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Lectin binding to carcinoma-in situ cells of the testis

A comparative study of CIS germ cells and seminoma cells

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Summary. Seven patients with carcinoma-in-situ of the testis were studied. Testicular biopsies were treated with eight fluorescein isothiocyanate conjugated lectins, and particular attention was paid to the similarities between CIS germ cells, normal germ cells and seminoma cells. In the cytoplasm of CIS cells a large number of granularly distributed Con A and LCA binding sites was noticed, indicating the presence of mannose and N-acetyl-glucosamine in these cells. The perinuclear fluorescence observed by WGA and RCA I suggests the incorporation of N-acetyl-glucosamine and galactose into glycoproteins in cytoplasmic cell organelles of these cells. The distribution of glycoconjugates in CIS germ cells is similar to that of invasive seminoma cells confirming the malignant nature of CIS cells. However, as there are differences in lectin binding of spermatogenetic cells and CIS cells, no conclusions regarding the origin of CIS cells can be drawn.

Key words: Lectins – Glycoconjugates – Human testis – Carcinoma-in-situ cells – Seminoma

Introduction

According to present concepts, both seminomatous and nonseminomatous germ cell tumours are derived from intratubular carcinoma-in-situ (CIS) cells (Skakkebaek and Berthelsen 1981). The CIS germ cells are large, abnormal cells with coarse nuclear chromatin and several nucleoli, and in typical cases, a single row of CIS cells is found between the basement membrane and normal Sertoli cells. The CIS cells are shown to be rich in glycogen and placental-like alkaline phosphatase (Skakkebaek et al. 1986). They have been observed in the testes antecedent to the appearance of various testicular germ cell tumours (Skakkebaek 1972; Skakkebaek 1978), and they are also present in the periphery of many germ cell tumours in histological sections (Jacobsen et al. 1981; Skakkebaek 1975). Light and electron microscopic studies indicate that the CIS cells within the seminiferous tubules resemble morphologically and are possibly derived from germ cells at early stages of differentiation (Gondos et al. 1983; Skakkebaek et al. 1986).

Previous studies have shown that both spermatogenetic cells and seminoma cells have a characteristic distribution of lectin binding glycoconjugates (Lee and Damjanov 1984; Lee et al. 1985; Malmi and Söderström 1985; Söderström et al. 1984). In order to investigate the nature and origin of CIS germ cells, we stained testicular biopsy specimens containing tubules with CIS, with eight fluorescein conjugated lectins of various carbohydrate binding specificities. Particular attention was paid to the similarities in the distribution of glycoconjugates between normal germ cells, CIS germ cells and seminoma cells.

Materials and methods

Testicular specimens from seven different patients were taken during investigation for infertility. The age of the patients ranged from 18 to 38 years, with a mean of 28 years. With conventional histological staining methods, carcinoma-in-situ cells were found inside the seminiferous tubules of all biopsies. In three cases tubules with normal spermatogenesis were also present in the biopsy, and in two cases there was invasive seminoma in addition to the tubules with carcinoma-in-situ.

For routine histology testicular tissue was fixed in Cleland's fluid (Rowley and Heller 1966), embedded in paraffin, and sections 4 μm thick were stained with iron haematoxylin and eosin. For lectin histochemistry, the sections were deparaffinized, covered with a drop of lectin solution and incubated in a moist chamber at room temperature. The following fluorescein isothiocyanate conjugated (FITC) lectins (Vector Laboratories, CA)

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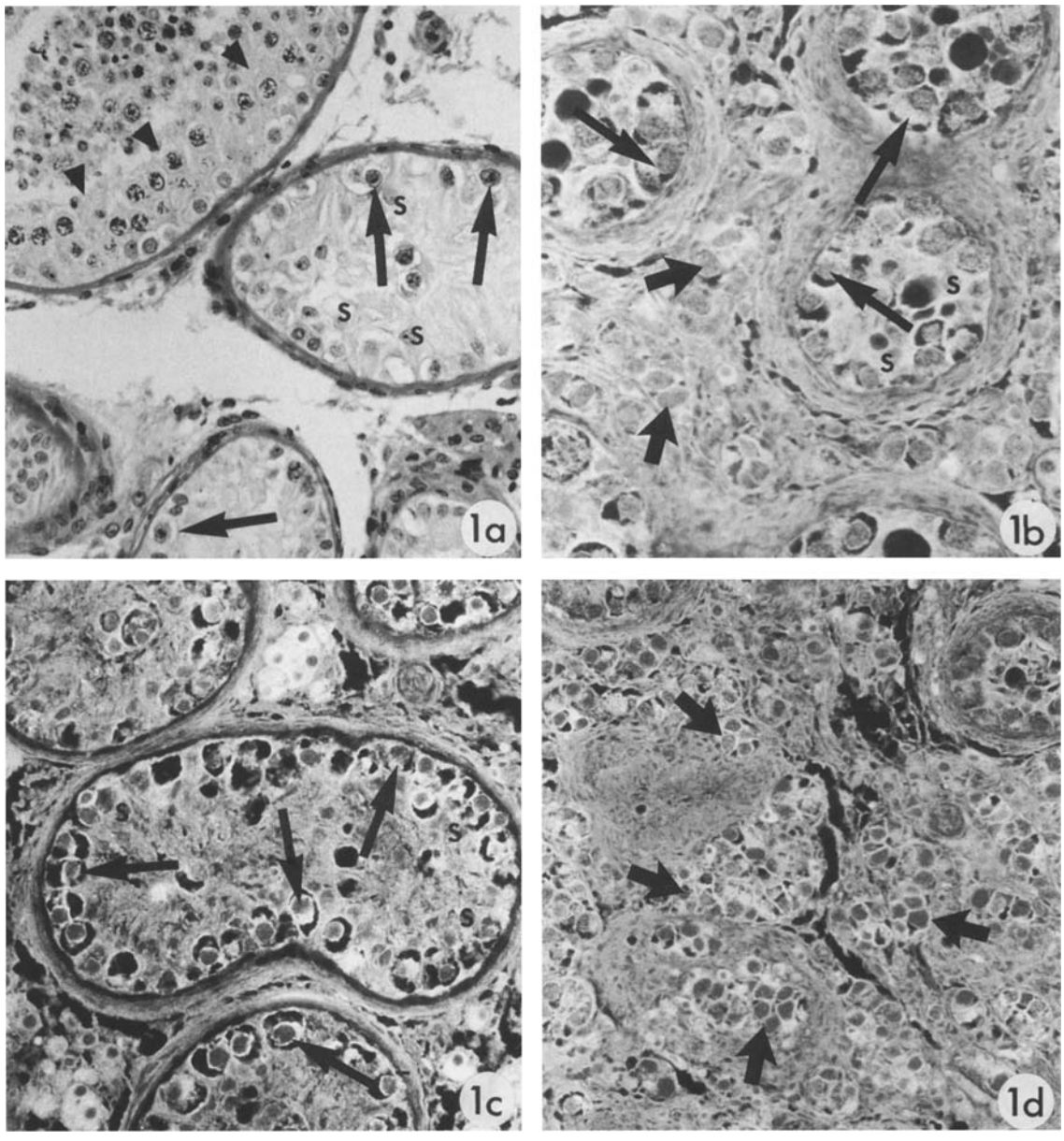

Fig. 1. (**a**) Section of testicular specimen with CIS, showing two seminiferous tubules with large atypical germ cells (*arrows*) and Sertoli cells (*s*). In one tubule normal spermatogenesis is noticed (*arrowheads*). Cleland's fixative, iron-haematoxylin stain. ×210. (**b**) FITC-LCA was bound to the cytoplasm of CIS cells (*arrows*) as well as to invading seminoma cells (*thick arrows*) in a similar manner. Sertoli cells (*s*) are brightly stained. ×240. (**c**) FITC-CON A shows a strong granular fluorescence in the cytoplasm of CIS cells, especially in the perinuclear region and along the cell borders (*arrows*). Sertoli cells (*s*) are also stained. ×240. (**d**) A similar granular fluorescence is expressed in seminoma cells (*thick arrows*) when stained by FITC-CON A. ×160

were used: Concanavalin A (jackbean; Con A), Lens culinaris (LCA), Triticum vulgaris (wheat germ; WGA), Ricinus communis I (castor bean; RCA I), Arachis hypogeae (peanut; PNA), Glycine maximum (soybean; SBA), Helix pomatia (HPA) and Ulex europaeus I (gorse; UEA I). The lectin conjugates were used at the concentration of 100–200 μg/ml, diluted in phosphate buffered saline, pH 7.4. After 30 min incubation the sections were thoroughly washed in PBS, twice in distilled water and mounted with Aquamount (Gurr BDH Chemicals, England).

The specificity of staining was tested by preincubation of the lectin in a solution of the appropriate inhibitory sugar: α-methylmannoside for Con A and LCA, N-acetylglucosamine for SBA and HPA, D-galactose for PNA, α-lactose for RCA I, N-acetyl-glucosamine for WGA and fucose for UEA I. The concentration of the inhibitor was 0.2 M, except 0.3 M for α-methylmannoside (Sigma Chemical Co., St. Louis, MO).

The tissue sections were examined with a Leitz Dialux epi-fluorescence microscope, equipped with the appropriate filter combination for FITC-fluorescence.

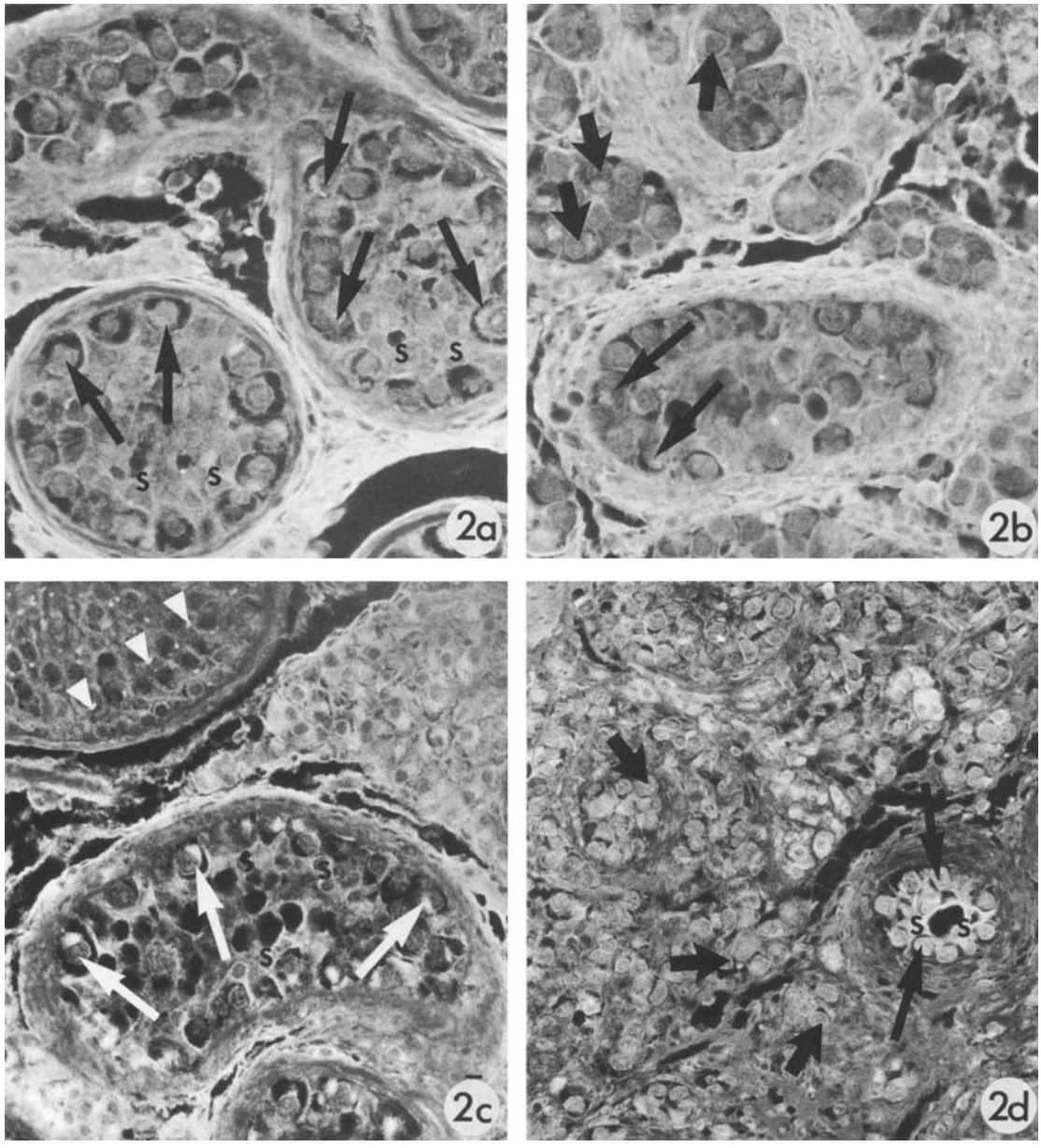

Fig. 2. (**a**) CIS germ cells are stained by FITC-WGA with the prominent perinuclear region (*arrows*), and Sertoli cells (*s*) are also stained. (**b**) A similar staining is observed in invading seminoma cells (*thick arrows*). Notice the strong fluorescence of tubular walls and fibrous tissue in both (**a**) and (**b**). a × 240, b × 240. (**c**) FITC-RCA I labels spermatid acrosomes (*arrowheads*) in normal seminiferous tubules. In CIS tubules the atypical germ cells are fluorescent in their perinuclear cytoplasm (*arrows*), and there is a faint reaction in Sertoli cells (*s*), tubular walls and interstitial cells. × 220. (**d**) FITC-SBA labels both CIS cells (*arrows*) and Sertoli cells (*s*). A similar delicate lectin staining is observed in seminoma cells (*thick arrrows*). × 160

Results

The histological examination showed CIS germ cells in all seven testicular specimens (Fig. 1 a). In addition, in three specimens with CIS seminiferous tubules with normal spermatogenesis were found, and in two cases with CIS there was invasive seminoma, thus providing internal controls with which the staining of CIS cells could be compared.

Each fluorochrome-labelled lectin presented a typical binding pattern on the seven biopsy specimens. Minor variations in the intensity of fluorescence was noticed among individual testes. With all the lectins used, the binding could be inhibited by preincubation of the lectin with their haptenic monosaccharides, except WGA. With WGA the specific reaction products decreased but did not disappear completely as with the other lectins.

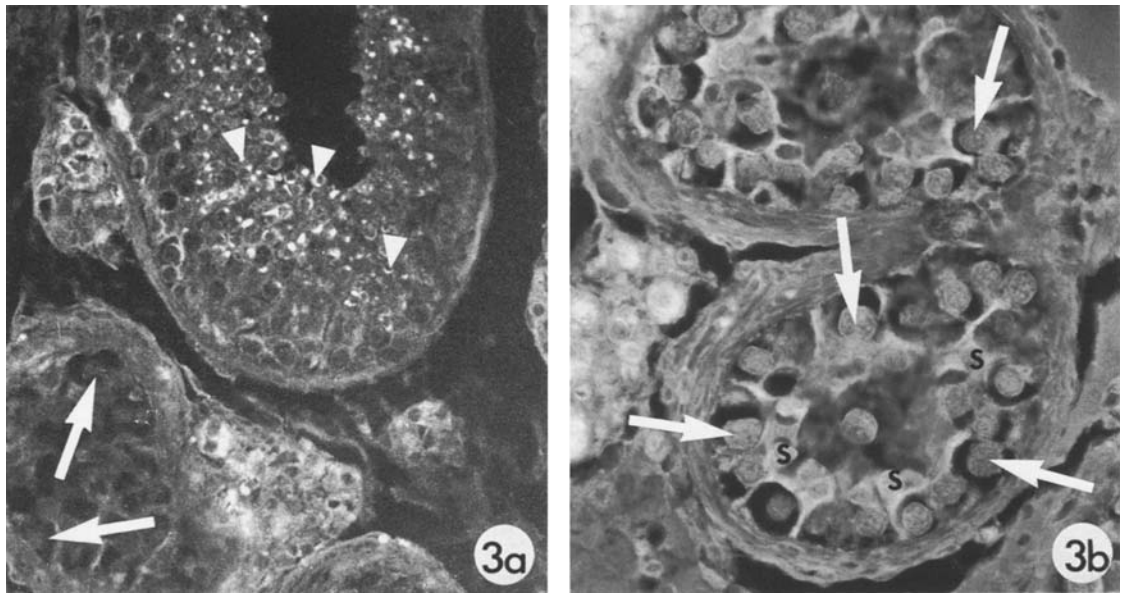

Fig. 3. (**a**) In three cases there was no positive reaction of CIS germ cells by FITC-PNA (*arrows*). In the seminiferous tubule with normal spermatogenic differentiation slight staining of all germ cell types was noticed, and in particular the acrosomes of developing spermatids (*arrowheads*) were brightly fluorescent. In four cases a faint staining of CIS cells was observed. ×240. (**b**) Both CIS cells (*arrows*) and Sertoli cells (*s*) presented a weak or moderate fluorescence by FITC-UEA I. ×240

In all the seven cases FITC-Con A stained brightly the cytoplasm and the cell borders of CIS germ cells; the fluorescence frequently appeared in a granular pattern (Fig. 1c). Sertoli cells, the lamina propria of the tubules and interstitial cells were also stained, but the reaction was even and less intense. Invading malignant cells in the two seminomas presented an identical granular staining to that found in CIS cells (Fig. 1d).

FITC-LCA also gave a strong, often granular staining reaction in the cytoplasm of CIS cells, but in some cases the fluorescence was less intense than with Con A. Sertoli cells, tubular walls and interstitial cells were also stained. Seminoma cells reacted in a similar manner as CIS cells (Fig. 1b).

FITC-WGA was bound to the cytoplasm of CIS and seminoma cells, especially in the perinuclear region, as well as to Sertoli cells and some interstitial cells. The most intense reaction was observed in the fibrous stroma of interstitial tissue and seminoma (Fig. 2a, b).

FITC-RCA I stained the perinuclear area in the cytoplasm of CIS germ cells (Fig. 2c), but was also bound either faintly or moderately to the tubular basement membrane, some interstitial cells and Sertoli cells. The seminoma cells were clearly stained. Slight differences in lectin staining pattern between various specimens were noted.

FITC-UEA I showed some variability in the intensity of lectin binding reaction, and a mostly faint or moderate staining of CIS cells, Sertoli cells, tubular walls, some interstitial cells and vascular walls was observed (Fig. 3b). The CIS and seminoma cells were labelled in a similar way.

In three cases FITC-PNA failed to stain testicular tissue with CIS, although the adjacent seminiferous tubules with normal spermatogenesis presented acrosomal binding characteristic for PNA (Fig. 3a). In the other four cases PNA reacted faintly with CIS cell cytoplasm and Sertoli cells. In the two seminomas a few malignant cells were positively stained, but the majority of seminoma cells were negative.

FITC-SBA was bound faintly to CIS germ cell cytoplasm and Sertoli cells in six cases (Fig. 2d), and was completely negative in one case. Seminoma cells were faintly positive. The lectin also interacted weakly with some interstitial cells, but was not bound to the tubular basement membrane.

A completely negative reaction in testicular tissue showing the CIS pattern was obtained by FITC-HPA, but selective binding to spermatid acrosomes was noted by this lectin during normal germ cell maturation. Seminoma cells were negative.

In three cases, in addition to CIS, there were seminiferous tubules with normal spermatogenesis in the biopsy specimens. Among germ cells, the staining of spermatogonia (the most primitive cells present in the adult seminiferous epithelium) and

Table 1. Staining of normal spermatogonia, normal spermatids, CIS cells and seminoma cells with lectins

	Spermatogonia	Spermatids (acrosomes)	CIS cells	Seminoma cells
Con A	+	++	++	++
LCA	+	++	++	++
WGA	+	++	+	+
RCA I	−	++	+	+
PNA	+	++	+/−	+/−
SBA	−	+	+/−	+/−
HPA	−	+ (late)	−	−
UEA I	+/−	+/−	+	+/−

the staining of spermatids were investigated especially. The cytoplasm of the spermatogonia expressed binding sites for Con A, LCA, WGA, UEA I and PNA. The staining of the seminiferous epithelium with normal spermatogenesis and CIS in adjacent tubules was similar to that found in normal testes (Malmi and Söderström 1985). In addition to the specific binding of interstitial cells by some lectins, moderate autofluorescence was observed in a number of these cells. However, the reddish colour of the autofluorescence is easily distinguishable from the greenish colour of fluorescein. The results of the lectin staining are summarized in Table 1.

Discussion

Changes in the carbohydrate composition of cells during malignant transformation (Nicolson and Poste 1976; Warren et al. 1978) have recently been studied by several authors using lectin histochemistry (Fischer et al. 1984; Holthöfer et al. 1983; Newman et al. 1979; Vierbuchen et al. 1980). The abnormal germ cells of seminiferous epithelium, presenting the carcinoma-in-situ pattern (Skakkebaek 1972; Skakkebaek 1975) are suggested as the common precursor cells for both seminomatous and nonseminomatous germ cell tumours, according to the revised germ cell theory (Skakkebaek and Berthelsen 1981).

When the lectin staining patterns of normal spermatogonia, spermatids, CIS germ cells and seminoma cells are compared (Table 1), an identical labelling of carcinoma-in-situ cells and seminoma cells supports the suggestion that CIS cells are precursors of invasive seminoma, as both cell types have similar carbohydrate composition. However, the staining of CIS cells is different from that of germ cells, and the carcinoma-in-situ cells express binding sites for RCA I and SBA which are not found in spermatogonia. However, the two lectins are positive in spermatid acrosomes during normal germ cell maturation. Therefore, glucosyl moieties not observed in spermatogonia are expressed during the malignant transformation in the seminiferous epithelium. Although it is probable that the changes in the composition of cellular glycoconjugates would follow the malignant transformation of spermatogonia to CIS germ cells, the lectin binding differences between the two cell types also suggest that the adult spermatogonia are not necessarily the direct precursors of CIS cells, but a more primitive cell type, possibly an embryonal or prepuberal spermatogonium may be involved.

In our study, the highly granular fluorescence in the cytoplasm and along the cell borders of CIS cells, observed with Con A, indicates an abundance of mannose and N-acetylglucosamine containing glycoconjugates in these cells, as the lectin binds to polymannosyl cores of carbohydrate chains (Lotan and Nicholson 1979). As the reaction was similar in seminoma cells both with regard to the staining intensity and the granular distribution of binding sites, the malignant nature of the carcinoma-in-situ cells is further confirmed. In contrast with this, normal seminiferous epithelium presents an even cytoplasmic fluorescence with Con A which is particularly bright in developing acrosomes. The presence of a high number of mannose and glucose containing glycoconjugates in CIS germ cells is also demonstrated by the strong, often granular reaction with LCA, a lectin specific for mannose and glucose containing residues but with a more rigid binding specificity than Con A (DeBray et al. 1981).

In addition to the high affinity for connective tissue in the tubular wall of testicular tissue and in fibrous stroma of seminoma, WGA was bound to the perinuclear region of the carcinoma-in-situ cells and seminoma cells. By electron microscopy, the CIS cells are reported to exhibit localized perinuclear collections of various cell organelles while the cytoplasm elsewhere is largely devoid of organelles (Gondos et al. 1983). Our results speak in favour of the similar nature of the abnormal cells and seminoma cells and suggest the presence of certain N-acetylglucosamine and sialic acid containing glycocompounds in the perinuclear area, specifically bound by WGA (Bhanvandan and Katlic 1979; Goldstein and Hayes 1978). However, these cells are different from the germ cells undergoing spermatogenic differentiation as there is a less prominent reaction all over the cytoplasm of all germ cell types, and, in particular, the acrosomes of developing spermatids are labelled.

Perinuclear staining of CIS germ cells was also observed by RCA I which lectin is expressed on germ cell surfaces and developing acrosomes among normally differentiating cells, thus presenting a different distribution of lectin binding carbohydrates in the CIS cells. RCA I is specific for terminal galactose and the galactose residues in penultimate position when substituted by sialic acids (DeBray et al. 1981). Apparently, the CIS cells differ in their activity from spermatogenic cells, and the carbohydrate chains in these cells are processed by adding galactose and N-acetylglucosamine residues in the perinuclearly located cell organelles by the action of various enzymes.

Another galactose specific lectin conjugate, peanut agglutinin, gave negative results in three cases and faintly positive in four. PNA binds β-D-galactose-(1–3)-N-acetylgalactosamine more avidly than D-galactose (Goldstein and Hayes 1978). Among spermatogenetic cells, the lectin is particularly bound to developing acrosomes which are known to contain some carbohydrate containing enzymes, essential for fertilization. In our study, the more limited binding specificity of PNA when compared to that of RCA I, explains the absence of labelling in the negative cases, but the faint positive staining in four cases may reflect the alterations in the functional activity among carcinoma-in-situ cells, and differs from the acrosomal binding observed during normal germ cell differentiation.

The faint or moderate staining of carcinoma-in-situ cells obtained by SBA, specific for N-acetylgalactosamine and galactose (Goldstein and Hayes 1978), suggests the presence of some N-acetylgalactosamine and galactose containing glycoconjugates in these cells. During normal germ cell maturation, binding sites for SBA appear fairly late in developing spermatids. Another N-acetylgalactosamine specific lectin HPA (Goldstein and Hayes 1978) was negative in CIS cells but during spermatogenic differentiation is expressed in the acrosomes of late spermatids.

The cytoplasm of Sertoli cells was stained with all the lectins in the study, except HPA. The Sertoli cells are known to phagocytize degraded cellular material, for example degenerating spermatogenic cells as well as residual bodies during spermatogenesis (Hugon and Rogers 1966; Söderström and Nikkanen 1979). The positive staining reaction may be due to the phagocytized material from carcinoma-in-situ cells. Probably, during the degradation process, sugar residues which are normally masked, are exposed for the binding of most lectins.

The results of this study show the specific character of carcinoma-in-situ cells to be close to seminoma cells, as they express the same glycocompounds in a similar manner. CIS cells are different from normal germ cells of human testis when studied by lectin histochemistry and their origin should be further clarified. In diagnostic use, lectin histochemistry is of less significance for germ cell tumours as the cells of the interstitial compartment are also specifically stained by some lectins, limiting their use as probes to investigate the invasive growth of seminoma cells. However, the use of lectin histochemistry in combination with other cellular markers, semithin section histology and electron microscopy, offers an approach for obtaining additional information on changes characterizing malignant transformation in the human seminiferous epithelium.

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