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Short note

Inhibition by TGF-β_1 of the in vitro thymulin-stimulated proliferation of gonocytes from fetal rat testes

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Summary — The cytokine transforming growth factor β_1 (TGF-β_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-β_1 to the culture medium blocked the response of the fetal male rat germ cells to thymulin. We suggest that TGF-β_1 and thymulin may thus influence the same metabolic chain of events.

gonocyte / proliferation / TGF-β_1 / thymulin / fetus

Résumé — Le TGF-β_1 inhibe la prolifération des gonocytes induite par la thymuline. Étude in vitro chez le fœtus de rat mâle. Le TGF-β_1 (transforming growth factor β_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-β_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-β_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 apeptide 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 incubates with thymulin, despite a rapid incorporation of ^{3}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 TGF-β_s are polypeptide growth factors that are multifonctional regulators of both growth and development in many different tissues. To date, three forms of TGF-β_s have been identified in mammals. TGF-β_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). TGF-β_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 TGF-β_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 TGF-β_1 on fetal male gonocyte proliferation after 1 day in vitro and identify any inhibitory effects of TGF-β_1 on proliferation stimulated by thymulin, so as to understand more clearly how thymulin acts.

MATERIALS AND METHODS

Animals

Wister 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 mesonophroi 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 µg streptomycin sulphate/mL (Specia) in dishes (Falcon Plastics, no 3037), at 36 ± 1 °C in 95% air + 5% CO_2, for 24 h. The explants were cultured in medium alone (controls) or in medium containing 25 µg/mL thymulin (Bachem, Switzerland), or 12 ng/mL TGF-β_1 (Sigma, France), or in medium containing both thymulin and TGF-β_1.

Counting gonocytes

The germ cells were identified by their large size and round lightly staining nuclei (6-7 µm diameter). The cultured explants were fixed in Bouin's fluid and serial sections (5 µm 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-β_1 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 no shown). Explants cultured in medium supplemented with both thymulin and TGF-β_1 contained the same number of gonocytes as the controls or testes cultured in medium containing TGF-β_1. This number was significantly lower than the number of gonocytes in testes cultured in medium with thymulin.

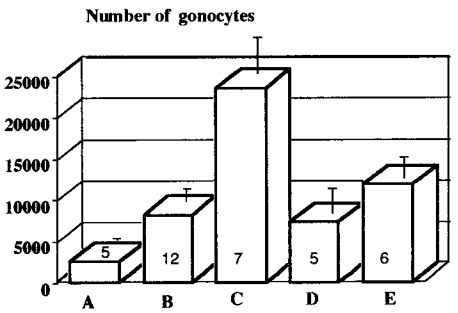

Fig 1. Effects of thymulin and TGF-β_1 on the mean numbers of gonocytes in both testes of fetal rats. The mean number ± SEM is indicated. **A** = on day 13.5, day of explantation. After 1 day of explant culture : **B** = in non-supplemented medium (controls); **C** = in medium supplemented with thymulin; **D** = in medium supplemented with TGF-β_1; **E** = in medium supplemented with thymulin and TGF-β_1. The numbers within the columns indicate the numbers of explants.

DISCUSSION

TGF-β_1 did not significantly inhibit the proliferation of gonocytes in testes grown as explants for 24 h and TGF-β_1 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 gonia 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-β_1, was also reported by Godin and Wylie (1991). But, TGF-β_1 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-β_1 to inhibit stimulation of gonia proliferation by thymulin. TGF-β_1 is known to prevent the proliferation of lung epithelial cells by inhibiting phosphorylation of protein P34 ^{cdc2} 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-β_1 and thymulin act on the same target, or on the same chain of processes leading to gonia proliferation. Further studies will be needed to confirm this.

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REFERENCES

Beaumont HM, Mandl AM (1963) A quantitative study of primordial germ cells in the male rat. *J Embryol Exp Morphol* 11, 715-740

Gautier C, Levacher C, Avallet O, Vigier M, Rouiller-Fabre V, Lecerf L, Saez J, Habert R (1994) Immunohistochemical localisation of transforming growth factor-β_1 in the fetal and neonatal rat testis. *Mol Cell Endocrinol* 99, 55-61

Godin I, Wylie CC (1991) TGF-β_1 inhibits proliferation and has a chemotropic effect on mouse primordial germ cells in culture. *Development* 113, 1451-1457

Howe P, Draeta G, Leof ED (1991) Transforming growth factor β_1 inhibition of P34 cdc^2 phosphorylation and histone H1 kinase activity is associated with G1/S-phase growth arrest. *Mol Cell Biol* 11, 1185-1194

Jost A, Picon L (1970) Hormonal control of fetal development and metabolism. In: *Advances in Metabolic Disorders* (Levine R, Luft E eds), Academic Press, New York, USA, 123-184

Prépin J (1993a) Prolifération des cellules germinales fœtales de mammifères, état en 1993, *Médecine/Sciences* 12, 1362-1366

Prépin J (1993b) Le thymus fœtal et la thymuline stimulent la prolifération des gonocytes dans le testicule fœtal de rat in vitro. *CR Acad Sci (Paris), Sciences de la vie/Life science* 316, 451-454

Prépin J, Jost A (1991) Augmentation du nombre des ovogonies dans l'ovaire de fœtus de lapin hypophysectomisé par décapitation. Rôle probable du thymus. *CR Acad Sci (Paris)* 313, 81-85

Prépin J, Le Vigouroux P, Dadoune JP (1994) Effects of thymulin on in vitro incorporation of ^{3}H-thymidine into gonocytes of newborn rat testes. *Reprod Nutr Dev* 34, 289-294