–15, but decreased markedly to non-pregnant expression levels by gestation Day 18. Erythroid precursor populations were also examined in the mouse spleen. Total cell counts per spleen were positively correlated with pregnancy dependent splenic changes in weight (Fig. 7a). Erythroid progenitors were detected by simultaneous immunostaining of Ter119 (glycophorin A) and CD71 (transferrin receptor; Socolovsky *et al.* 2001). Erythroid progenitors increased 3.71-fold by gestation Day 13 in the mouse spleen (Fig. 7b, c).

The maternal spleen of both the mouse and rat is affected by pregnancy in the form of altered growth and erythroid-associated gene expression. However, the magnitude of the

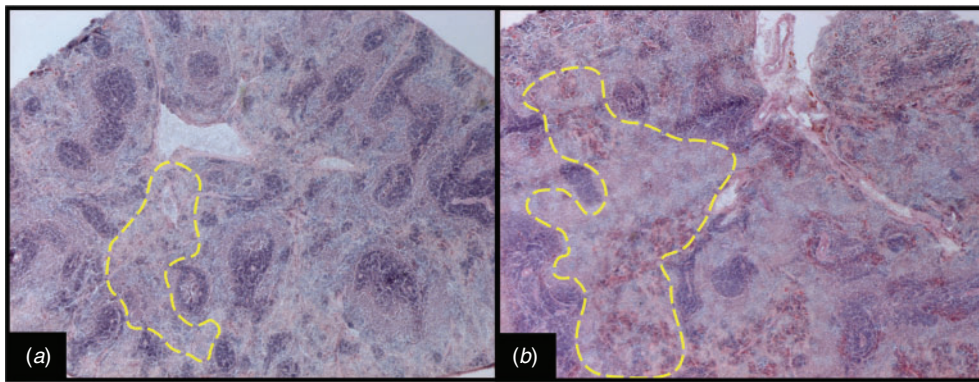


Fig. 3. Histological analysis of maternal spleen tissue from non-pregnant and pregnant rats. Cryostat sectioned spleens from non-pregnant (a) and gestation Day 18 (b) rats were stained with methyl green–pyronin solution. (Original magnification $\times 25$.) The outline indicates examples of areas containing a majority of red pulp.

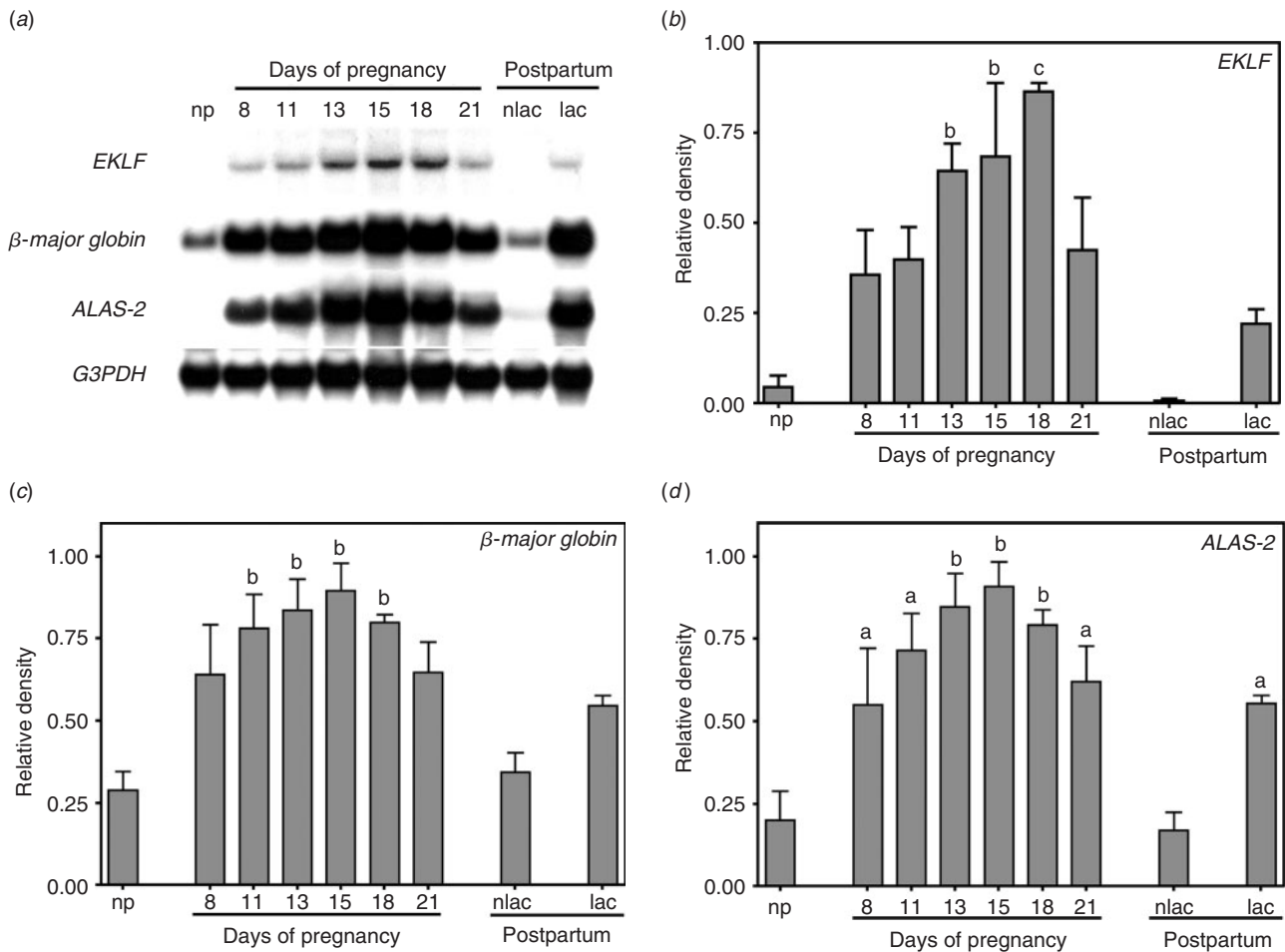


Fig. 4. Northern blot analysis of erythroid-associated gene expression in rat spleen. (a) Total RNA was prepared from spleen tissue of non-pregnant (np), pregnant (gestation Days 8, 11, 13, 15, 18, 21) and Day 10 post partum non-lactating (nlac) and lactating (lac) rats. Total RNA (20 μ g) was loaded and mRNA expression levels in the spleen were evaluated by northern blot analysis using erythroid Krüppel-like factor (EKLf), β -major globin and erythroid 5-aminolevulinic synthase-2 (ALAS-2) cDNA probes labelled with [32 P]-dCTP. Glyceraldehyde-3-phosphate dehydrogenase (G3PDH) served as an internal control to monitor loading and RNA integrity. Densitometric analysis of the multiple northern blots of (b) EKLf, (c) β -major globin and (d) ALAS-2 was normalised against G3PDH. The analysis was performed in triplicate. ^a $P < 0.05$, ^b $P < 0.01$, ^c $P < 0.001$ compared with non-pregnant animals.

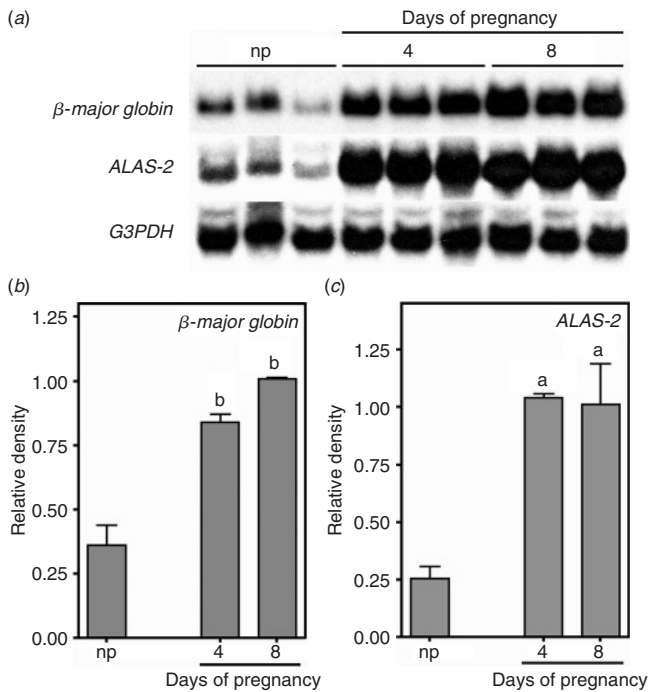


Fig. 5. Northern blot analysis of upregulation of erythroid-associated genes in the spleen before implantation. (a) Total RNA was prepared from spleen tissue of non-pregnant (np) and pregnant (gestation Days 4 and 8) rats. Total RNA (20 μ g) was loaded and mRNA expression levels in the spleen were evaluated by northern blot analysis using β -major globin and erythroid 5-aminolevulinic acid synthase-2 (ALAS-2) cDNA probes labelled with [32 P]-dCTP. Glyceraldehyde-3-phosphate dehydrogenase (G3PDH) served as an internal control to monitor loading and RNA integrity. Densitometric analysis of the northern blot of (b) β -major globin and (c) ALAS-2 was normalised against G3PDH. The analysis was performed in triplicate. ^a $P < 0.01$, ^b $P < 0.001$ compared with non-pregnant animals.

splenic gravimetric response and temporal patterns of the splenic responses are species specific. The mouse spleen also exhibited a significant increase in erythroid progenitors.

Discussion

Maternal adaptations to pregnancy are a necessary prerequisite to ensure the health of the mother and the developing fetus. In the present study, we investigated the impact of pregnancy and lactation on the maternal spleen of the rat and mouse. Maternal spleen structure and function were affected by gestation and lactation in both species. Elements of the responses to pregnancy and lactation showed species specificity. In the rat, the increase in spleen size peaked at gestation Day 18 and was maintained until parturition, whereas maternal spleen growth in the mouse peaked on gestation Day 13 and regressed to non-pregnant sizes by gestation Day 18. Similar gestation-dependent maternal splenic growth patterns have been observed for the mouse (Fowler and Nash 1968; Maroni and de Sousa 1973; Hetherington and Humber 1977; Mattsson *et al.* 1979; Chatterjee-Hasrouni

et al. 1980). Lactation had differing effects on rat and mouse maternal spleen development. During the post partum period, the maternal rat spleen regressed in size to its non-pregnant size, independent of lactation. In contrast, the post partum mouse spleen was significantly larger in lactating compared with non-lactating animals. Mechanisms underlying the species differences in splenic responses to pregnancy and lactation are unknown.

Pregnancy dependent increases in splenic growth in the mouse are associated with an increase in red pulp (Fowler and Nash 1968; Sasaki *et al.* 1981; Mattsson *et al.* 1984). We similarly observed expansions in splenic red pulp in pregnant rats. In addition, a significant accumulation of mRNAs associated with the erythroid cell lineage was observed. These genes included *EKLF*, a transcriptional regulator of the erythroid lineage (Miller and Bieker 1993; Perry and Soreq 2002), *ALAS-2*, a key enzyme involved in heme synthesis (Dzikaite *et al.* 2003), and β -major globin, which encodes the protein chains comprising haemoglobin (Espersen *et al.* 2002). Unlike pregnancy in the rat, erythroid gene expression in spleens of lactating rats showed no correlation with spleen size. We also observed a significant pregnancy dependent increase in erythroid progenitors in the mouse spleen. These results correlate with increases in circulating reticulocytes in the rat during pregnancy (de Rijk *et al.* 2002).

The impact of pregnancy and lactation on the physiology of the maternal spleen may be related to established splenic stress responses. Under stressful physiological conditions, the spleen acts as a reservoir for erythrocytes, which can be released back into the circulation (Fowler and Nash 1968; Peterson 2003). The increase in spleen size and accumulation of erythroid-associated mRNAs during gestation may be due, in part, to the accrual of red blood cells. During exposure to physiological stressors, the spleen can also serve as a secondary site of erythropoiesis (Mattsson *et al.* 1984; Welniak *et al.* 2001). Pregnancy represents a physiological stress and is associated with changes in blood volume resulting in haemodilution, which could serve as an effective activator of splenic erythropoiesis (Fowler and Nash 1968; Mattsson *et al.* 1984; de Rijk *et al.* 2002).

Prolactin (PRL) has been implicated as a regulator of haematopoiesis (Jepson and Lowenstein 1964, 1965; Bellone *et al.* 1997; Abkowitz *et al.* 2002; Akiyama *et al.* 2005). Early studies demonstrated that PRL stimulates erythropoiesis in non-pregnant, pregnant and lactating mice (Jepson and Lowenstein 1964, 1965). In addition, *in vivo* and *in vitro* studies have shown that PRL can increase the number of erythroid progenitors in haematopoietic tissue (Bellone *et al.* 1997; Woody *et al.* 1999; Richards and Murphy 2000; Welniak *et al.* 2001). In the rat and mouse, mating activates twice-daily PRL surges for the first half of pregnancy; in addition, suckling during lactation is a well-established stimulus for PRL secretion (Erskine 1995; Soares 2004; Andrews 2005). These mechanisms for increasing circulating PRL may contribute to activation of the splenic erythroid lineage during early pregnancy and lactation. However, during most of the second half of pregnancy, circulating PRL levels are very low. Maternal splenic adaptations to pregnancy may involve the endocrine activities of the placenta. The placenta produces true PRL mimetics (placental lactogens; Soares 2004) and other

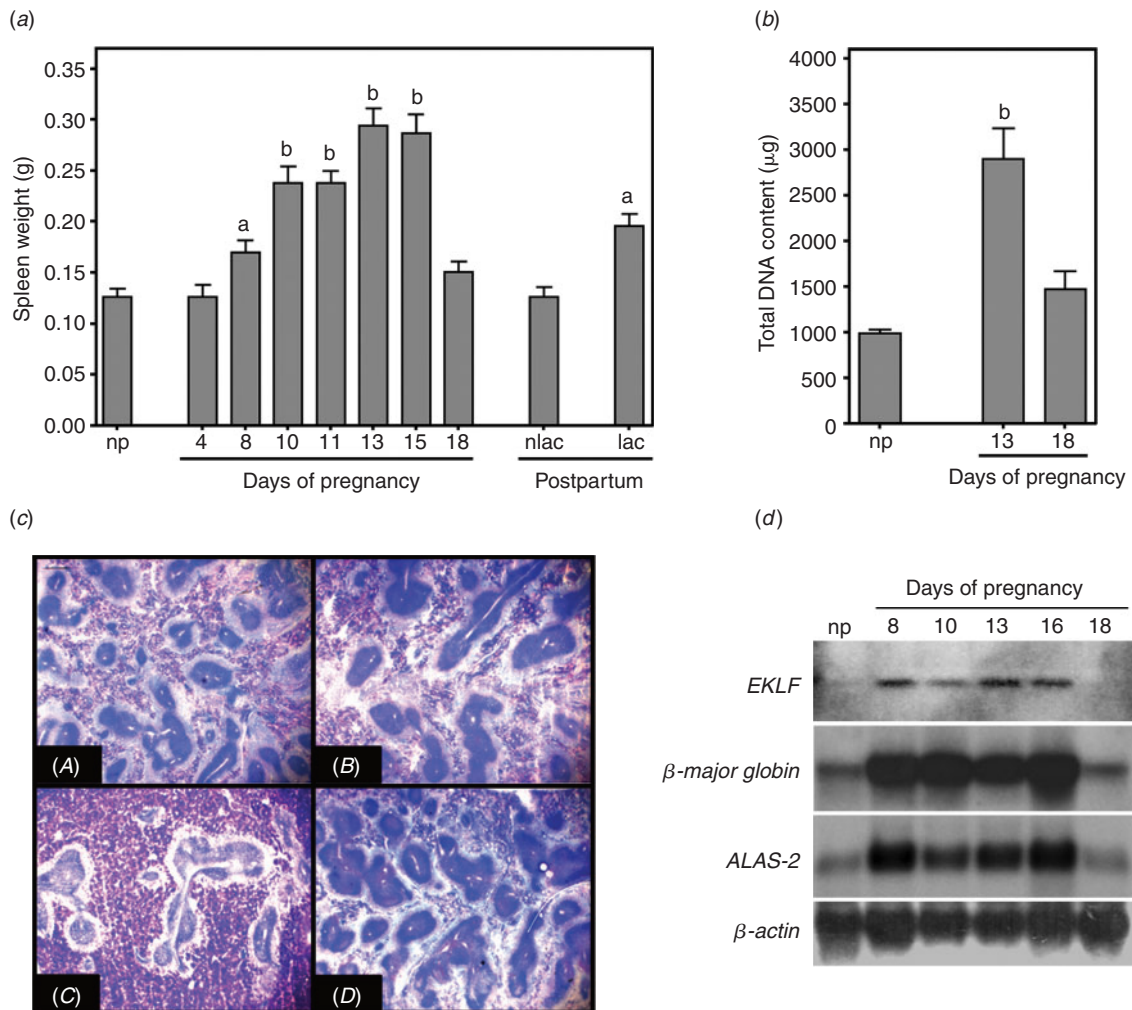


Fig. 6. Pregnancy and lactation-dependent changes in mouse spleen. (a) Maternal weight responses of the spleen to pregnancy and lactation. Spleen tissues were collected from non-pregnant (np), pregnant (gestation Days 4 ($n = 5$), 8 ($n = 16$), 10 ($n = 7$), 11 ($n = 7$), 13 ($n = 17$), 15 ($n = 10$) and 18 ($n = 16$)) and Day 10 post partum without pups (non-lactating; nlac; $n = 11$) and with pups (lactating; lac; $n = 7$) CD-1 mice and weighed. (b) Measurement of spleen DNA content from non-pregnant and pregnant CD-1 mice. Spleen tissues collected from non-pregnant and pregnant (gestation Days 13 and 18) CD-1 mice were completely digested overnight. The DNA content was measured using a Quant-iT PicoGreen dsDNA Kit (Molecular Probes, Eugene, OR, USA). DNA concentrations were determined using a standard curve of fluorescence emission intensity plotted *v.* DNA concentration. The analysis was performed in replicates of four. (c) Histological analysis of maternal spleen tissue from non-pregnant and pregnant CD-1 mice. Cryostat sectioned spleens from non-pregnant (A) and pregnant (gestation Days 10 (B), 13 (C), and 18 (D)) CD-1 mice were stained with methyl green–pyronin solution. (Original magnification $\times 40$.) (d) Northern blot analysis of erythroid-associated gene expression in mouse spleen. Total RNA was prepared from spleen tissue of non-pregnant (np) and pregnant (gestation Days 8, 10, 13, 15 and 18) CD-1 mice. Total RNA (20 μ g) was loaded and mRNA expression levels in the spleen were evaluated by northern blot analysis using erythroid Krüppel-like factor (EKLf), β -major globin and erythroid 5-aminolevulinic synthase-2 (ALAS-2). cDNA probes labelled with [32 P]-dCTP. β -Actin served as an internal control demonstrating loading and RNA integrity. ^a $P < 0.05$, ^b $P < 0.001$ compared with non-pregnant animals.

PRL-related proteins known to regulate haematopoiesis (PRL-like protein (PLP)-E/F subfamily; Lin *et al.* 1997; Müller *et al.* 1998; Lin and Linzer 1999; Sahgal *et al.* 2000; Bhattacharyya *et al.* 2002; Zhou *et al.* 2002, 2005; Alam *et al.* 2006; Ho-Chen *et al.* 2007). PLP-E effectively stimulates erythroid cell proliferation and differentiation (Bittorf *et al.* 2000; Lin *et al.* 2000). The precise roles attributed to PRL and members of the

placental PRL family in regulating maternal splenic physiology are yet to be established.

In summary, the demands of pregnancy cause marked adaptations in the maternal spleen. The maternal spleen increases in size, most prominently within the red pulp, and exhibits an expansion of the erythroid lineage. Active lactation maintains pregnancy associated stimulation of the erythroid lineage.

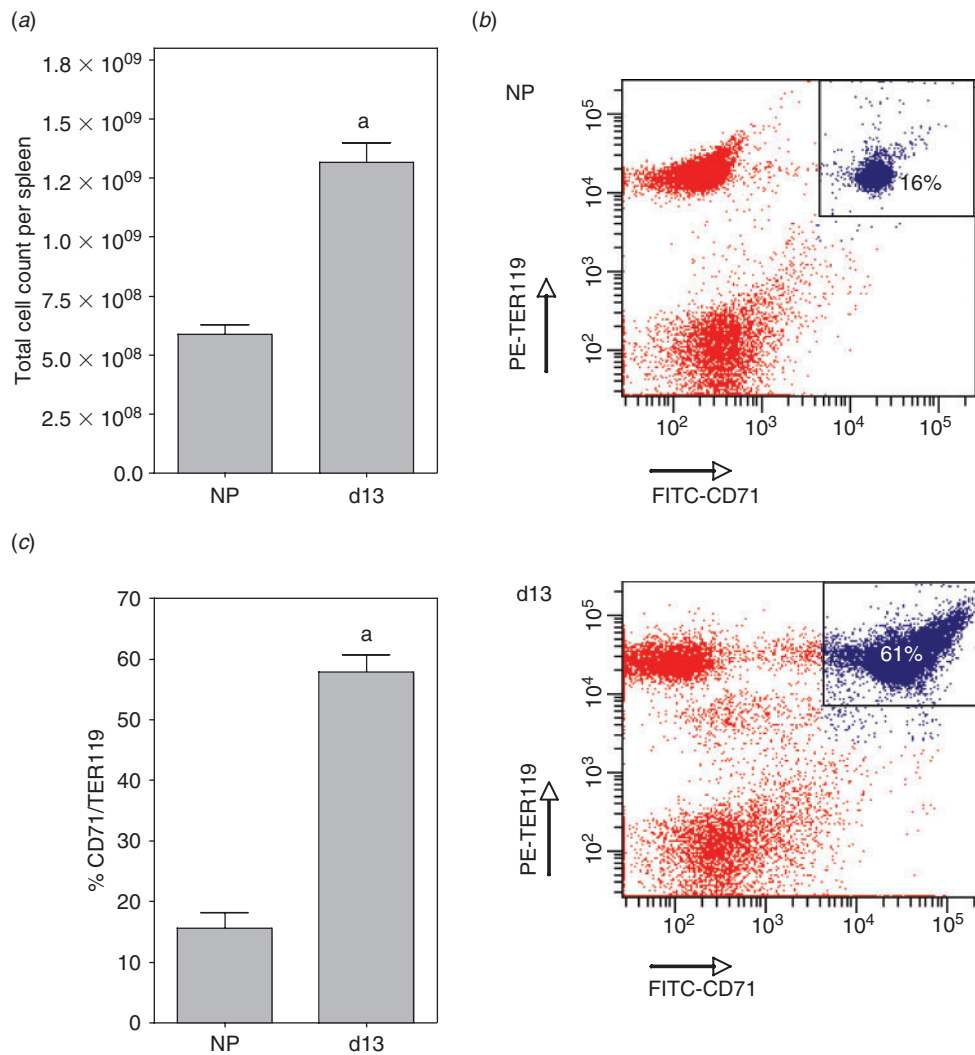


Fig. 7. Pregnancy dependent increase of splenic erythroid progenitors in mice. Spleens were collected from non-pregnant and pregnant (gestation Day 13) CD1 mice. (a) Total cell count was determined for each spleen. Spleen cells were analysed for the presence erythroid progenitors using flow cytometry. (b) Staining pattern of spleen cells for antibodies against CD71 and TER119. (c) Analysis of the double-stained CD71- and TER119-positive spleen cells. The analysis was performed in triplicate. ^a $P < 0.0001$ compared with non-pregnant animals.

Acknowledgements

The authors thank Mr Adam Alt and Ms Mary Jane Peal for their technical assistance. The authors thank Joyce Slusser for her assistance with the flow cytometry analysis. This work was supported by the National Institutes of Health (HD20676, HD39878, HD48861, HD49503, HD55523) and the Hall Family Foundation.

References

- Abkowitz, J. L., Schaison, G., Boulad, F., Brown, D. L., Buchanan, G. R., Johnson, C. A., Murray, J. C., and Sabo, K. M. (2002). Response of Diamond–Blackfan anemia to metoclopramide: evidence for a role for prolactin in erythropoiesis. *Blood* **100**, 2687–2691. doi:10.1182/BLOOD.V100.8.2687
- Akiyama, M., Yanagisawa, T., Yuza, Y., Yokoi, K., Ariga, M., Fujisawa, K., Hoshi, Y., and Eto, Y. (2005). Successful treatment of Diamond–Blackfan anemia with metoclopramide. *Am. J. Hematol.* **78**, 295–298. doi:10.1002/AJH.20278
- Alam, S. M. K., Ain, R., Konno, T., Ho-Chen, J. K., and Soares, M. J. (2006). The rat prolactin gene family locus: species-specific gene family expansion. *Mamm. Genome* **17**, 858–877. doi:10.1007/S00335-006-0010-1
- Andrews, Z. B. (2005). Neuroendocrine regulation of prolactin secretion during late pregnancy: easing the transition into lactation. *J. Neuroendocrinol.* **17**, 466–473. doi:10.1111/J.1365-2826.2005.01327.X
- Bellone, G., Astarita, P., Artusio, E., Silvestri, S., Mareschi, K., Turletti, A., Buttiglieri, S., Emanuelli, G., and Matera, L. (1997). Bone marrow stroma-derived prolactin is involved in basal and platelet-activating factor-stimulated *in vitro* erythropoiesis. *Blood* **90**, 21–27.
- Bhattacharyya, S., Lin, J., and Linzer, D. I. H. (2002). Reactivation of a hematopoietic endocrine program of pregnancy contributes to

- recovery from thrombocytopenia. *Mol. Endocrinol.* **16**, 1386–1393. doi:10.1210/ME.16.6.1386
- Bittorf, T., Jaster, R., Soares, M. J., Seiler, J., Brock, J., Friese, K., and Müller, H. (2000). Induction of erythroid proliferation and differentiation by a trophoblast-specific cytokine involves activation of the JAK/STAT pathway. *J. Mol. Endocrinol.* **25**, 253–262. doi:10.1677/JME.0.0250253
- Chatterjee-Hasrouni, S., Santer, V., and Lala, P. K. (1980). Characterization of maternal small lymphocyte subsets during allogeneic pregnancy in the mouse. *Cell. Immunol.* **50**, 290–304. doi:10.1016/0008-8749(80)90284-1
- Chomczynski, P., and Sacchi, N. (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction. *Anal. Biochem.* **162**, 156–159. doi:10.1016/0003-2697(87)90021-2
- de Rijk, E. P. C. T., van Esch, E., and Flik, G. (2002). Pregnancy dating in the rat: placental morphology and maternal blood parameters. *Toxicol. Pathol.* **30**, 271–282. doi:10.1080/019262302753559614
- Dzikaite, V., Hultcrantz, R., and Melefors, O. (2003). The regulatory effect of heme on erythroid aminolevulinic synthase in natural erythroid cells. *Biochim. Biophys. Acta* **1630**, 19–24.
- Erskine, M. S. (1995). Prolactin release after mating and genitosensory stimulation in females. *Endocr. Rev.* **16**, 508–528. doi:10.1210/ER.16.4.508
- Espersen, K., Frandsen, H., Lorentzen, T., Kanstrup, I. L., and Christensen, N. J. (2002). The human spleen as an erythrocyte reservoir in diving-related interventions. *J. Appl. Physiol.* **92**, 2071–2079.
- Faria, T. N., Deb, S., Kwok, S. C., Talamantes, F., and Soares, M. J. (1990). Ontogeny of placental lactogen-I and placental lactogen-II expression in the developing rat placenta. *Dev. Biol.* **141**, 279–291. doi:10.1016/0012-1606(90)90384-U
- Fowler, J. H., and Nash, D. J. (1968). Erythropoiesis in the spleen and bone marrow of the pregnant mouse. *Dev. Biol.* **18**, 331–353. doi:10.1016/0012-1606(68)90045-6
- Hetherington, C. M., and Humber, D. P. (1977). The effect of pregnancy on lymph node weight in the mouse. *J. Immunogenet.* **4**, 271–276. doi:10.1111/J.1744-313X.1977.TB00909.X
- Ho-Chen, J. K., Bustamante, J. J., and Soares, M. J. (2007). Prolactin-like protein-F subfamily of placental hormones/cytokines: responsiveness to maternal hypoxia. *Endocrinology* **148**, 559–565. doi:10.1210/EN.2006-1146
- Jepson, J. H., and Lowenstein, L. (1964). Effect of prolactin on erythropoiesis in the mouse. *Blood* **24**, 726–738.
- Jepson, J. H., and Lowenstein, L. (1965). Erythropoiesis during pregnancy and lactation. I. Effect of various hormones on erythropoiesis during lactation. *Proc. Soc. Exp. Biol. Med.* **120**, 500–504.
- Lenox, L. E., Perry, J. M., and Paulson, R. F. (2005). BMP4 and Madh5 regulate the erythroid response to acute anemia. *Blood* **105**, 2741–2748. doi:10.1182/BLOOD-2004-02-0703
- Lin, J., and Linzer, D. I. H. (1999). Induction of megakaryocytes differentiation by a novel pregnancy-specific hormone. *J. Biol. Chem.* **274**, 21485–21489. doi:10.1074/JBC.274.30.21485
- Lin, J., Poole, J., and Linzer, D. I. H. (1997). Two novel members of the prolactin/growth hormone family are expressed in the mouse placenta. *Endocrinology* **138**, 5535–5540. doi:10.1210/EN.138.12.5535
- Lin, J., Toft, D. J., Bengston, N. W., and Linzer, D. I. (2000). Placental prolactins and the physiology of pregnancy. *Recent Prog. Horm. Res.* **55**, 37–51.
- Maroni, E. S., and de Sousa, M. A. (1973). The lymphoid organs during pregnancy in the mouse. A comparison between a syngeneic and an allogeneic mating. *Clin. Exp. Immunol.* **13**, 107–124.
- Mattsson, R., Nilsson, B., and Lindahl-Kiessling, K. (1979). An investigation of splenic enlargement in pregnant mice. *Dev. Comp. Immunol.* **3**, 683–695. doi:10.1016/S0145-305X(79)80062-2
- Mattsson, R., Mattsson, A., and Lindahl-Kiessling, K. (1984). Anemia causes erythropoiesis and increased antibody synthesis in the spleen of the pregnant mouse. *Dev. Comp. Immunol.* **8**, 169–178. doi:10.1016/0145-305X(84)90020-X
- Mebius, R. E., and Kraal, G. (2005). Structure and function of the spleen. *Nat. Rev. Immunol.* **5**, 606–616. doi:10.1038/NRI1669
- Miller, I. J., and Bieker, J. J. (1993). A novel, erythroid cell-specific murine transcription factor that binds to the CACCC element and is related to the Krüppel family of nuclear proteins. *Mol. Cell. Biol.* **13**, 2776–2786.
- Müller, H., Orwig, K. E., and Soares, M. J. (1998). Identification of two new members of the mouse prolactin gene family. *Biochim. Biophys. Acta* **1396**, 251–258.
- Obinata, M., and Yanai, N. (1999). Cellular and molecular regulation of an erythropoietic inductive microenvironment (EIM). *Cell Struct. Funct.* **24**, 171–179. doi:10.1247/CSF.24.171
- Perry, C., and Soreq, H. (2002). Transcriptional regulation of erythropoiesis. Fine tuning of combinatorial multi-domain elements. *Eur. J. Biochem.* **269**, 3607–3618. doi:10.1046/J.1432-1033.2002.02999.X
- Peterson, K. R. (2003). Hemoglobin switching: new insights. *Curr. Opin. Hematol.* **10**, 123–129. doi:10.1097/00062752-200303000-00004
- Richards, S. M., and Murphy, W. J. (2000). Use of human prolactin as a therapeutic protein to potentiate immunohematopoietic function. *J. Neuroimmunol.* **109**, 56–62. doi:10.1016/S0165-5728(00)00303-9
- Sahgal, N., Knipp, G. T., Liu, B., Chapman, B. M., Dai, G., and Soares, M. J. (2000). Identification of two new nonclassical members of the rat prolactin family. *J. Mol. Endocrinol.* **24**, 95–108. doi:10.1677/JME.0.0240095
- Sasaki, K., Matsumura, G., and Ito, T. (1981). Effects of pregnancy on erythropoiesis in the splenic red pulp of the mouse: a quantitative electron microscopic study. *Arch. Histol. Jpn.* **44**, 429–438.
- Soares, M. J. (2004). The prolactin and growth hormone families: pregnancy-specific hormones/cytokines at the maternal–fetal interface. *Reprod. Biol. Endocrinol.* **2**, 51. doi:10.1186/1477-7827-2-51
- Socolovsky, M., Nam, H.-S., Fleming, M. D., Haase, V. H., Brugnara, C., and Lodish, H. F. (2001). Ineffective erythropoiesis in Stat5a^{-/-}5b^{-/-} mice due to decreased survival of early erythroblasts. *Blood* **98**, 3261–3273. doi:10.1182/BLOOD.V98.12.3261
- Welniak, L. A., Richards, S. M., and Murphy, W. J. (2001). Effects of prolactin in hematopoiesis. *Lupus* **10**, 700–705. doi:10.1191/096120301717164930
- Woody, M. A., Welniak, L. A., Sun, R., Tian, Z. G., Henry, M., Richards, S., Raziuddin, A., Longo, D. L., and Murphy, W. J. (1999). Prolactin exerts hematopoietic growth-promoting effects *in vivo* and partially counteracts myelosuppression by azidothymidine. *Exp. Hematol.* **27**, 811–816. doi:10.1016/S0301-472X(99)00019-3
- Zhou, B., Lum, H. E., Lin, J., and Linzer, D. I. H. (2002). Two placental hormones are agonists in stimulating megakaryocyte growth and differentiation. *Endocrinology* **143**, 4281–4286. doi:10.1210/EN.2002-220447
- Zhou, B., Kong, X., and Linzer, D. I. H. (2005). Enhanced recovery from thrombocytopenia and neutropenia in mice constitutively expressing a placental hematopoietic cytokine. *Endocrinology* **146**, 64–70. doi:10.1210/EN.2004-1011

Manuscript received 7 July 2007, accepted 3 December 2007