Arch. Biol. Med. Exp. *13*, 321-324, 1980

Control of Differentiation and Proliferation of Germ Cells by Somatic Cells in the Developing Gonad*

Control de la diferenciación y proliferación de células germinales por células somáticas en la gónada en desarrollo

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(Recibido el 20 de octubre de 1980)

WARTENBERG, H. Control of differentiation and proliferation of germ cells by somatic cells in the developing gonad (Control de la diferenciación y proliferación de células germinales por células somáticas en la gónada en desarrollo). *Arch. Biol. Med. Exp. 13,* 321-324, 1980.

In the preceding paper (Wartenberg, 1980c) the role of two different types of somatic cells during development of the gonadal anlage and during sexual differentiation was discussed. There is good evidence that differentiation into a male or female organ, into a testis or an ovary, depends on the distribution of one of the two blastemal parts in relation to the other. The early period of differentiation, characterized by a sexually indifferent gonad, shows an interrelation between two gonadal blastemal parts which leads to the formation of a common gonadal blastema. One of the blastemal parts derives from superficial epithelium, the former coelomic epithelium, which proliferates under the influence of the other blastemal part originating from the mesonephros (Wartenberg, 1978, 1979, 1980a, 1980b).

During the course of sexual differentiation in the developing ovary, mesonephrogenic cells and the derivatives of the superficial epithelium remain apart, the latter forming the ovarian cortex. In the developing testis, the epithelial blastema becomes displaced from its superficial position into the medulla, since mesonephrogenic cells move peripherally and gain dominance at the surface of the testis. In the medulla, both types of blastemal cells are intermingled, and from this mixed somatic blastema, testicular cords and interstitial tissue differentiate.

During further development, somatic cells of dual origin fill the testicular cords and differentiate into supporting cells which establish an interactive relationship with the enclosed germ cells. In the ovary mesonephric blastemal cells penetrate the epithelial cortex. Due to the increasing number of mesonephrogenic cells within the cortex, germ cells come under the influence of these. The conclusion seems justified that the proclivity of the germ cell to become male or female is incorporated in the interactive relationship established by two different types of supporting cells, those in the testis representing the future Sertoli cells and those in the ovary the future granulosa cells. This interaction between sex and soma, between germ cells and supporting cells, and its influence on germ cell differentiation, will be the subject of this paper.

Proliferation of the male germ cell and its differentiation during the prespermatogenic period

During gonadal development germ cells are characterized by certain peculiarities. They show close contact to somatic cells and under the influence of an inductive system

* Presented at the Ist Regional Meeting of the Sección Biología de la Reproducción y del Desarrollo (Sociedad de Biología de Chile), Antofagasta, 14-17, August, 1980.

they start the proliferate. Male and female germ cells run through a similar sequence of differential periods and phases of structural changes. The male germ cell, however, differs in one important point from the female one: The latter enters the prophase of meiosis, while the male germ cell seems to be prevented from acting in same way. On the other hand, it has been demonstrated that male and female germ cells enter their proliferating phase at the same time. In human testes the period of rapid multiplication starts on about day 64 and results in the appearance of prospermatogonia (Wartenberg, 1976). These fetal gonia increase numerically in a manner similar to the mechanism of spermatogonial proliferation in the adult testis. They are linked by intercellular bridges (Wartenberg *et al.*, 1971). The mitotic activity of the fetal spermatogonia is highly synchronized and results in a rapid multiplication of the germ cells.

In view of these differences and because the cells are morphologically dissimilar to their primordial stage, a new terminology has been introduced by Hilscher *et al.*, (1974), who refer to these germ cells as M-prospermatogonia (M=multiplying). Fig. 1a summarizes these events and demonstrates that female germ cells undergo a very similar period of mitotic activity. Oogonia divide as rapidly as their male counterpart. If oogonia have terminated their multiplication period they enter the oocyte stage. Immediately afterwards, meiotic prophase commences. The M-prospermatogonia, on the other hand, on terminating their mitotic activity enter a resting phase instead of commencing meiotic prophase. These so called T-prospermatogonia (T=transitional) appear in human testes on about day 85 (Wartenberg, 1978). After conclusion of meiotic prophase, when the oocytes begin forming the first follicles, the mitotic activity of the male germ cells is restored. According to Hilscher *et al.* (1974), these cells are termed T_1 –prospermatogonia. They subsequently become the A-spermatogonia of the adult testis. This similarity between the differential processes of the male and female germ cell lines is characteristic not only for the mouse but for most of the small mammalian species (Wartenberg, 1976).

Due to the long duration of prenatal development, the differentiation of human germ cells and that of other large mammalian species exhibits a rather different mechanism. The time of germ cell differentiation becomes indistinct due to an overlap of the single periods (Fig. 1b). Populations of different types of germ cells may exist side by side in the testis or in the ovary: this might explain why primordial germ cells can be localized between the most advanced stages of the meiotic prophase, or T_2 –prospermatogonia.

The dual system of supporting cells regulating male germ cell differentiation

To explain the induction of the meiotic process in the female gonad and the inhibiting effect that prevents the male germ cell from entering meiosis during fetal life, inducing and inhibiting agents have been postulated (Byskov, 1974, Byskov and Saxén, 1976). This concept of an antagonistic mechanism, enabling two substances to induce or prevent meiosis, has been adapted to a concept of two separate types of supporting cells in the developing testis (Wartenberg, 1978). Dark and light supporting cells as observed in the fetal testis may represent this dualistic system. Their stimulating or inhibiting effect might act positively on the mitotic proliferation of the male gonial stage and, on the other hand, prevent onset of meiosis. The fact that the appearance of mitotic prospermatogonia together with that of dark supporting cells and with the existence of primordial germ cells or resting prospermatogonia coincides with an increasing number of light supporting cells corroborates the concept of an antagonistic influence mediated by two different somatic cell populations.

In the human testis the number of dark supporting cells increases at the beginning of the 10th week. The dark cells show close contact to proliferating prospermatogonia. The number of dark somatic cells increases

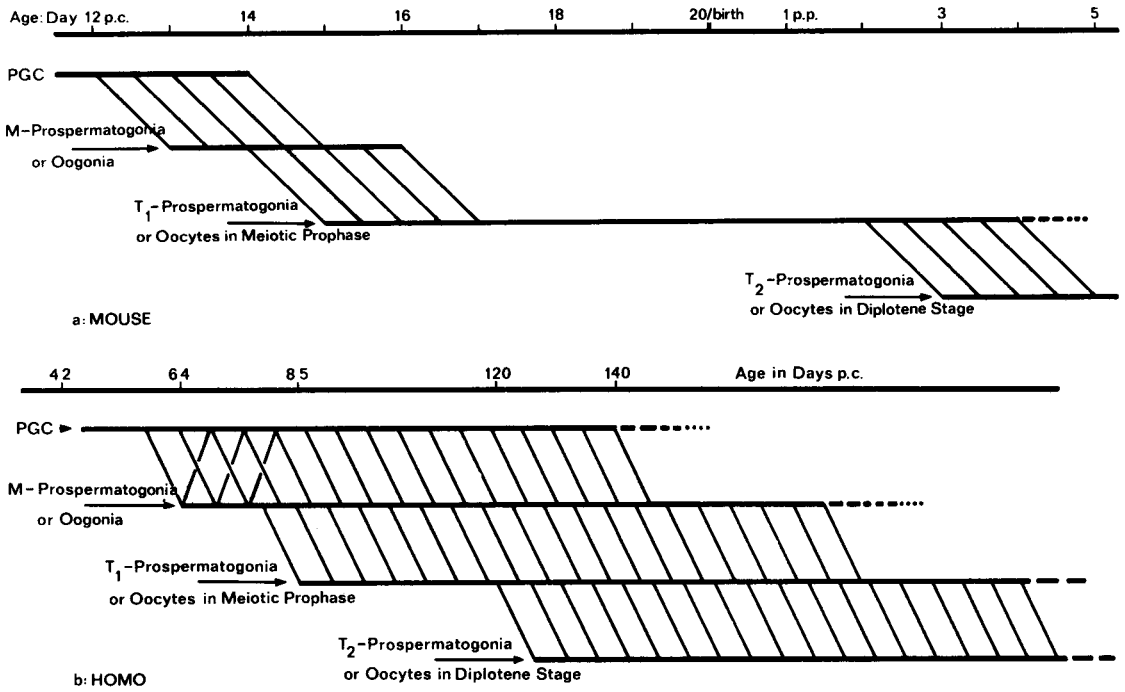

Fig. 1. Periods of male and female germ cell differentiation during prenatal development of the mouse (a) and human (b) embryogenesis. (from Wartenberg 1980 a).

rapidly until the 12th week of testicular development and the number of germ cells has also increased. On the other hand, light supporting cells dominate from the 7th until 9th week.

Under these conditions the primordial germ cells persist in their undifferentiated stage. The electron micrographs demonstrate these differences. Non-proliferating germ cells (primordial germ cells and resting prospermatogonia) are surrounded by light supporting cells that do not exhibit any peculiarities. More advanced prospermatogonia are enveloped by dark supporting cells or their cell processes which can be extremely thin and long (Wartenberg, 1978, 1979, 1980).

The parallelism of events in the developing testis and ovary lends additional support to the idea that the male germ cells, like the female germ cells, prepare for the onset of meiotic prophase by entering premeiotic proliferation. They do this under the activating influence of dark mitosis-and meiosis-inducing cells. But, unlike conditions in the female gonad, the light mitosis-and meiosis-preventing cells regain dominance in the testicular cords, and meiosis is arrested before it starts.

The dual system of supporting cells regulating female germ cell differentiation in the ovarian cortex

It has been shown that the ovarian cortex is primarily formed by most of proliferating superficial epithelium, which contains most of the germ cells. The mesonephrogenic blastema is concentrated below the cortex. During further differentiation, the mesonephrogenic dark cells penetrate the cortex and the two blastemas become mixed. During the beginning of the 10th week, majority of the dark cells are still located in the deeper part of the cortex and below it. A few dark cells, however, have penetrated the cortex and are visible in the upper part. Many of the germ cells in the lower region are surrounded by dark supporting cells and these have started to proliferate.

These are oogonia, while germ cells close to the superficial epithelium stay in their primordial stage. Three weeks later, the number of dark supporting cells has increased and they are concentrated even in

the most superficial region. Germ cells in the lower part have entered the meiotic prophase while germ cells in the upper part proliferate rapidly.

Summarizing the points so far discussed, it may be postulated from evidence of primarily morpholcgical nature that the germ cells are regulated, in respect of their capability of proliferating and of entering meiotic prophase by the numerical ratio of inducing to preventing cells. Whether or not a germ cell enters mitotic proliferation depends on the amount of cellular contact between germ cell and the inducing cellular system. As long as the inductive influence dominates the preventing influence of the antagonistic cellular system, the germ cell divides mitotically and may even enter meiosis. The topographical arrangement in the testicular cords, which differs from that in the ovarian cortex, might be due to the fact that male germ cells do not enter meiosis during the prenatal and prepuberal period.

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