# $\alpha_2$-Macroglobulin expression in the liver in response to inflammation is mediated by the testis

**Wing-Yee Lui**[1], **Yan Ho Cheng**[1], **Dolores D Mruk**[1], **Chin Ho Cheng**[1], **Meng Yun Mo**[1], **Will M Lee**[2] and **C Yan Cheng**[1]

[1]Population Council, Center for Biomedical Research, 1230 York Avenue, New York, New York 10021, USA

[2]Department of Zoology, University of Hong Kong, Hong Kong, China

(Requests for offprints should be addressed to C Yan Cheng; Email: Y-Cheng@popcbr.rockefeller.edu)

Wing-Yee Lui's present address is Department of Zoology, Faculty of Science, University of Hong Kong, Hong Kong, China

## Abstract

Earlier studies have shown that germ cells or germ cell-conditioned media are capable of regulating $\alpha_2$-macroglobulin ($\alpha_2$-MG, a non-specific protease inhibitor) expression by Sertoli cells and hepatocytes cultured *in vitro*. These results illustrate a possible physiological link between testes and liver regarding $\alpha_2$-MG production. Using a series of surgical procedures including castration, hemicastration, and hepatectomy coupled with Northern blot and immunoblot analyses, we report herein that the surge in $\alpha_2$-MG expression in the liver in response to inflammation is indeed regulated, at least in part, by the testis via testosterone. It was found that hepatectomy induced at least a tenfold increase in the steady-state mRNA and protein production of $\alpha_2$-MG in the liver. However, castration induced a mild but not statistically significant induction of $\alpha_2$-MG in the liver in contrast to sham operation or hemicastration alone, when hemicastration alone could induce liver $\alpha_2$-MG production by almost fourfold. Perhaps most important of all, hepatectomy accompanied by castration significantly reduced the liver $\alpha_2$-MG response to the surgery-induced inflammation compared with hepatectomy alone, illustrating that the removal of the testicles can induce a loss of signal communications between the testis and the liver, rendering a significant loss of the $\alpha_2$-MG response to experimentally induced inflammation in the liver. Interestingly, this lack of response of the liver to surgery-induced inflammation regarding $\alpha_2$-MG production following castration could be restored, at least in part, by using testosterone implants placed subdermally 6 days prior to orchiectomy. Collectively, these results illustrate that a physiological link does indeed exist between the testis and the liver, and that testes *per se* can influence the liver $\alpha_2$-MG expression in response to inflammation *in vivo* possibly via testosterone or testosterone-induced biological factor(s).

*Journal of Endocrinology* (2005) **185,** 497–505

## Introduction

$\alpha_2$-Macroglobulin ($\alpha_2$-MG) is a 720 kDa glycoprotein consisting of four identical subunits of ~180 kDa for each subunit. It functions as a non-specific protease inhibitor in mammals which is predominantly produced in the liver, and it is one of the major acute-phase serum proteins associated with inflammatory response (Kushner 1982). Serum $\alpha_2$-MG is also capable of binding to a broad spectrum of cytokines, such as transforming growth factor (Huang *et al.* 1988) and basic fibroblast growth factor (Dennis *et al.* 1989). This $\alpha_2$-MG–cytokine complex formation is believed to provide a mechanism to regulate the bioavailability of cytokines and to clear excessive cytokines during inflammation and wound repair (Gaddy-Kurten *et al.* 1989). Apart from the liver, other tissues such as ovary, uterus, placenta and testes have also been shown to synthesize $\alpha_2$-MG. For instance, Sertoli cells are the source of $\alpha_2$-MG in the rat testis (Cheng *et al.* 1990), and $\alpha_2$-MG in the testis can bind to TGF-β3 (Wang *et al.* 2004). Unlike the liver, testicular $\alpha_2$-MG is not induced in response to inflammation, such as during experimental inflammation following administration of fermented yeast *in vivo* (Li *et al.* 1994). Interestingly, the steady-state mRNA or the protein level of $\alpha_2$-MG in the testis is several orders of magnitude higher than in the liver (Li *et al.* 1994), illustrating that its level must be maintained at a relatively high level for spermatogenesis. Indeed, recent studies have shown that $\alpha_2$-MG in the testis may be used to prevent unchecked proteolytic activity induced by degenerating germ cells, implicating its involvement in tissue remodeling pertinent to spermatogenesis in the


0022–0795/05/0185–497
© 2005 Society for Endocrinology *Printed in Great Britain*
DOI: 10.1677/joe.1.06136
Online version via http://www.endocrinology-journals.org

Downloaded from Bioscientifica.com at 11/06/2024 05:16:59PM
via free access

seminiferous epithelium (Zhu *et al.* 1994). Additionally, $\alpha_2$-MG is apparently being used to protect the testis from the assaults of environmental toxicants, such as cadmium toxicity, via the c-Jun N-terminal protein kinase signaling pathway (Wong *et al.* 2004, 2005). Even though hepatocytes and Sertoli cells both synthesize and secrete $\alpha_2$-MG, its production by Sertoli cells, unlike hepatocytes, does not respond to interleukin-6 treatment *in vitro*, confirming that $\alpha_2$-MG is not an acute phase protein in the testis (Zwain *et al.* 1993). Yet $\alpha_2$-MG production by Sertoli cells could be stimulated by germ cells and germ cell-conditioned medium (GCCM) (Braghiroli *et al.* 1998), which seemingly suggests that germ cells may release a soluble factor(s) to regulate Sertoli cell $\alpha_2$-MG expression. Interestingly, GCCM was also shown to stimulate hepatocyte $\alpha_2$-MG production *in vitro* in this earlier study (Braghiroli *et al.* 1998). However, a physiological link between the testis and the liver *in vivo* is not known. Although there is no visible anatomical linkage between testis and liver, it is plausible that germ cells secrete soluble factors apically to regulate Sertoli cells as well as releasing these factors basally to regulate hepatocyte $\alpha_2$-MG via the systemic circulation. In this study, certain surgical operations, such as hepatectomy and castration, were performed in adult rats to assess whether a physiological link exists between these two organs *in vivo*.

## Materials and Methods

### *Animals*

Male Sprague-Dawley rats at 60 days of age were obtained from Charles River Laboratories (Kingston, New York, USA). Rats in groups of three at specific time points for a particular surgery were killed following treatment and/or surgery by $CO_2$ asphyxiation. Testes and livers were removed immediately and kept at −80 °C until used. The use of animals for these studies was approved by the Rockefeller University Animal Care and Use Committee with protocol numbers 00111, 95129-R and 03017.

### *Partial hepatectomy*

Adult rats ranging between 380 and 400 g body weight (bw) were anesthetized with ketamine HCl (Ketaset, Fort Dodge Animal Health, Forth Dodge, IA, USA) at 50–60 mg/kg bw administered intramuscularly (i.m.) near the thigh using a 26-gauge needle. Hair at the surgical site was removed using an electric shaver, and the area was carefully cleansed with 70% ethanol and Betadine (twice each). A small incision, ∼2·5–3 cm, was made using a sterile surgical blade at the surgical site to expose the liver, and two-thirds of the liver was removed as described earlier (Higgins & Anderson 1931). After bleeding stopped, the abdomen was closed using sterile nylon black monofilament suture (Ethicon Inc., Somerville, NJ, USA), and rats were allowed to recover. Sham operations were carried out via an abdominal incision (∼2·5 cm long) with manipulation of the liver lobes instead of an incision in the liver. Animals were killed at 6 h, 24 h, 48 h, 72 h, 1 week and 4 weeks after operation. Liver and testes were removed immediately, frozen in liquid nitrogen, and stored at −80 °C until used for RNA extraction or protein lysates preparation.

### *Castration and hemicastration*

Animals were anesthetized with Metofane (2,2-dichloro-1,1-difluoro-ethyl methyl ether; Mallinckrodt Veterinary Inc., Mundelein, IL, USA). The scrotum was cleansed with 70% ethanol and Betadine (twice each). A small incision (∼1 cm) was made to expose the testis. The testicular vein and artery were tied with sterile and absorbable surgical suture (Ethicon 3–0 chromic gut suture) above the testis to minimize blood loss. Thereafter, one (hemicastration) or both (castration) testes were removed. Sham operations were performed by making a scrotum incision of ∼1 cm without the removal of the testis. Thereafter, the surgical site was cleansed with 70% ethanol, and stitched with nylon black monofilament suture, and the rats were allowed to recover. Thereafter, animals were killed at 6 h, 24 h, 48 h, 72 h, 1 week and 4 weeks by $CO_2$ asphyxiation. Liver and/or testis were removed, frozen in liquid nitrogen immediately, and stored at −80 °C until used for RNA extraction and/or lysate preparation.

### *Preparation of steroid implants and their placement subdermally in adult rats*

To assess if testosterone replacement could restore the inflammatory response in the liver regarding $\alpha_2$-MG production after orchiectomy, the following experiment was performed. Testosterone implants were prepared and their subdermal placement in adult rats (∼400 g bw) was performed as described by Zhang *et al.* (2005). In brief, 4-cm implants were prepared just before surgery by filling ethylene and vinyl acetate (EVA) tubing (Elvax 770, 9% VA; 2·15 mm i.d. × 2·4 mm o.d.; Du Pont, Wilmington, DE, USA) with testosterone (Sigma); both ends were sealed by heat using a soldering iron, and cleansed with 70% ethanol prior to their use. Earlier studies have shown that implants using EVA had a steroid release rate (e.g. of 7α-methyl-19-nortestosterone) of ∼90 μg/cm/day *in vivo* when placed under the skin in humans (Noe *et al.* 1999, von Eckardstein *et al.* 2003). Animal surgery was performed in adult rats (∼400 g bw) under anesthesia using ketamine HCl at 60 mg/kg bw (i.m.). Hair at the surgical site on the dorsal side of the rat was removed, and the skin cleansed with 70% ethanol and Betadine (twice each). A small incision was immediately opened (∼2 cm), and four



Downloaded from Bioscientifica.com at 11/06/2024 05:16:59PM
via free access

4-cm testosterone implants were inserted subdermally. The surgical site was then cleansed with 70% ethanol, and stitched with nylon black monofilament suture. Rats were allowed to recover for 6 days until the level of $\alpha_2$-MG subsided (see Fig. 1). On day 7, castration was performed with both testes removed as described above. Rats were killed at 6 and 24 h thereafter, and livers were removed and stored at −80 °C until used for protein lysate preparation. Because the testosterone implants sustained the endogenous testosterone levels, the loss of androgens in the systemic circulation following orchiectomy was negligible.

### *RNA extraction and Northern analysis*

Total RNA was extracted from liver and testis using RNA STAT-60 (Tel-Test Inc., Friendswood, TX, USA) as described by Braghiroli *et al.* (1998). Northern blot analysis was performed as previously described using an α-$^{32}$P-labeled $\alpha_2$-MG cDNA probe by nick translation for hybridization (Braghiroli *et al.* 1998). To ensure that equal amounts of RNA were loaded into each lane within an experimental group, agarose gels were stained with ethidium bromide. In experiments reported in Fig. 1, the blots were stripped and rehybridized with an α-$^{32}$P-labeled S-16 cDNA probe, and data were normalized after densitometric scanning analysis to correct for possible uneven loading.

### *Preparation of liver lysates*

Liver was lysed in SDS lysis buffer (0·125 M Tris, pH 6·8, 22 °C, containing 1% SDS, 1·6% 2-mercaptoethanol, 2 mM PMSF, 1 mM EDTA). Samples were homogenized using a Polytron or sonicated using a sonicator (15 s, 2 times, interspaced by 30 s on ice), vortexed, and centrifuged at 15 000 ***g*** for 10 min at 4 °C. Supernatants were collected and used as liver lysates. Protein estimation was performed by the Coomassie blue-dye binding assay (Bradford 1976) using BSA as a standard.

### *Immunoblotting analysis*

Protein, 50 µg per sample, was resolved onto 7·5% *T* SDS-polyacrylamide gels by SDS-PAGE under reducing conditions as described by Cheng and Bardin (1987). Following electrophoresis, proteins were transferred to nitrocellulose membranes (Schleicher & Schuell Inc, Keene, NH, USA). Blots were probed with an $\alpha_2$-MG antibody which was characterized earlier (Cheng *et al.* 1990, Stahler *et al.* 1991), followed by bovine anti-rabbit IgG conjugated to horseradish peroxidase and developed using ECL chemiluminiscent kits from Amersham-Pharmacia-Biotec as described (Lui *et al.* 2003, 2005).

## Results

### *Changes in $\alpha_2$-MG steady-state mRNA level in the liver in response to experimental inflammation*

In this study, hepatectomy or castration was used to induce an inflammatory response. It was noted that the basal steady-state mRNA level of $\alpha_2$-MG, an acute-phase protein, was barely detectable in the liver of normal rats. However, its mRNA level was induced considerably in response to inflammation as early as 6 h after surgery (Fig. 1A–D), and this 6-h time point was then used as the baseline for densitometic scanning analysis. As such, the fold stimulation of $\alpha_2$-MG in response to hepatectomy as reported herein is an underestimate of the actual increase. The steady-state mRNA level of $\alpha_2$-MG 24 h after hepatectomy was induced by at least 12-fold over the level at 6 h (Fig. 1A, D). However, castration alone had a much milder effect on the liver $\alpha_2$-MG levels compared with hepatectomy (Fig. 1B, D vs 1A, D). For instance, if the increase in $\alpha_2$-MG levels in the liver by 6 h post operation was used as the baseline, castration *per se* failed to induce any changes in the liver $\alpha_2$-MG level (Fig. 1B, D). More importantly, when castration was performed prior to hepatectomy with a lapse of about 15–50 min, liver $\alpha_2$-MG mRNA levels were stimulated by only 7-fold compared with 12-fold for hepatectomy alone (Fig. 1C, D vs 1A, D), illustrating a significant loss of response to hepatectomy-induced inflammation when both testicles were removed, and implying that the testes were releasing a substance(s) that modulates the liver acute-phase response.

### *Changes in testicular $\alpha_2$-MG steady-state mRNA levels in response to inflammation*

In this study, the steady-state $\alpha_2$-MG mRNA levels in the testis after hepatectomy and hemicastration were also examined and compared with two sham operation procedures. However, we failed to detect any significant changes in testicular $\alpha_2$-MG mRNA levels after hepatectomy (Fig. 2A, E) or hemicastration (Fig. 2B, E) when compared with the corresponding controls (Fig. 2C, D and E), suggesting that $\alpha_2$-MG is not an acute-phase protein in the testis, consistent with the results of an earlier report (Stahler *et al.* 1991). Furthermore, in contrast to the liver, the basal $\alpha_2$-MG level in the testis is significantly higher than in the liver, suggesting that this protein is apparently being activated in the testis for normal functioning pertinent to spermatogenesis.

### *The testis is crucial in the regulation of liver $\alpha_2$-MG expression in response to inflammation*

To further confirm that the testis does indeed play a significant physiological role in regulating liver $\alpha_2$-MG



Downloaded from Bioscientifica.com at 11/06/2024 05:16:59PM
via free access

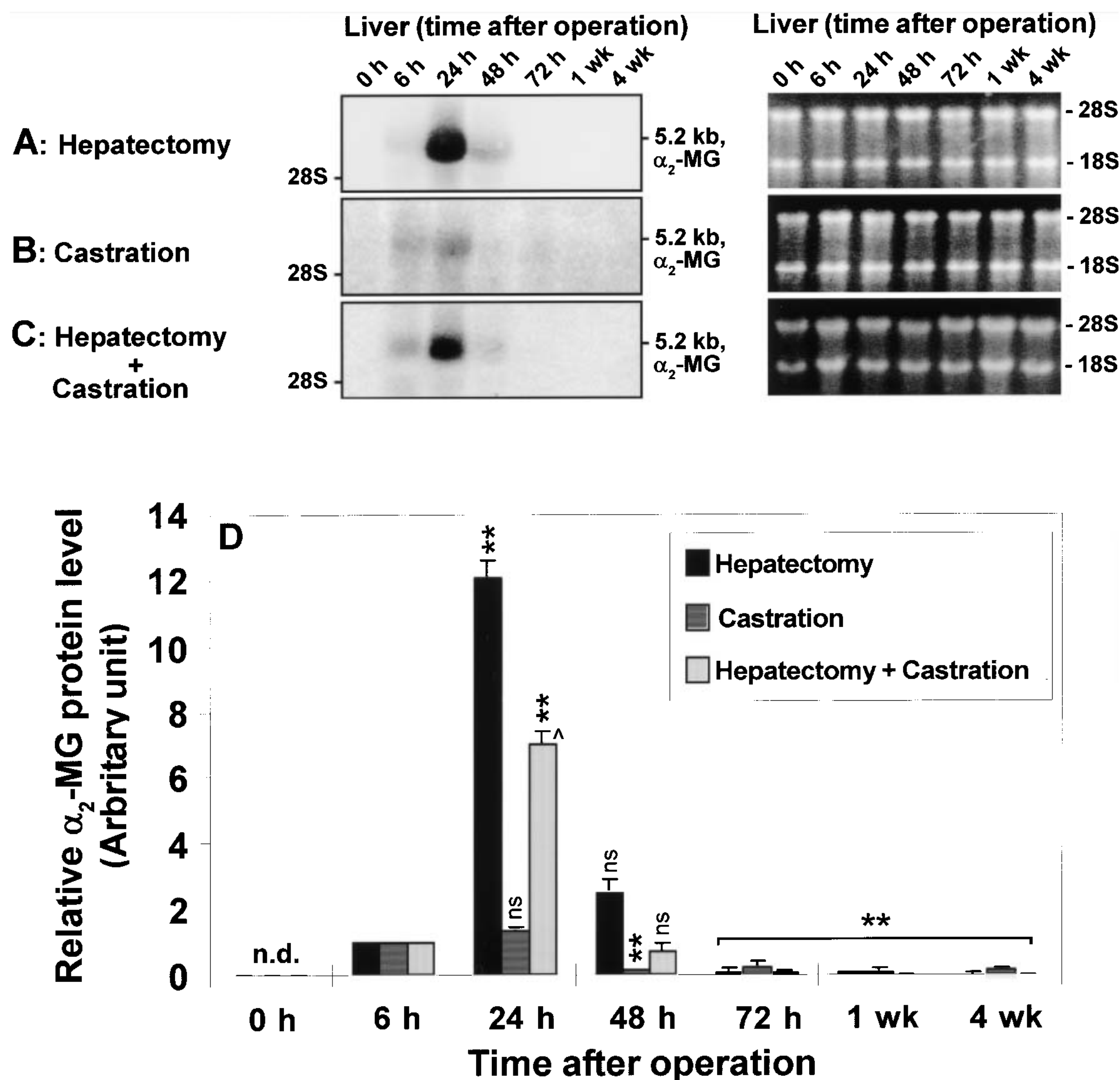


**Figure 1** Changes in the $\alpha_2$-MG steady-state mRNA levels in the liver in response to different surgical procedures. (A-C) Northern blots (left panel) illustrating changes in the steady-state $\alpha_2$-MG mRNA levels in the liver after (A) hepatectomy by removing 2/3 of the liver, (B) castration and (C) hepatectomy plus castration. The right panel shows the corresponding ethidium bromide-stained gels of the left panel, illustrating similar amounts of total RNA (15 µg total RNA per lane) were used for this experiment. (D) The corresponding densitometrically scanned results using autoradiograms such as those shown in A-C are shown. Each bar represents the mean ± S.D. of samples from three adult Sprague-Dawley rats (~300 g bw). Since the basal level of $\alpha_2$-MG in the normal liver is virtually undetectable, the level at 6 h after operation was arbitrarily set at 1, against which the increase in $\alpha_2$-MG levels at other time points was compared. ns, not significantly different from control (6 h), *$P$<0·05, **$P$<0·01 (by Student's $t$-test). ^$P$<0·05 (by ANOVA), comparing data in rats with hepatectomy and castration vs rats with hepatectomy only and rats with castration. h, hour; wk, week; n.d., not detectable.

levels during inflammation, liver $\alpha_2$-MG steady-state protein levels were quantified following hemicastration and were compared with the levels following other surgical procedures. Similar to the data shown in Fig. 1, the $\alpha_2$-MG protein level was significantly induced in the liver 24 h after hepatectomy (Fig. 3A, E). Interestingly, it was found that hemicastration could stimulate a significant increase in liver $\alpha_2$-MG protein levels similar to the sham operation (scrotum incision) by 24 h after operation (Fig. 3B, C, E). Yet no significant increase in liver $\alpha_2$-MG protein levels was detected by 24 h in rats when both testicles were removed (Fig. 3D, E). These results suggest that there is a possible physiological link between the liver and the testis, and that this linkage is important in maintaining the inflammatory response in the liver regarding its $\alpha_2$-MG production.



Downloaded from Bioscientifica.com at 11/06/2024 05:16:59PM
via free access

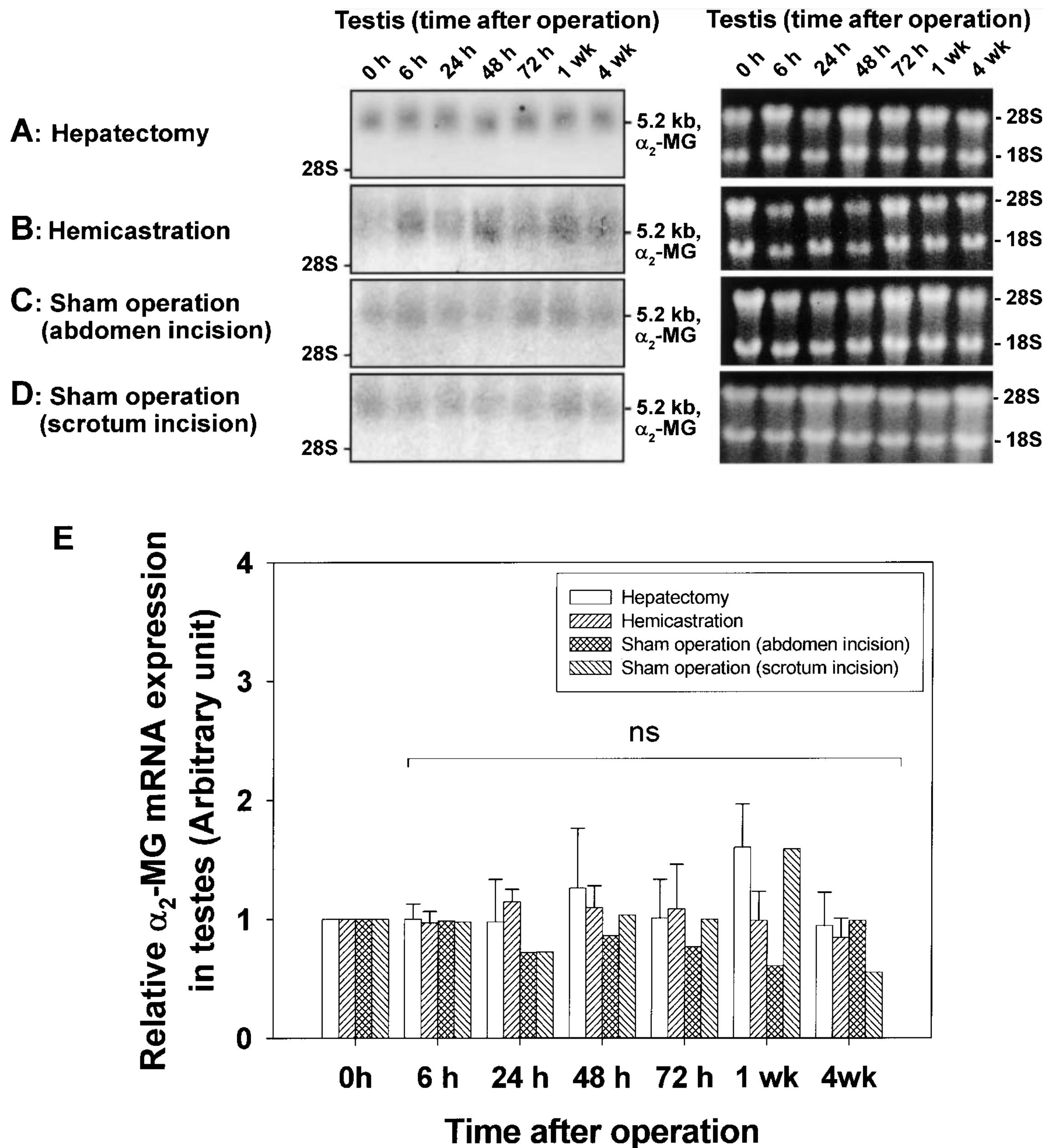


**Figure 2** Changes in the $\alpha_2$-MG steady-state mRNA levels in testes in response to different surgical procedures. (A-D) Northern blots (left panel) illustrating $\alpha_2$-MG expression in testes after (A) hepatectomy, (B) hemicastration, (C) sham operation (abdomen incision) and (D) sham operation (scrotum incision). The right panel shows the corresponding eithidium bromide-stained gels of the left panel, illustrating that similar amounts of total RNA (15 µg total RNA per lane) were used for this experiment. (E) Histogram showing the corresponding densitometrically scanned results using autoradiograms such as those shown in A-D. Each bar is the mean ± S.D. of samples from three rats. ns, not significantly different from control (6 h) (by Student's *t*-test). h, hour; wk, week.

*The reduced $\alpha_2$-MG production in the liver in response to castration-induced inflammation can be restored by using testosterone implants*

To assess if the loss of response in the liver regarding $\alpha_2$-MG production during surgery-induced inflammation by removing both testicles (total castration) is mediated by testosterone, the following study was conducted. In order to replace the loss of testosterone following orchiectomy, four 4-cm testosterone implants were placed subdermally on the dorsal side of adult rats 6 days prior to castration which was performed on day 7. In this way, the



Downloaded from Bioscientifica.com at 11/06/2024 05:16:59PM
via free access

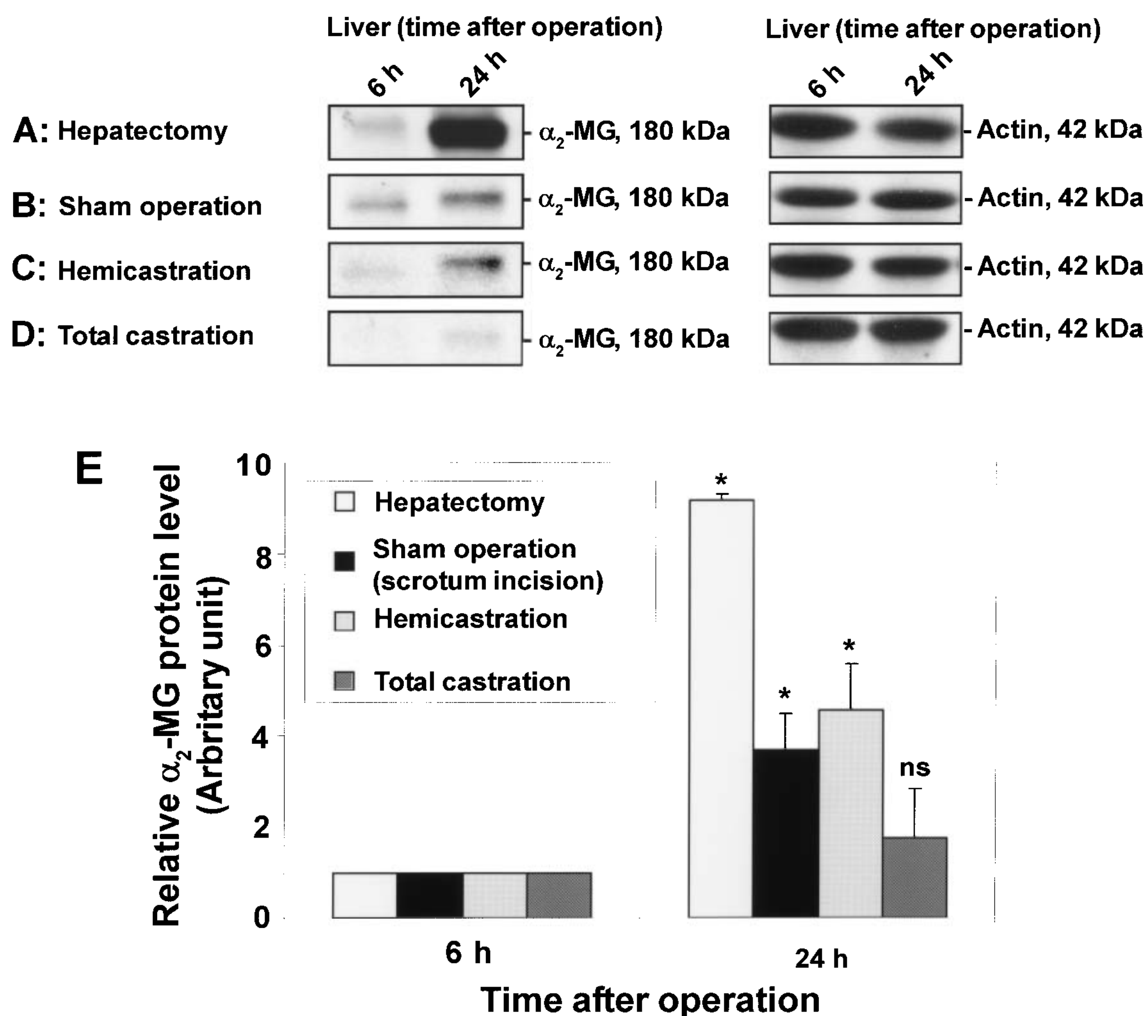


**Figure 3** Changes in liver $\alpha_2$-MG protein level in response to different surgical procedures in rats with and without testes, or with one of the two testes. (A-D) Immunoblots (left panel) illustrating the steady-state $\alpha_2$-MG protein levels in liver after (A) hepatectomy, (B) sham operation (scrotum incision), (C) hemicastration and (D) total castration. The right panel shows the corresponding blots stained for actin using an anti-actin antibody, illustrating equal protein loading between samples. (E) Histogram showing the corresponding densitometrically scanned results using chemiluminograms such as those shown in A-D. The level of $\alpha_2$-MG in the liver at 6 h was arbitrarily set at 1 since the basal level of $\alpha_2$-MG in normal liver was virtually undetectable (see Fig. 1). Results are expressed as the mean ± S.D. of three separate experiments, using samples from different rats. ns, not significantly different from control (6 h); *$P<0{\cdot}01$ (by Student's *t*-test). h, hour.

endogenous testosterone level can be maintained after orchiectomy. Following different surgical procedures (either hepatectomy or castration with and without four 4-cm testosterone implants), rats ($n$=3 per time point) were killed after 6 h or 24 h and two-thirds of the liver was removed for lysate preparation. As expected, the endogenous $\alpha_2$-MG steady-state protein levels in the liver were induced by ~ninefold following hepatectomy (Fig. 4A, D). Likewise, total castration (without testosterone implants) rendered the rats non-responsive to surgery-induced inflammation regarding $\alpha_2$-MG production (Fig. 4B, D vs Fig. 4A, D). Yet the presence of four 4-cm testosterone implants could restore, at least in part, the liver response to surgery-induced inflammation (Fig. 4C, D vs 4B, D), illustrating that androgen is one of the crucial physiological links between the testis and the liver.

## Discussion

In this study, we have demonstrated that $\alpha_2$-MG is an acute-phase protein in the liver, but not in the testis, which is consistent with our previous observations (Braghiroli *et al.* 1998, Stahler *et al.* 1991). For instance, the liver $\alpha_2$-MG mRNA levels increased dramatically by 24 h after hepatectomy, yet no such increase was detected



Downloaded from Bioscientifica.com at 11/06/2024 05:16:59PM
via free access

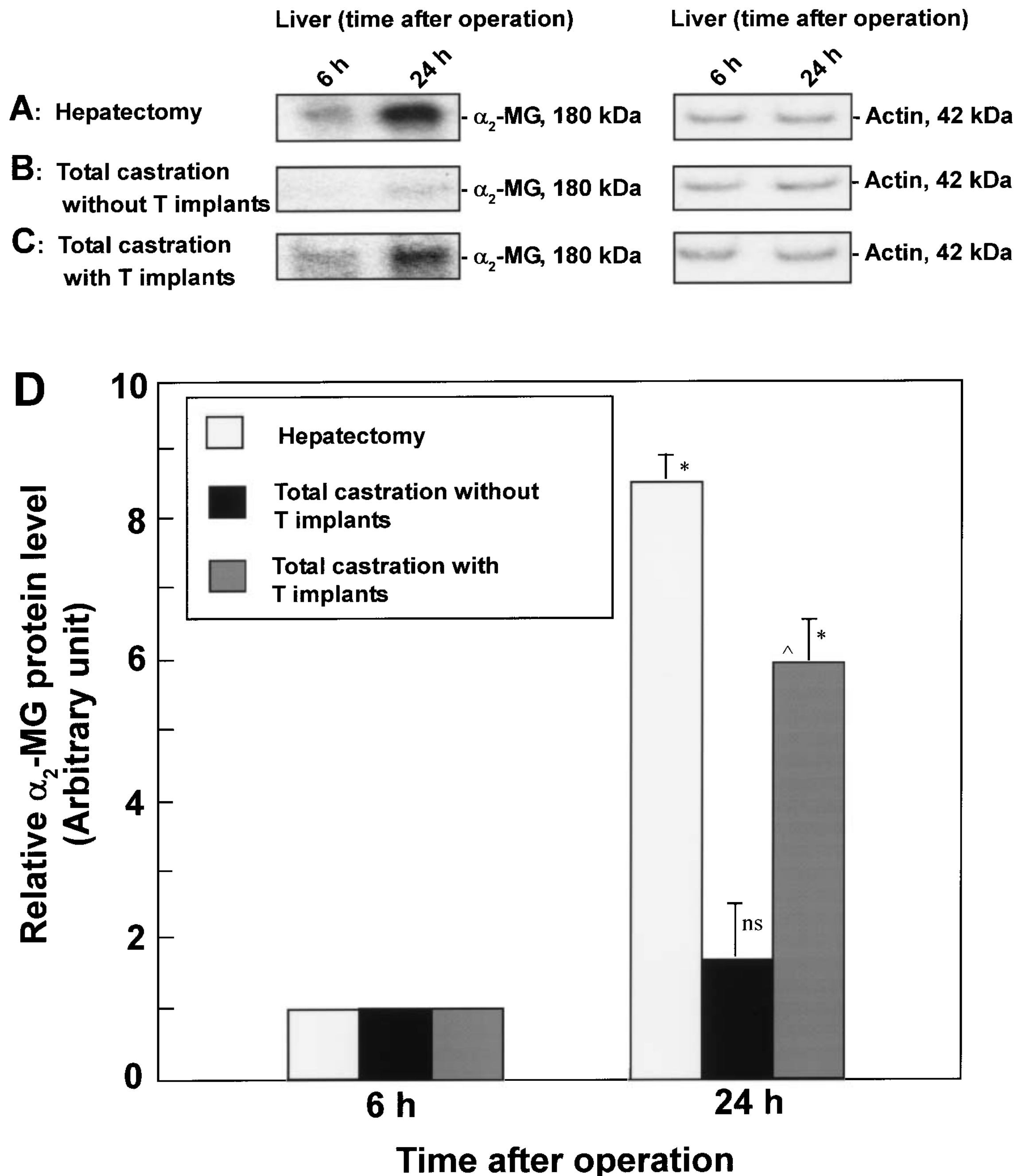


**Figure 4** A study to assess the use of testosterone implants to restore the liver responsiveness to castration-induced $\alpha_2$-MG production during inflammation. (A-C) Immunoblots (left panel) illustrating the steady-state $\alpha_2$-MG levels in the liver at 6 and 24 h following (A) hepatectomy, (B) total castration without any testosterone implants and (C) total castration in rats in which four 4-cm testosterone implants were placed subdermally for 6 days prior to orchiectomy (on day 7) to maintain the endogenous testosterone level. Each time point had 3 adult rats. The right panel shows the same blots as the left panel after these blots were stripped and re-probed with an anti-actin antibody to assess equal protein loading and uniform protein transfer from gels to nitrocellulose membranes. (D) Histogram summarizing the densitometically scanned results using chemiluminograms such as those shown in A-C and normalized against actin. The level of $\alpha_2$-MG in the liver at 6 h was arbitrarily set at 1 since the basal level of $\alpha_2$-MG at the time of orchiectomy (time 0) was virtually non-detectable (see Fig. 1). Results are expressed as the mean ± s.d. of 3 rats. ns, not significantly different from the 6 h time point; *$P$<0·01 (by Student's $t$-test). ^$P$<0·01 (by ANOVA), comparing data in rats with testosterone implants and rats without implants.



Downloaded from Bioscientifica.com at 11/06/2024 05:16:59PM
via free access

in the testis after any surgical procedures. However, the basal $\alpha_2$-MG level in the testis is significantly higher than that in the liver, and is possibly being used to maintain spermatogenesis in the seminiferous epithelium, for example by protecting the seminiferous epithelium from the unwanted proteolysis associated with germ cell development during the epithelial cycle. Indeed, recent studies have shown that the testis is utilizing proteolysis to facilitate junction restructuring in the seminiferous epithelium pertinent to germ cell migration during spermatogenesis (Mruk *et al.* 1997, Longin *et al.* 2001, Siu & Cheng 2004, Wong *et al.* 2004, 2005) in much the same way as cell adhesion complexes are used to facilitate cell movement at the cell–matrix interface (for reviews, see McCawley & Matrisian 2001, Mruk & Cheng 2004).

Interestingly, the liver $\alpha_2$-MG levels were induced more significantly after sham operation (e.g. scrotum incision) than after total castration. While the general inflammatory response caused by total castration is more severe than that caused by sham operation, the inflammatory response of the liver is significantly lower when both testicles have been removed than after sham operation or hemicastration. These results have provided a strong argument for the fact that the testis may be releasing a hormonal substance(s) that can modulate liver $\alpha_2$-MG levels in response to inflammation induced by the surgical procedures. It is possible that the testis secretes biological factor(s) into the systemic circulation, which, in turn, accelerates the liver inflammatory response. When both testes are removed in total castration, they can no longer synthesize and secrete these biological factors, resulting in a significantly lowered inflammatory response. This observation also explains our earlier studies that germ cells and GCCM can upregulate $\alpha_2$-MG levels in hepatocyte cultures. Since the testis is the principal source of androgens in mammals, we next sought to investigate if the endogenous testosterone level that can be maintained by using testosterone implants when both testicles are removed can restore the liver's responsiveness to castration-induced inflammation regarding its $\alpha_2$-MG production. Indeed, the presence of testosterone implants can significantly revive the inflammation-induced $\alpha_2$-MG production in the liver following orchiectomy, and this strongly suggests that testosterone is the crucial regulator released from the testis that can regulate acute-phase response in the liver.

While these findings are preliminary in nature regarding the presence of a physiological relationship between the liver and the testis, they are significant in many ways. For instance, they provide proof that there is physiological cross-talk between the liver and the testis via androgens, at least in terms of the acute-phase response in the liver. Furthermore, these results illustrate that androgens are crucial regulators of acute phase response in the liver. It is also likely that testosterone is one of the factors that maintain a high level of $\alpha_2$-MG in the seminiferous epithelium to support spermatogenesis. Nonetheless, there are many questions that remain to be addressed. For example: are these yet-to-be identified testis factors (e.g. testosterone) that can modulate the inflammatory response in the liver, limited only to regulating the level of $\alpha_2$-MG? Do these factors regulate other liver functions *in vivo* under normal physiological conditions in addition to the protein secretory function such as $\alpha_2$-MG? These questions must be carefully addressed in future studies. These future studies will also shed more light on the physiological link between the testis and the liver.

## Funding

This work was supported in part by grants from the National Institutes of Health (NICHD, U01 HD045908 to C Y C, U54 HD029990 Project 3 to C Y C), the CONRAD Program (CICCR CIG-01–72 to C Y C, and CIG-01–74 to D D M) and Hong Kong Research Grant Council (HKU 7194/01 M and 7413/04 M to W M L). The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

## References

Bradford MM 1976 A rapid and sensitive method for the quantitation of microgram quantites of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72** 248–254.

Braghiroli L, Silvestrini B, Sorrentino C, Grima J, Mruk D & Cheng CY 1998 Regulation of $\alpha_2$-macroglobulin expression in rat Sertoli cells and hepatocytes by germ cells *in vitro*. *Biology of Reproduction* **59** 111–123.

Cheng CY & Bardin CW 1987 Identification of two testosterone-responsive testicular proteins in Sertoli cell-enriched culture medium whose secretion is suppressed by cells of the intact seminiferous tubule. *Journal of Biological Chemistry* **262** 12768–12779.

Cheng CY, Grima J, Stahler MS, Guglielmotti A, Silvestrini B & Bardin CW 1990 Sertoli cell synthesizes and secretes a protease inhibitor, $\alpha_2$-macroglobulin. *Biochemistry* **29** 1063–1068.

Dennis PA, Saksela O, Harpel P & Rifkin DB 1989 $\alpha_2$-Macroglobulin is a binding protein for basic fibroblast growth factor. *Journal of Biological Chemistry* **264** 7210–7216.

von Eckardstein S, Noe G, Brache V, Nieschlag E, Croxatto H, Alvarez F, Moo-Young A, Sivin I, Kumar N, Small M & Sundaram K 2003 A clinical trial of 7$\alpha$-methyl-19-nortestosterone implants for possible use as a long-acting contraceptive for men. *Journal of Clinical Endocrinology and Metabolism* **88** 5232–5239.

Gaddy-Kurten D, Hickey GJ, Gauldie J & Richards JS 1989 Hormonal regulation and tissue-specific localization of $\alpha_2$-macroglobulin in rat ovarian follicles and corpora lutea. *Endocrinology* **125** 2985–2995.

Higgins GM & Anderson RM 1931 Experimental pathology of liver: restoration of liver of white rat following partial surgical removal. *Archives in Pathology and Laboratory Medicine* **12** 186–202.

Huang SS, O'Grady P & Huang JS 1988 Human transforming growth factor $\beta$-$\alpha_2$–macroglobulin complex is a latent form of transforming growth factor $\beta$. *Journal of Biological Chemistry* **263** 1535–1541.

Kushner I 1982 The phenomenon of the acute phase response. *Annals of the New York Academy of Sciences* **389** 39–49.

Li AHY, Zwain IH, Pineau C, Cazzolla N, Saso L, Silvestrini B, Bardin CW & Cheng CY 1994 Response of $\alpha_2$-macroglobulin



Downloaded from Bioscientifica.com at 11/06/2024 05:16:59PM
via free access

messenger ribonucleic acid expression to acute inflammation in the testis is different from the response in the liver and brain. *Biology of Reproduction* **50** 1287–1296.

Longin J, Guillaumot P, Chauvin MA, Morera AM & Le Magueresse-Battistoni B 2001 MT1-MMP in rat testicular development and the control of Sertoli cell proMMP-2 activation. *Journal of Cell Science* **114** 2125–2134.

Lui WY, Lee WM & Cheng CY 2003 Sertoli-germ cell adherens junction dynamics in the testis are regulated by RhoB GTPase via the ROCK/LIMK signaling pathway. *Biology of Reproduction* **68** 2189–2206.

Lui WY, Mruk DD & Cheng CY 2005 Interactions among IQGAP1, Cdc42, and the cadherin/catenin protein complex regulate Sertoli-germ cell adherens junction dynamics in the testis. *Journal of Cellular Physiology* **202** 49–66.

McCawley LJ & Matrisian LM 2001 Matrix metalloproteinases: they're not just for matrix anymore! *Current Opinions in Cell Biology* **13** 534–540.

Mruk DD & Cheng CY 2004 Sertoli–Sertoli and Sertoli–germ cell interactions and their significance in germ cell movement in the seminiferous epithelium during spermatogenesis. *Endocrine Reviews* **25** 747–806.

Mruk D, Zhu LJ, Silvestrini B, Lee WM & Cheng CY 1997 Interactions of proteases and protease inhibitors in Sertoli-germ cell co-cultures preceding the formation of specialized Sertoli-germ cell junctions *in vitro*. *Journal of Andrology* **18** 612–622.

Noe G, Suvisaari J, Martin C, Moo-Young A, Sundaram K, Saleh SI, Quintero E, Croxatto HB & Lahteenmaki P 1999 Gonadotropin and testosterone suppression by 7α-methyl-19-nortestosterone acetate administered by subdermal implant to healthy men. *Human Reproduction* **14** 2200–2206.

Siu MKY & Cheng CY 2004 Interactions of proteases, protease inhibitors, and the β1 integrin/laminin γ3 protein complex in the regulation of ectoplasmic specialization dynamics in the rat testis. *Biology of Reproduction* **70** 945–964.

Stahler MS, Schlegel P, Bardin CW, Silvestrini B & Cheng CY 1991 $\alpha_2$-Macroglobulin is not an acute-phase protein in the rat testis. *Endocrinology* **128** 2805–2814.

Wong CH, Mruk DD, Lui WY & Cheng CY 2004 Regulation of blood–testis barrier dynamics: an *in vivo* study. *Journal of Cell Science* **117** 783–798.

Wong CH, Mruk DD, Siu MKY & Cheng CY 2005 Blood–testis barrier dynamics are regulated by $\alpha_2$-macroglobulin via the c-Jun N-terminal protein kinase pathway. *Endocrinology* **146** 1893–1908.

Zhang J, Wong CH, Xia W, Mruk DD, Lee NPY, Lee WM & Cheng CY 2005 Regulation of Sertoli-germ cell adherens junction dynamics via changes in protein–protein interactions of the N-cadherin-β-catenin protein complex which are possibly mediated by c-Src and myotubularin-related protein 2: an *in vivo* study using an androgen suppression model. *Endocrinology* **146** 1268–1284.

Zhu LJ, Cheng CY, Phillips DM & Bardin CW 1994 The immunohistochemical localization of $\alpha_2$-macroglobulin in rat testes is consistent with its role in germ cell movement and spermiation. *Journal of Andrology* **15** 575–582.

Zwain IH, Grima J, Stahler MS, Saso L, Cailleau J, Verhoeven G, Bardin CW & Cheng CY 1993 Regulation of Sertoli cell $\alpha_2$-macroglobulin and clusterin (SGP-2) secretion by peritubular myoid cells. *Biology of Reproduction* **48** 180–187.

Received 28 December 2004
Accepted 16 March 2005



Downloaded from Bioscientifica.com at 11/06/2024 05:16:59PM
via free access