Dynamics of Leydig Cell Regeneration After EDS
A Model for Postnatal Leydig Cell Development

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SUMMARY

Synthesis and secretion of androgens is the most important function of Leydig cells in the testis. The levels of androgens produced by these cells not only depend on their capacity to produce these steroids, but also on the number of Leydig cells present in the testis. Therefore, it is essential to understand how the formation of the Leydig cell population is regulated and to identify the factors, which play a role in this developmental process. However, the initial studies to investigate the regulation of Leydig cell development were not undertaken in the (pre)pubertal testis but in the adult testis. With the identification of ethane-1,2-dimethyl sulphoxide (EDS) as a specific Leydig cell toxicant a large number of studies were initiated. The latter was because of the fact that following EDS administration a completely new Leydig cell population was formed. This chapter summarizes more than 20 yr of research on Leydig cell development in the adult testis using EDS as a model. The sensitivity of Leydig cells in different species for the cytotoxic action of EDS is discussed as well as the possible mechanism of action of this cytotoxic compound. A comparison is made between Leydig cell development in the (pre)pubertal testis and the adult testis during the regeneration process following EDS administration. Specific emphasis is paid to the regulatory role of the gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH) as well as other systemic and locally produced factors, such as thyroid hormone, insulin-like growth factor (IGF)-1, and transforming growth factors (TGF)-α and TGF-β, in this developmental process. It is concluded that there appear to be many similarities and hardly any discrepancies in the regulation of the development of precursor cells into mature adult-type Leydig cells during (pre)puberty and in the adult rat following EDS administration.

In the perinatal period when the stem cells become committed to lineage-specific differentiation, there are also differentiated Leydig cells present in the interstitium, namely, the fetal-type Leydig cells, which could influence the development of the adult-type Leydig cell population. Moreover, the intratesticular microenvironment of the (pre)pubertal testis is presumably rich in growth and differentiation inducing factors, whereas not only adult-type Leydig cells are developing but also other somatic cells are undergoing growth and differentiation. In contrast, following EDS administration in the adult animal, all differentiated Leydig cells are eliminated; the only undifferentiated cells left are presumably the stem cells/precursor cells. Taking into account the aforementioned, although EDS is a toxic compound which might influence the testicular microenvironment, the similarities between adult-type Leydig cell development in the (pre)pubertal testis and Leydig cell regeneration after EDS, make it tempting to speculate that the EDS-treated adult rat is better model for the study of the regulation of adult-type Leydig cell development than the (pre)pubertal testis. It is easier to follow cellular differentiation and ontogeny when no other mature cells are around.

Key Words: Adult-type Leydig cell development; EDS; Leydig cell regeneration; LH; FSH; LH receptor; steroidogenic enzyme.

INTRODUCTION

The most important function of Leydig cells in the (pre)pubertal and adult testis is the synthesis and secretion of steroids, 5α-reduced androgens and testosterone, which are essential for the progression of spermatogenesis. In the 1970s and 1980s, much emphasis was put on the regulation of androgen synthesis by the Leydig cells. However, the levels of androgens produced by the testis not only depend on the capacity of these cells to produce steroids, but also on the number of Leydig cells present in the interstitium.
Therefore, it is essential to understand how the Leydig cell population in the adult testis is formed, and which factors play a role in the regulation of this developmental process. However, the initial studies concerning the regulation of Leydig cell development in the testis were not undertaken in the (pre)pubertal testis but in the adult testis. In the early 1980s, it became apparent that it was possible to selectively destroy the complete Leydig cell population in the adult rat testis within 3 d by treating the animals with a single dose of the alkylating agent EDS. This process was followed by a complete regeneration of the original Leydig cell population within a few weeks (1–3). In this chapter a comparison will be made between the regeneration of the Leydig cell population after EDS administration and the development of the adult-type Leydig cell population in the (pre)pubertal testis. Although, it has been demonstrated in the past 10 yr that EDS also acts as a Leydig cell toxicant in other species, such as mouse (4–7), guinea pig (7), hamster (7–9), monkey (10), rabbit (11), frog (12,13), and lizard (14), the main emphasis in this chapter will be on the rat. Evidence will be presented that Leydig cell regeneration in the adult testis following EDS, and the formation of the adult-type Leydig cell population in the (pre)pubertal testis develop along the same lineage.

**Gonadotropins and Leydig Cell Development**

Generally, in the rodent testis it has been accepted that two waves of proliferation and differentiation can be discerned during the development of the Leydig cell population. The first wave takes place during the fetal life independent of LH (15–18), whereas the second wave occurs during the (pre)pubertal period and is largely dependent on LH (19–22). After birth the population of fetal-type Leydig cells decreases in size, although some fetal-type cells persist even in the adult testis (23). The second generation of Leydig cells, the so-called adult-type Leydig cells, develops through several steps of proliferation and differentiation (15,20,21). Stem cells which are platelet-derived growth factor (PDGF) receptor-α-positive, but do not express steroidogenic enzymes or LH receptors, are believed to differentiate into mesenchymal-like precursor cells (24). These precursor cells also do not express steroidogenic enzymes but do acquire LH receptors before they differentiate into 3β-HSD hydroxysteroid dehydrogenase positive Leydig cell progenitors (Teerds et al., manuscript submitted). Subsequently, these cells undergo further proliferation and differentiation, and develop into immature adult-type Leydig cells, in which proliferation is limited. These immature Leydig cells undergo further differentiation into mature adult-type Leydig cells (20,21,25). In the rat, this wave of adult-type Leydig cell development is initiated around day 14 after birth with the formation of the first Leydig cell progenitors. These cells undergo proliferation and differentiation and around day 35 postpartum become immature adult-type Leydig cells, which still undergo some mitotic divisions and finally, when proliferation ceases around the age of 60 d, become mature adult-type Leydig cells (Fig. 1; refs. 20,26,27). Each step in this developmental process is characterized by specific morphological aspects of the developing cells (21,22,25,28) and the expression of specific steroidogenic enzymes, such as 3β-HSD, 17β-HSD/17-ketosteroid reductase, and 5α-reductase (29,30).

Although, some controversy exists whether mesenchymal-like Leydig cell precursors at the onset of differentiating into progenitor cells express LH receptors, in vivo studies have indicated that the conversion of precursor cells into Leydig cell progenitors and the subsequent proliferation and differentiation of the progenitors and immature adult-type Leydig cells into mature adult-type Leydig cells, is a gonadotropin-dependent process (refs. 21,31–37; Teerds et al., manuscript submitted). Treatment of hypophysectomized immature rats with highly purified preparations of pituitary FSH or human recombinant FSH, had a stimulatory effect on the differentiation of mesenchymal-like precursor cells into Leydig cell progenitors, without affecting the proliferative capacity of these progenitors (34–37).

Treatment with highly purified LH stimulated the differentiation of the precursors into progenitors as well as the proliferation of these newly formed Leydig cell progenitors (36). Administration of the LH analog human chorionic gonadotropin (hCG) to (pre)pubertal boys whose testes were devoid of morphologically recognizable Leydig cells, also enhanced the differentiation of precursor cells into progenitors and immature adult-type Leydig cells (32,33). When gonadotropin levels were very low or negligible, such as in hypogonadal mice (38), or in case of disruption of LH signaling in LH receptor knockout mice (39), adult-type Leydig cell development appeared to be severely affected. Taken together, these data indicate that the generation of a full complement of steroidogenically active mature adult-type Leydig cells is dependent on the presence of gonadotropins and functional gonadotropin receptors (Fig. 1). The mature adult-type Leydig cell population formed in this way is a stable population of cells that has long been presumed to be relatively insensitive to cytotoxic compounds and to irradiation (40–43).