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Relaxin-like Factor – a Marker of the Rat Leydig Cell Differentiation Status

Y. Koeva, M. Bakalska, N. Atanassova*, M. Davidoff***

Department of Anatomy, Histology and Embryology, Medical University, Plovdiv, Bulgaria,
**Institute of Experimental Morphology and Anthropology with Museum,*
Bulgarian Academy of Sciences, Sofia, Bulgaria
***Institute of Anatomy, University Hospital Hamburg, Germany*

The cytotoxic agent ethane dimethanesulphonate (EDS) specifically destroys Leydig cells (LC) in the adult testis, followed by a complete regeneration. The process of LC renewal after EDS shows homology to the development of the adult – type LC population in prepubertal testis. After EDS treatment, the immunoreactivity for relaxin-like factor (RLF), a new marker for LC maturation, disappeared from the testis and reappeared again at the time of regeneration of the first LC. Concomitant with the increase of the LC repopulation, the number of RLF –positive cells increased. The present findings support the hypothesis that EDS-treated rats can serve as a model for studying the LC development in the prepubertal rat testis and indicate a specific role of hormonal factors like a RLF in this process.

Key words: RLF- EDS- testis- Leydig cells.

Introduction

Ethane-1,2-dimethanesulphonate (EDS) is a unique testicular toxicant which selectively and temporary destroyed testicular Leydig cells (LC). Later a new population of LC regenerates, apparently from mesenchymal fibroblast-like precursors [2, 8]. The restoration of new LC population after EDS passed through the same intermediate stages occurred during normal postnatal development [8]. For this reason EDS model has been used extensively to investigate the physiological role of different hormonal and non-hormonal factors in the processes of LC regeneration and differentiation.

The Relaxin-like factor (RLF) or insulin-like 3 (INSL3) is peptide hormone, a novel member of the insulin- relaxin- insulin-like growth factor family, and seems to be localized predominantly in gonadal tissues [3]. RLF mRNA is expressed in the LC in a constitutive fashion and RLF/INSL3 thus seems to be a useful marker of LC differentiation status. The present study was aimed to establish the chronology and dynamic of RLF/INSL3 expression in the LC repopulation after exposure with EDS of mature rats.

Material and Methods

Testes of mature Wistar rats that received single intraperitoneal (i.p.) injection of EDS (75 mg/kg body weight) dissolved in dimethyl sulphoxide (DMSO): water (1:3 v/v) were used. A second group of rats received a single i.p. injection only of DMSO: water. The animals were killed 1, 7, 14, 21 and 35 days after initial treatment (n=10 per group). The pattern of RLF/INSL3 expression in newly formed LC after EDS treatment was established using a specific polyclonal anti-RLF/INSL3 antibody (Phoenix Peptide, USA, 1:200) and high sensitive immunohistohemical polymer detection kit (Zymed, USA).

Results and Discussion

In male mammals, RLF/INSL3 is a majority secretory product of the LC, where it appears to be expressed in a differentiation – dependent manner [3]. In the present study we found that on the 1st and especially 7 days after EDS, RLF- positive LC disappeared from the testicular interstitium consisting with the total loss of LC (Figs. 1, 2). Routine histological analysis showed that between 14 and 21 days after EDS injection a few LC with oval or spherical nuclei were observed within the intertubular space in a peritubular or perivascular position. During this period the number of LC was progressively increased and they formed large clusters usually in the vicinity of blood capillaries. According to previously reported data [1 ,9] our results revealed that the appearance of RLF/INSL3-immunoreactive cells coincided with the restoration of the LC population 14 days after EDS. On the 21st day after EDS treatment intensive immunolabeling for RLF was found in the regenerating LC (Fig. 3). 35 days after EDS destruction a larger number of RLF positive Leydig cells were seen in form of clusters corresponding with the regeneration of adult type LC population (Fig. 4). The chronology and dynamic of RLF/INSL3 expression in the present work is very similar to that seen in studies in rat postnatal development [5, 6, 7] and its pattern of expression correlates temporally with the development of steroidogenic func-

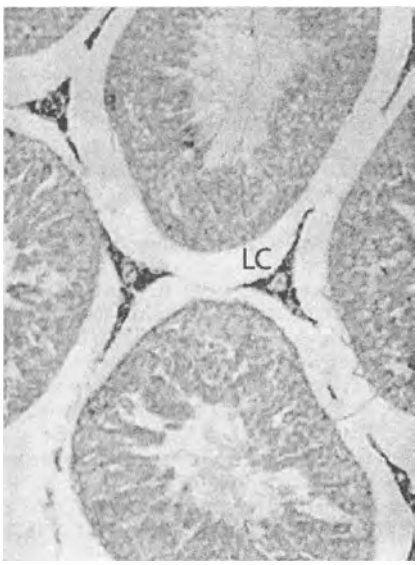

Fig. 1. Control group. INSL3 immunoreactivity in the Leydig cells (**LC**). × 200

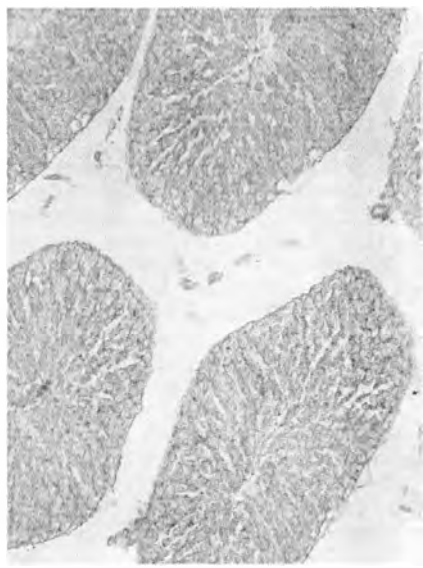

Fig. 2. 7 days after EDS. INSL3 positive cells disappeared from the testicular interstitium. × 200

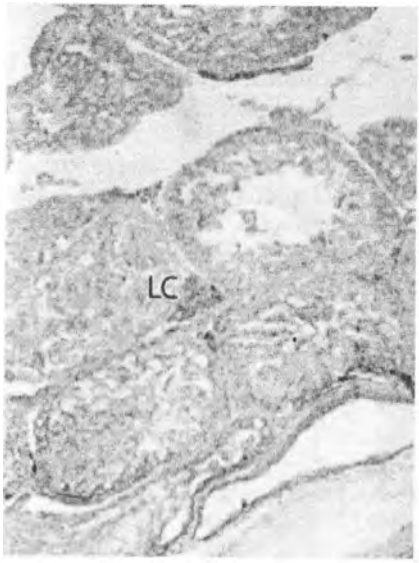

Fig. 3. 21 days after treatment with EDS. Immunolabeling for INSL3 was found in the newly formed Leydig cells (**LC**). × 200

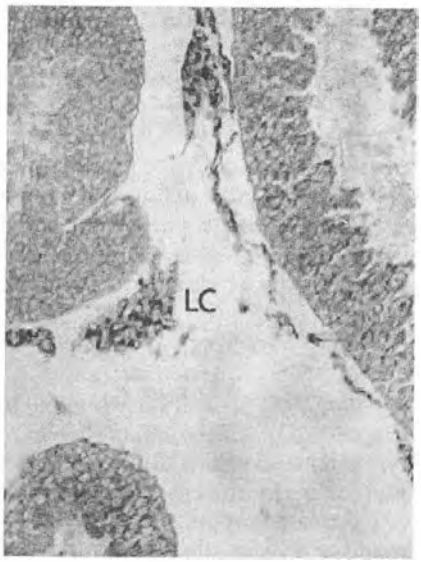

Fig. 4. 35 days after EDS. Clusters of strong INSL3 positive Leydig cells (**LC**). × 200

tion and spermatogenesis [6]. Our data are in agreement that in the mature testis, RLF expression is a good marker for adult type LC, but is weakly expressed in immature LC [4].

The results obtained are step forwards in elucidating the differentiation of adult LC in prepubertal testis and indicate a specific role of hormonal factors like a RLF/INSL3 in this process.

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