

## Sertoli cell expression of galectin-1 and -3 and accessible binding sites in normal human testis and Sertoli cell only-syndrome

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**Summary.** Galectins are vertebrate lectins interacting with  $\beta$ -galactosides and derivatives thereof such as blood group A, B and H determinants. The expression of galectin-1 and -3 and galectin-specific binding sites by human Sertoli cells was analyzed in normal human testis and Sertoli cell only-syndrome (SCOS). Staining intensity was scored semiquantitatively on a 4-grade scale. Sertoli cells in normal testes displayed a moderate cytoplasmic and weak nuclear staining for galectin-1-specific binding sites. Galectin-3-specific binding sites were expressed in Sertoli cells less intensely than accessible ligands for galectin-1 (mean score 2.25 for galectin-1 and 1.50 for galectin-3). Germ cells were only weakly reactive. Tubular walls were negative for both classes of galectin-specific binding sites. In SCOS, galectin-1 binding was moderate to strong and more pronounced than galectin-3 binding by Sertoli cells (mean scores 4.00 and 2.25). Tubular walls were negative for galectin-staining. The ratio for galectin-1-/galectin-3-specific binding (staining score ratio) was 1.50 from normal testis and 1.78 for SCOS disclosing a relative increase of galectin-3 binding sites in the latter. Staining with galectin-1- and -3-specific antisera showed a strong cytoplasmic galectin-1 immunoreactivity in Sertoli cells of normal and SCOS testis (score 4.00 for both). Anti-galectin-3 did not stain Sertoli cells or germ cells in normal testis. Only Leydig cells were labeled (score 3.00). In SCOS a weak to moderate nuclear staining of Sertoli cells was noted (score 2.00).

Galectin-3 expression and galectin-1-specific binding sites were found to be increased in Sertoli cells of SCOS. This modulation of reactivity can have implications for Sertoli cell interactions with galectin-reactive extracellular matrix components like laminin and for anti-apoptotic effects.

**Key words:** Sertoli cell only-syndrome, Galectin, Human testis, Leydig cells

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### Introduction

Galectins constitute a protein family with conserved sequence elements in the carbohydrate-binding site. They are characterized as vertebrate lectins binding to gal- $\beta$ 1,4(3)-GlcNAc cores and extended structures establishing A, B or H-blood group epitopes in a species- and galectin type-dependent manner, as documented by determination of the ligand features in specific assays as well as by NMR spectroscopy and X-ray analysis of galectin/ligand-complexes (Hirabayashi, 1997; Hughes, 1997; Gabius, 1997a, 1998; von der Lieth et al., 1998). By interaction with  $\beta$ -galactosides on glycans of glycolipids they are involved in cell-cell interactions, cell adhesion to extracellular matrix components and proliferation control (Gabius, 1997a; Zanetta, 1997; Kaltner and Stierstorfer, 1998). Moreover, additional capacity to be engaged in protein contacts can play a role for the activity of galectin-1 and -3 in pre-mRNA processing (Patterson et al., 1997).

Glycohistochemical tissue analysis is commonly based on the monitoring with plant/invertebrate lectins, providing a detailed mapping of the expression of carbohydrate epitopes. Lectin application has only recently been extended to the use of endogenous lectins as tools (Bardosi et al., 1990; Gabius et al., 1993; Brinck et al., 1995; Gabius, 1997b). While lectin histochemical studies on glycoconjugate expression in vertebrate testes including human are available (Arya and Vanha-Perttula, 1984; Malmi et al., 1987; Wollina et al., 1989a; Lemaire and Heinlein, 1992; Wine and Chapin, 1997), expression of galectins and galectin-specific binding sites have not been studied so far. Galectin-1 and -3 are the best characterized members of the galectin family. Amongst activities galectin-1 appears to be involved in the organization of extracellular matrix, in cell contact formation and in biosignaling. In human cytotrophoblasts, galectin-1 is colocalized with laminin (Vicovac et al., 1998). When interacting with the ganglioside GM1 on human neuroblastoma cells, their proliferative activity is reduced and differentiation is triggered (Kopitz et al., 1998). Another intriguing role in growth

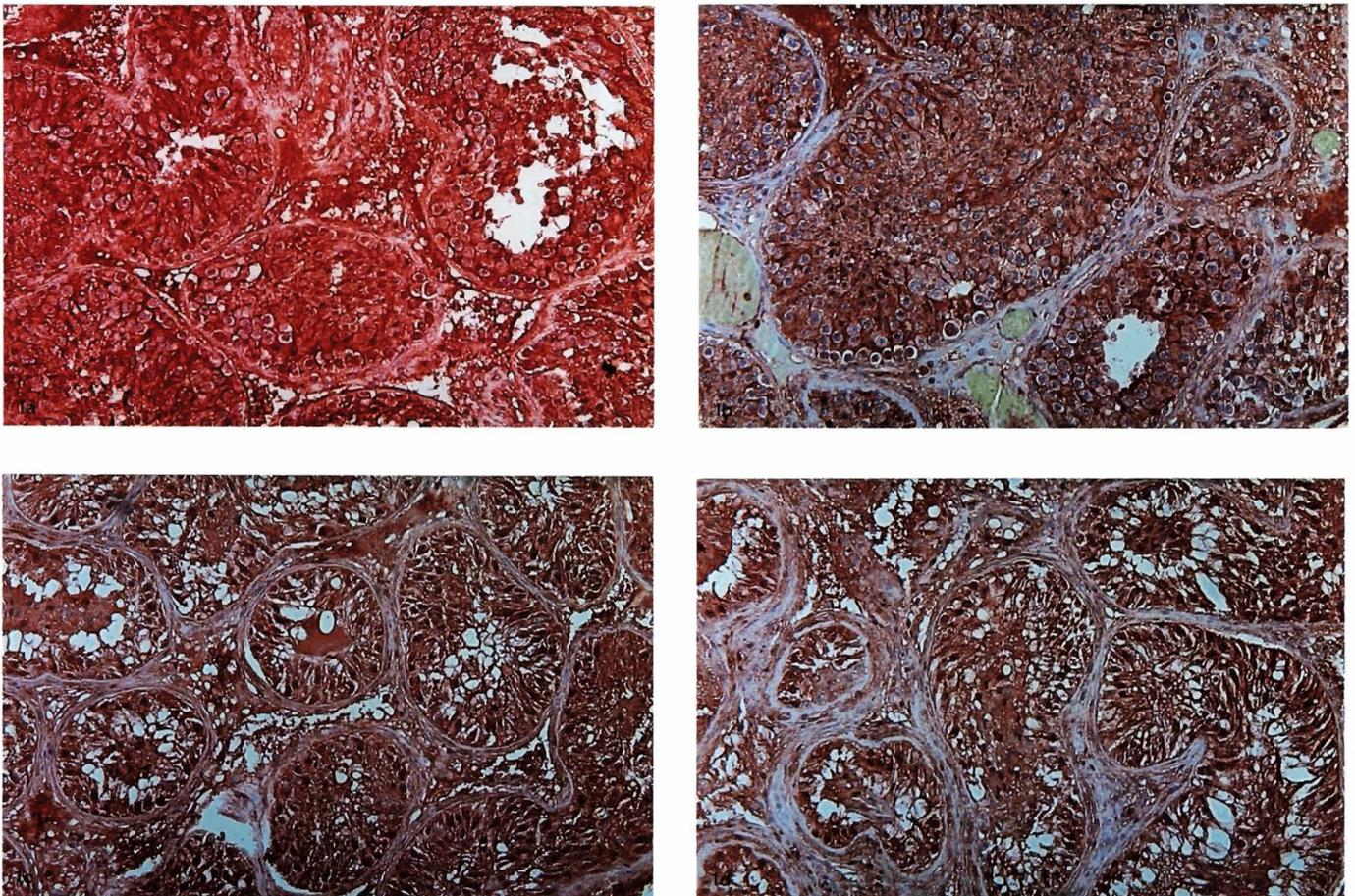
control is played by galectin-3. It inhibits apoptosis through a cysteine protease pathway, without altering Bcl-2, Bcl-X(L), or Bax expression (Yang et al., 1996; Akahani et al., 1997). Interestingly, the pattern of expression of galectins is not necessarily identical, as initially demonstrated in human and murine tumors and in normal tissue such as the reproductive tract of pregnant mice or trophoblasts (Gabius et al., 1986a,b; Phillips et al., 1996; Gabius, 1997b; Maquoi et al., 1997; Ohannesian and Lotam, 1997). The present study compares the expression and distribution of two galectins, namely galectin-1 and -3, and their specific binding sites in normal human testes and in Sertoli cell only-syndrome (SCOS).

#### Materials and methods

Tissue samples from normal human testes (n=4) and SCOS (n=4) have been obtained from the files of the Andrology Unit at the Department of Dermatology, University of Jena. The patient's mean age was 29.8 years for normal testes (range 27 to 32 years) and 26.7 years (range 21 to 31 years) for SCOS. The tissue

specimens were fixed in Bouin's solution and embedded in paraffin after common dehydration.

Galectin-1 and -3 have been prepared as described recently (Gabius et al., 1991; Siebert et al., 1997). They were biotinylated with biotinyl-N-hydroxysuccinimide ester in presence of lactose to protect the active site (Bardosi et al., 1990). Galectin-1- and -3-specific polyclonal antisera were raised in rabbits and controlled rigorously for the lack of cross-reactivity (Kopitz et al., 1998). Lectin- and immunohistochemical staining were performed on 4  $\mu$ m-thick sections, dewaxed, rehydrated and treated subsequently with 1% methanolic H<sub>2</sub>O<sub>2</sub> for 30 min to block endogenous peroxidase activity. The sections were rinsed in phosphate-buffered saline (PBS buffer; pH 7.4) and incubated with 0.1% bovine serum albumin in PBS buffer for 30 min to block nonspecific protein-binding sites. Biotinylated galectins-1 and -3 were used at a final concentration of 1  $\mu$ g/ml and applied overnight at 4 °C. Thorough washes, application of commercial avidin-peroxidase kit reagents (MaxiTags<sup>®</sup>, Quartett, Berlin/Germany), repetitive rinses and development of the chromogenic product from 9-aminoethylcarbazole as substrate concluded the processing.



**Fig. 1.** Galectin-accessible binding sites in testes. Normal human testes (a, b) and SCOS (c, d). Expression of galectin-1-specific binding sites (a, c) and galectin-3-specific binding sites (b, d). x 120

### Galectins in human testes

Control reactions included competitive inhibition with 0.2M lactose and 0.5 mg asialofetuin (ASF)/ml PBS buffer and omission of the incubation step with the labeled marker to exclude any nonspecific staining by binding of the kit reagents. Positive controls were performed with galactoside-specific *Viscum album* agglutinin (Gabius et al., 1991). In addition, antisera against galectin-1 and -3 have been used at a final dilution of 1:200 and an avidin-peroxidase kit as described above. To intensify immunostaining for galectin-specific binding sites, galectins-1 and -3 (0.1 to 1.0  $\mu\text{g/ml}$ ) have been used together with anti-galectins (1:200) and processed as described above. Using this procedure immunostaining was markedly increased. Staining intensity was semiquantitatively scored on a 4-grade scale. In the following section (Results) the use of the term "galectin-specific binding sites" will only refer to those identified by this procedure.

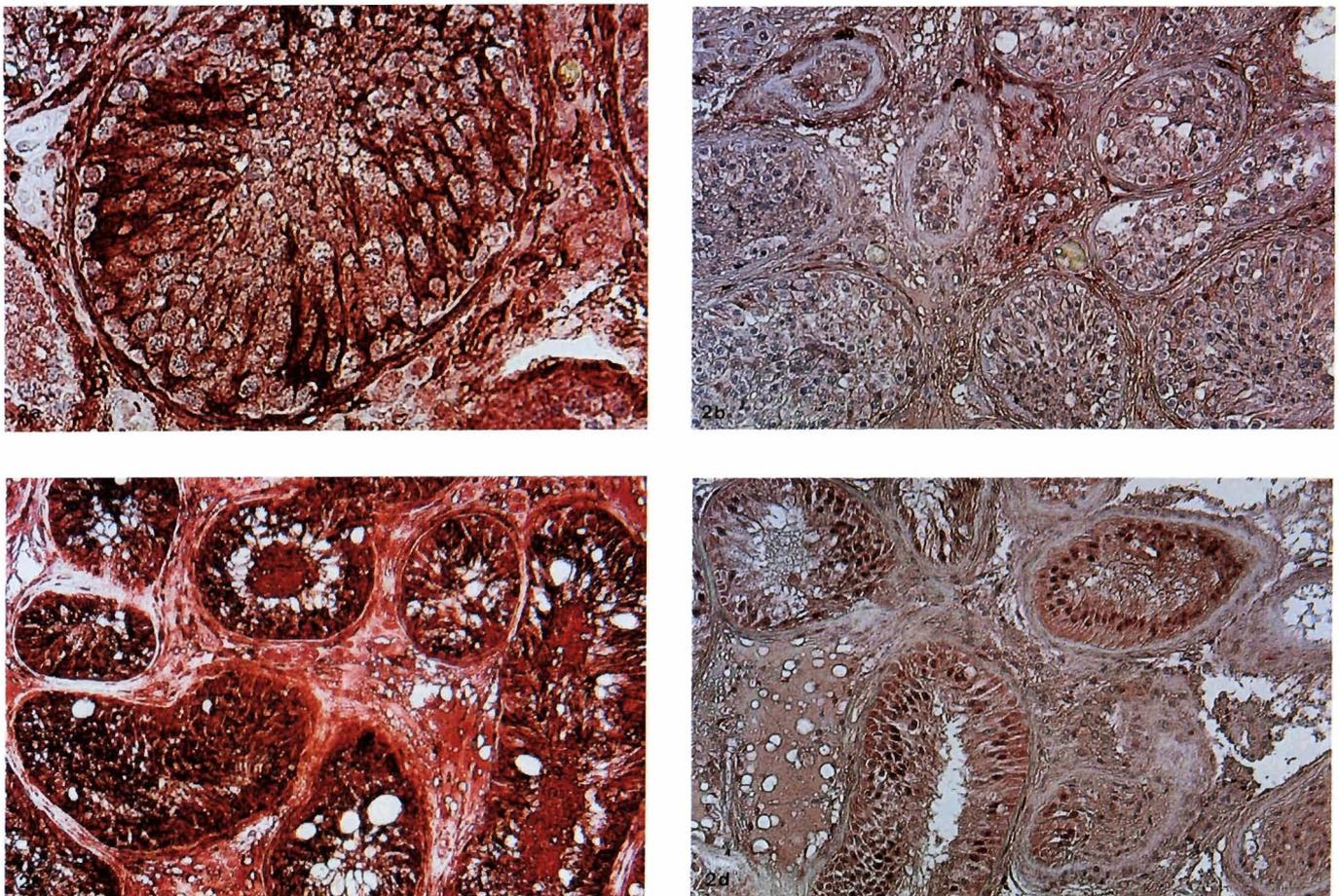
### Results

Sertoli cells in normal testes displayed a moderate cytoplasmic and weak nuclear staining for galectin-1-

specific binding sites compared to a weak cytoplasmic staining in germ cells. Galectin-3-specific binding sites were expressed in Sertoli cells less intensely than galectin-1-specific epitopes (mean score 2.25 for galectin-1 and 1.50 for galectin-3). Germ cells disclosed a weak expression. Tubular walls were devoid of accessible sites for both galectins (Fig. 1a, b).

In SCOS, galectin-1-specific binding was moderate to strong and more pronounced than galectin-3-binding by Sertoli cells (mean score 4.00 and 2.25). Tubular walls remained negative for galectin staining under these conditions. In Sertoli cells of normal testes galectin-1-specific binding sites were predominant, whereas an indication for cell-type specificity was underscored by the relative abundance of galectin-1 and -3 binding in SCOS (Fig. 1a-d). The ratio of galectin-1/galectin-3 staining intensity (score ratio) was 1.50 for normal testis and 1.78 for SCOS showing a relative increase of galectin-3 binding in the latter. Inhibition experiments with ASF/lactose markedly diminished the galectin staining ascertaining the dependence of signal generation on carbohydrate-specific binding of the galectins.

Additionally, a search for endogenous galectin



**Fig. 2.** Galectin expression in human testes. Normal human testes (a, b) and SCOS (c, d). Expression of galectin-1 (a, c) and galectin-3 (b, d). a, x 400; b, d, x 120; c x 240

immunoreactivity by the use of anti-galectin IgG fractions was performed. Galectin expression was measured with specific antisera against galectin-1 and -3. We observed a strong expression of galectin-1 in Sertoli cell cytoplasm of all samples (score 4.00). There was no difference between normal and SCOS specimens. The anti-galectin-3 stained Leydig cells only (score 3.00) in normal testis, but gave no positive signals on either Sertoli cells or germ cells. In SCOS, a weak to moderate nuclear staining of Sertoli cells was demonstrated (score 2.00; Fig. 2a-d). Galectin-1 immunoreactivity was found along the tubular wall in normal testis (Fig. 2a). In SCOS the tubular walls gave a discontinuous staining (Fig. 2c). Galectin-3 immunoreactivity was not found in the tubular walls of testes from normal and SCOS samples (Fig. 2b, d). Negative controls with omission of the antisera against galectins yielded no staining.

## Discussion

Since the original description of SCOS, numerous clinical and morphological studies have been published to improve the definition of features of this disease (Del Castillo et al., 1947). Though SCOS may occur as an idiopathic condition, Turek et al. (1995) found germ cell aplasia in 38% of their cases to be associated with an underlying disease (chemotherapeutic agents or radiotherapy for cancer treatment and others).

In SCOS contrasting with the normal human testes, different types of Sertoli cell differentiation have been described and associated with the severity of impairment of Sertoli cell function. Terada and Hatakeyama (1991) described two types of Sertoli cells in SCOS: one resembling the normal adult Sertoli cells and another with a vimentin distribution resembling fetal Sertoli cells. A transient expression of cytokeratins has been observed (Stosiek et al., 1990; Bergmann and Kliesch, 1994). Nistal et al. (1990) distinguished four types of Sertoli cells: (1) normal adult mature cells showing an indented nucleus, grossly triangular in shape with a prominent tripartite nucleolus; (2) immature cells with round, regularly shaped nuclei and immature cytoplasm; (3) dysgenetic cells harboring immature nuclei and a nearly mature cytoplasm with less developed organelles and (4) involuting cells with very irregularly shaped nuclei and a cytoplasm containing abundant lipid droplets and residual bodies with atypical inter-Sertoli junctions. They observed types (1), (3) and (4) in SCOS.

The morphological changes of Sertoli cells are not specific for SCOS but may occur in other pathological conditions as well (Schulze et al., 1976). The expression of plant lectin-reactive glycoconjugates by Sertoli cells of SCOS has not been found to be different from normal testis (Wollina et al., 1989a,b). However, endogenous lectins have so far not been introduced to study ligand presentative for all these types, as initiated in this study. Labeled galectins proved to be suitable glycohistochemical probes.

Upon comparing staining extents a relative increase of galectin binding by Sertoli cells in SCOS compared to normal testis was detectable. The galectin-1/galectin-3 binding ratio was higher in SCOS (1.78) vs. normal testis (1.50) (Fig. 1a-d). Staining for galectin-1 and -3 revealed differences between normal testis and SCOS. Whereas galectin-1-dependent cytoplasmic immunoreactivity was found in Sertoli cells of normal and pathological samples, galectin-3 expression was only seen in nuclei of Sertoli cells in SCOS (Fig. 2a-d). As already emphasized in the introduction, nuclear presence of a galectin can be an indication for a role in pre-mRNA processing (Patterson et al., 1997).

A further hallmark of SCOS is the thickening of the lamina propria of seminiferous tubules. Both in normal testis and in SCOS, laminin and collagen type IV are found in the tubular wall and the peritubular cell layers. Sertoli cells do not only contain laminin but the distribution of laminin and collagen IV suggests their secretion by Sertoli cells (Pollanen et al., 1985; Skinner et al., 1985). Their distribution is disturbed in SCOS (Pollanen et al., 1985).

Wollina et al. (1989b) described a selective loss of mannose residues in the adluminal part of tubular walls in SCOS. In the present investigation, we found neither galectin-1 or -3 expression nor specific binding sites in tubular walls of normal and SCOS testis. Galectin-1 immunoreactivity was expressed in normal and SCOS testis but was rather discontinuous in the latter.

Sertoli cells control germ cell number by secretion of the Fas ligand, which is bound to Fas on germ cells. The Fas system is a key pathway of germ cell apoptosis (Lee et al., 1997). Owing to a connection to the control of apoptosis an increased galectin-3 expression and a higher level of galectin-binding sites in Sertoli cells of SCOS compared to normal human testis deserves attention. This observation may be related to the disturbed laminin desposition in the former (Pollanen et al., 1985) and/or inhibition of apoptosis (Yang et al., 1996; Akahani et al., 1997). In culture, Sertoli cell apoptosis is mediated by contact inhibition or serum deprivation (Pogan et al., 1997). In vitro, basement membrane contact (laminin or Matrigel) prevents Sertoli cell apoptosis (Dirami et al., 1995). Since galectins may function as accessory adhesion molecules, changes of abundance of galectin-specific binding sites in SCOS may be associated with disturbed Sertoli cell interaction including galectin-reactive extracellular matrix components like laminin. Further studies with these non-integrin laminin-binding proteins are thus warranted.

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