

Short note

## Inhibition by TGF- $\beta_1$ of the *in vitro* thymulin-stimulated proliferation of gonocytes from fetal rat testes

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(Received 5 July 1996; accepted 27 November 1996)

**Summary** — The cytokine transforming growth factor  $\beta_1$  (TGF- $\beta_1$ ) inhibits the growth of certain cells and the differentiation of others. A thymus hormone, thymulin, stimulated the proliferation of fetal male germ cells in explants of testes from 13.5 gestation day rat fetuses. The way in which thymulin acts is unknown. Adding TGF- $\beta_1$  to the culture medium blocked the response of the fetal male rat germ cells to thymulin. We suggest that TGF- $\beta_1$  and thymulin may thus influence the same metabolic chain of events.

**gonocyte / prolifération / TGF- $\beta_1$  / thymulin / fœtus**

**Résumé** — Le TGF- $\beta_1$  inhibe la prolifération des gonocytes induite par la thymuline. Étude *in vitro* chez le fœtus de rat mâle. Le TGF- $\beta_1$  (transforming growth factor  $\beta_1$ ) est une cytokine modulatrice de prolifération qui inhibe la croissance de certaines lignées cellulaires et stimule la croissance ou la différenciation d'autres lignées. La thymuline, hormone thymique, stimule la prolifération des cellules germinales fœtales de rat mâle bien que les bases moléculaires de son mode d'action concernant cet effet ne soient pas connues. L'addition dans le milieu de culture de TGF- $\beta_1$  diminue considérablement les effets stimulateurs de la thymuline sur la prolifération des cellules germinales fœtales de rat. Une hypothèse est proposée concernant le mode d'action de la thymuline.

**gonocyte / prolifération / TGF- $\beta_1$  / thymuline / fœtus**

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## INTRODUCTION

Many experiments have been performed on the control of fetal and perinatal germ cells proliferation (Prépin, 1993a). We have reported that thymulin, a non peptide released by thymus epithelial cells, which plays an essential role in the development of immunological capacity increases germ cell proliferation in fetal male rat gonads. We have also shown that the hypophyseal-adrenal-thymus axis is involved in the regulation of fetal germ cell numbers (Prépin and Jost, 1991). But the number of mitoses remains low in gonocytes from male newborn rats incubated with thymulin, despite a rapid incorporation of  $^3\text{H}$ -thymidine. This suggests that thymulin affects DNA duplication in gonocytes but not proliferation (Prépin et al, 1994). The way in which thymulin acts on germ cell proliferation has not yet been elucidated.

In contrast, the  $\text{TGF-}\beta_s$  are polypeptide growth factors that are multifunctional regulators of both growth and development in many different tissues. To date, three forms of  $\text{TGF-}\beta_s$  have been identified in mammals.  $\text{TGF-}\beta_1$  is present in the fetal rat testis (Gautier et al, 1994) and it decreases the rate of isolated mouse primordial germ cells proliferation after 3 days in vitro (Godin and Wylie, 1991).  $\text{TGF-}\beta_1$  also prevents the multiplication of lung epithelial cells by blocking the late G1 phases of the cell cycle transition G1-phase / S-phase (Howe et al, 1991). Thus, thymulin and  $\text{TGF-}\beta_1$  may act by influencing the S-phase of the cell cycle in different cell types.

The studies described here were undertaken to determine the effects of  $\text{TGF-}\beta_1$  on fetal male gonocyte proliferation after 1 day in vitro and identify any inhibitory effects of  $\text{TGF-}\beta_1$  on proliferation stimulated by thymulin, so as to understand more clearly how thymulin acts.

## MATERIALS AND METHODS

### Animals

Wistar CF rats (stock from the CNRS, France) were used. The age of the fetus was based on the estimated time of ovulation, ie, 0200 h on the night of pairing (Jost and Picon, 1970). Pregnant females were identified by palpation 13 days later. Precisely 13 days plus 13 h after fertilization 1400 h-1500 h, the females were killed by cervical dislocation and the fetuses were rapidly removed from the uterus. Their sex was determined by applying the sex chromatin to cells of the aminotic membrane. Both gonads and their associated mesonephroi were aseptically dissected from the fetuses and placed in organ culture.

### Culture procedure

The explants were cultured immersed in 0.8 mL medium CMRL 1066 (Eurobio, Paris) containing 250 IU benzyl-penicillin/mL (Spenicilline G: Specia, France) and 100  $\mu\text{g}$  streptomycin sulphate/mL (Specia) in dishes (Falcon Plastics, no 3037), at  $36 \pm 1$  °C in 95% air + 5%  $\text{CO}_2$ , for 24 h. The explants were cultured in medium alone (controls) or in medium containing 25  $\mu\text{g}/\text{mL}$  thymulin (Bachem, Switzerland), or 12  $\text{ng}/\text{mL}$   $\text{TGF-}\beta_1$  (Sigma, France), or in medium containing both thymulin and  $\text{TGF-}\beta_1$ .

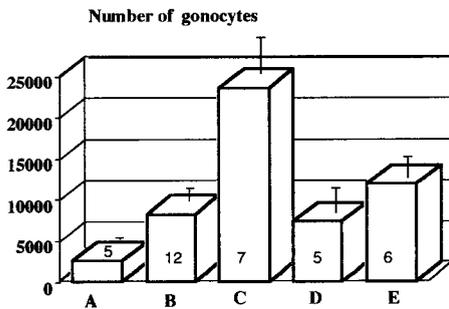
### Counting gonocytes

The germ cells were identified by their large size and round lightly staining nuclei (6-7  $\mu\text{m}$  diameter). The cultured explants were fixed in Bouin's fluid and serial sections (5  $\mu\text{m}$  thick) were cut and stained with haematoxylin-eosin. Germ cell were counted on serial sections of each pair of the testicular explants. Significance was accepted as  $P < 0.05$  and determined using one way analysis of variance (PLSD of Fischer, F of Scheffé and t of Dunnett).

## RESULTS

The results are shown in figure 1. The two testes taken from fetuses on day 13.5 pc

(post-coitus) contained 2 550 gonocytes prior to organ culture. The number of germ cells increased 3.3-fold after culture in control medium for 1 day. The number of gonocytes in explants cultured in medium supplemented with thymulin was about 3-fold greater than that of testes grown in medium alone. In contrast, the number of gonocytes in testes cultured in medium containing TGF- $\beta$ <sub>1</sub> was slightly smaller than in the cultured controls, but the difference was not significant. The decrease in the number of germ cells appeared after only 2 days in vitro (data not shown). Explants cultured in medium supplemented with both thymulin and TGF- $\beta$ <sub>1</sub> contained the same number of gonocytes as the controls or testes cultured in medium containing TGF- $\beta$ <sub>1</sub>. This number was significantly lower than the number of gonocytes in testes cultured in medium with thymulin.



**Fig 1.** Effects of thymulin and TGF- $\beta$ <sub>1</sub> on the mean numbers of gonocytes in both testes of fetal rats. The mean number  $\pm$  SEM is indicated. A = on day 13.5, day of explantation. After 1 day of explant culture: B = in non-supplemented medium (controls); C = in medium supplemented with thymulin; D = in medium supplemented with TGF- $\beta$ <sub>1</sub>; E = in medium supplemented with thymulin and TGF- $\beta$ <sub>1</sub>. The numbers within the columns indicate the numbers of explants.

## DISCUSSION

TGF- $\beta$ <sub>1</sub> did not significantly inhibit the proliferation of gonocytes in testes grown as explants for 24 h and TGF- $\beta$ <sub>1</sub> is present in Sertoli cells on day 14.5 pc and in the Leydig cell on day 16.5 pc (Gautier et al, 1994), shortly before the fetal male gonial stop proliferating (Beaumont and Mandl, 1963). A similar absence of inhibition of proliferation of fetal mouse primordial germ cells after 1 day in medium supplemented with TGF- $\beta$ <sub>1</sub>, was also reported by Godin and Wylie (1991). But, TGF- $\beta$ <sub>1</sub> inhibits the fetal male proliferation after 2 days in vitro in a dose dependent manner (personal data not shown), while thymulin stimulates gonocyte proliferation in male rat fetuses when explants of fetal testes and thymus are cultured together (Prépin, 1993b). The main finding from the present study is the capacity of TGF- $\beta$ <sub>1</sub> to inhibit stimulation of gonial proliferation by thymulin. TGF- $\beta$ <sub>1</sub> is known to prevent the proliferation of lung epithelial cells by inhibiting phosphorylation of protein P34<sup>cdc2</sup> in the MPF (M-phase Factor) (Howe et al, 1991), which is the universal regulator of cell division. Although the mechanism of thymulin action remains to be discovered, the present findings suggest that both TGF- $\beta$ <sub>1</sub> and thymulin act on the same target, or on the same chain of processes leading to gonial proliferation. Further studies will be needed to confirm this.

## ACKNOWLEDGMENTS

We thank Pr R Habert for his help and advice. The English text was checked by Dr Owen Parkes.

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