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Atypical germ cells of the testis

Comparative ultrastructural and immunohistochemical investigations

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Summary. It is uncertain whether the so called intratubular atypical germ cells (carcinoma in situ cells) demonstrable in the testicular tissue around different germ cell tumors and in testicular biopsies of patients with impaired fertility are identical with regard to their morphology and further development. Thus atypical germ cells of 18 patients with testicular germ cell tumors and of 3 patients with atypical germ cells in testicular biopsies without tumor were studied by electron microscopy and/or by immunohistochemistry. The atypical germ cells show characteristic alterations distinguishing them from normal germ cells, especially spermatogonia. However, there are no differences between atypical germ cells in the above mentioned groups. Immunohistochemical reactions are negative with anti-alpha-fetoprotein and anti-beta-human-chorionic-gonadotropin, but 6 of the 15 cases are positive with antiferritin. However, this positive reaction occurs in cases in different diagnostic groups. Atypical germ cells of the different groups cannot be distinguished by electron microscopy or immunohistochemical methods, but further investigations, including cell cultures, may provide more information.

Key words: Atypical germ cells – Carcinoma in situ of the testis – Normal germ cells – Ultrastructure – Differential diagnosis

Large cells with dark nuclei lined up in a row at the basement membrane of atrophic seminiferous tubules were first described in the residual testicular tissue surrounding germ cell tumors. They attained clinical importance when their occurrence was noted in testicular biopsies of patients with impaired fertility who developed germ cell tumors of the testis months to years later (Skakkebaek 1972). In 1965, Mark and Hedinger defined the light microscopic features of such cells which they called Atypical Germ Cells (AGC). Other terms, Abnormal Intratubular Germ Cells (Nielsen et al. 1974), Atypi-

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cal Spermatogonia (Nüesch and Hedinger 1977), Carcinoma-in-situ (Skakkebaek 1978), Atypical Cells (Pugh and Parkinson 1981) or Seminoma Cells (Schütte et al. 1981) express the importance attached to these cells in the more intensive investigations after 1965.

The original dual theory on the development of germ cell tumors of the testis is now largely rejected. According to this theory, seminomas are derived from intratubular germ cells, whereas non-seminomatous germ cell tumors (NSGCT) originate from dispersed blastomeres (Willis 1962). Today, most authors agree that both seminomas and NSGCT originate from germ cells (Dixon and Moore 1952; Mostofi and Price 1973).

Skakkebaek and Berthelsen (1981) consider the AGC as the possible stem cell of all these tumors. Pugh and Parkinson (1981), however, raised the question as to whether AGC in seminomas correspond to those found in the tissue surrounding NSGCT. Another problem which is still unsolved is whether AGC are capable of forming only the same histological type of tumor (i.e. a seminoma or a NSGCT) already present in the testis or whether in fact they represent totipotential cells, capable of forming both seminomas and NSGCT, as proposed by Berthelsen et al. 1979, Krabbe et al. 1979 and Mostofi 1980. In addition, migrating and possibly still undifferentiated tumor cells spreading out in testicular tubules around already existing tumors should not be confused with AGC. Finally, degenerating germ cells resembling AGC have to be considered in differential diagnosis (Sigg and Hedinger 1981 and 1983).

In order to clarify these questions, ultrastructural and histochemical investigations were undertaken on AGC from tubules surrounding testicular germ cell tumors and on biopsy specimens with AGC obtained from patients with impaired fertility.

Materials and methods

1. Materials and methods for electron microscopic study

Testicular tissue of 15 patients suffering from a germ cell tumor, 7 seminomas (group I), 6 NSGCT (group II) and 2 combined tumors (seminomas and NSGCT; group III) was investigated. Longitudinal sections of the testis were made immediately after orchiectomy followed by removal of macroscopically unremarkable testicular parenchyma surrounding the tumor. In addition, testicular tissue with AGC of 3 patients who underwent biopsy because of infertility (group IV) was investigated. On light microscopy, these cases revealed focal tubular atrophy, discrete interstitial fibrosis and clearly identifiable AGC in occasional groups of seminiferous tubules with thickened walls and diminished diameter. Tumor type and age of these 18 patients are listed in Table 1.

The testicular tissue was fixed in 2% phosphate-buffered glutaraldehyde for at least 2 h. Post fixation was done in two different ways: Half the material of each case was post-fixed in buffered 1% OsO_4, the other half in 1% OsO_4 with 1.5% $K_4Fe(CN)_6$. The slides cut on a LKB-Ultrotome were studied and photographed on a Philips EM 201.

2. Materials and methods for immunohistochemical study

Immunohistochemical studies were done in 12 of the above mentioned 18 cases: 10 cases with germ cell tumors and 2 of the 3 patients with biopsies (see Table 1). In addition, 3 cases with germ cell tumors of the testis (1 seminoma and 2 embryonal carcinoma) not included in the electron microscopic study were investigated immunohistochemically. In all these 15 cases the tissue was fixed in 4% buffered formaldehyde solution, embedded in paraffin and

Table 1.

No	Age	Tumor type	EM investigation	Immunohistochemical investigations
A. *Patients with testicular germ cell tumors and AGC*				
Group I				
1	41	seminoma	+	+
2	24	seminoma	+	+
3	31	seminoma	+	+
4	28	seminoma	+	+
5	37	seminoma	+	−
6	31	seminoma	+	−
7	27	seminoma	+	−
8	36	seminoma	−	+
Group II				
9	23	embryonal carcinoma	+	+
10	26	embryonal carcinoma	−	+
11	35	embryonal carcinoma	−	+
12	29	embryonal carcinoma choriocarcinoma + yolk sac tumor	+	+
13	22	embryonal carcinoma + yolk sac tumor + STGC[a]	+	+
14	30	embryonal carcinoma + immature teratoma	+	+
15	22	teratocarcinoma + yolk sac tumor + STGC[a]	+	+
16	27	teratocarcinoma + yolk sac tumor	+	−
Group III				
17	20	embryonal carcinoma + seminoma	+	+
18	24	embryonal carcinoma + seminoma + yolk sac tumor + STGC[a]	+	−
B. *Patients with AGC in testicular biopsies*				
Group IV				
19	33		+	+
20	27		+	−
21	29		+	+

[a] STGC = syncytiotrophoblastic giant cell

cut in 4 μm thick sections. In the immunohistochemical investigations for alpha-feto-protein (α-FP), beta-human-chorionic-gonadotropin (β-HCG) and ferritin a modified unlabeled antibody-PAP method of Sternberger et al. (1970) was applied. Dilution of the rabbit-anti-human α-FP (obtained from DAKO) was 1:100. Histosets from Immulok Inc. USA and Hypolab, Switzerland were used to demonstrate ferritin and β-HCG.

Results

1. Morphological investigations on AGC

The *diameter* of the AGC varies between 15 and 25 μm. The nuclei are usually located in the center of the cell whereas the organelles are generally

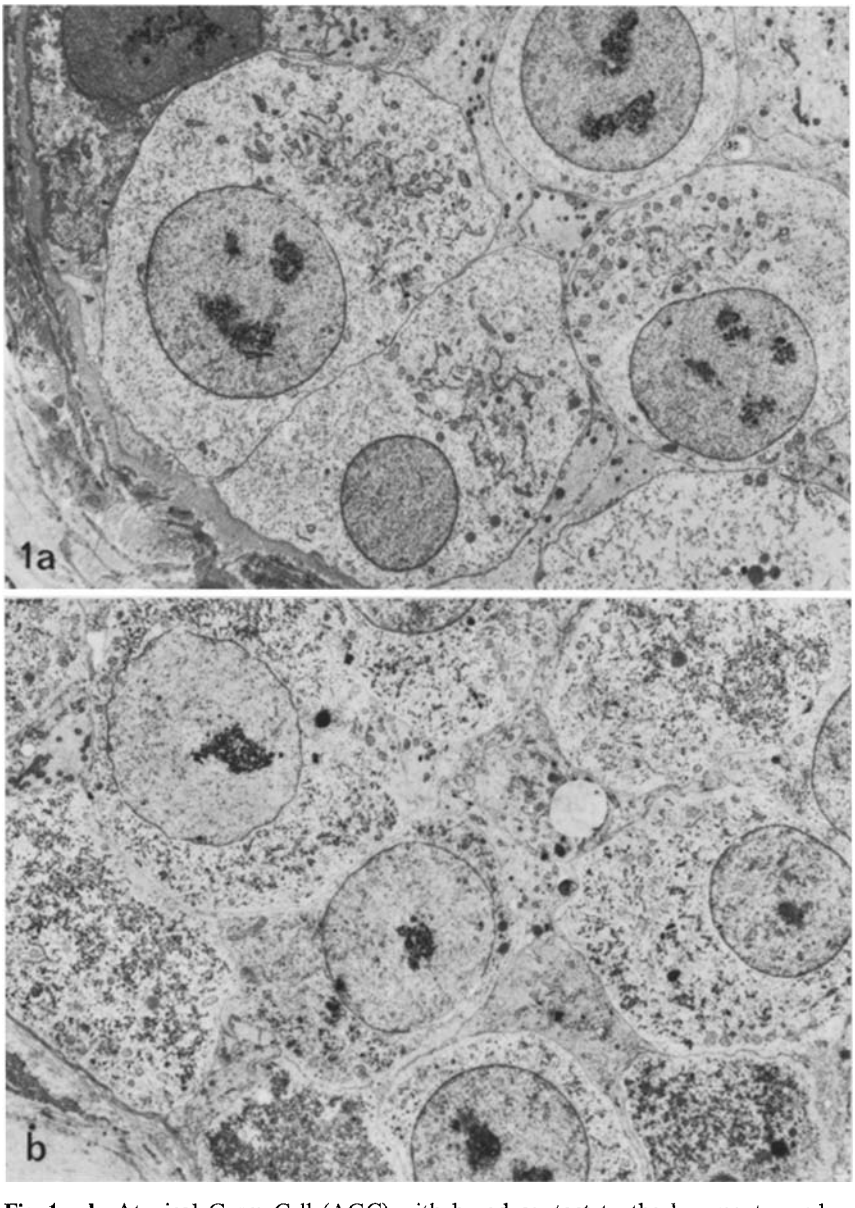

Fig. 1a, b. Atypical Germ Cell (AGC) with broad contact to the basement membrane and polarization of the cell organelles. **a** AGC in a seminiferous tubule near germ cell tumor. **b** AGC in testicular biopsy × 2250

grouped in the basal section (Figs. 1, 2). The nuclei of the Sertoli cells are displaced towards the center of the tubular lumen.

The *nuclei* of the AGC are round or slightly ovoid and vary in diameter between 12–20 μm. The inhomogeneously distributed chromatin is generally dense and finely granular. Nucleoli are mostly multiple and remarkably

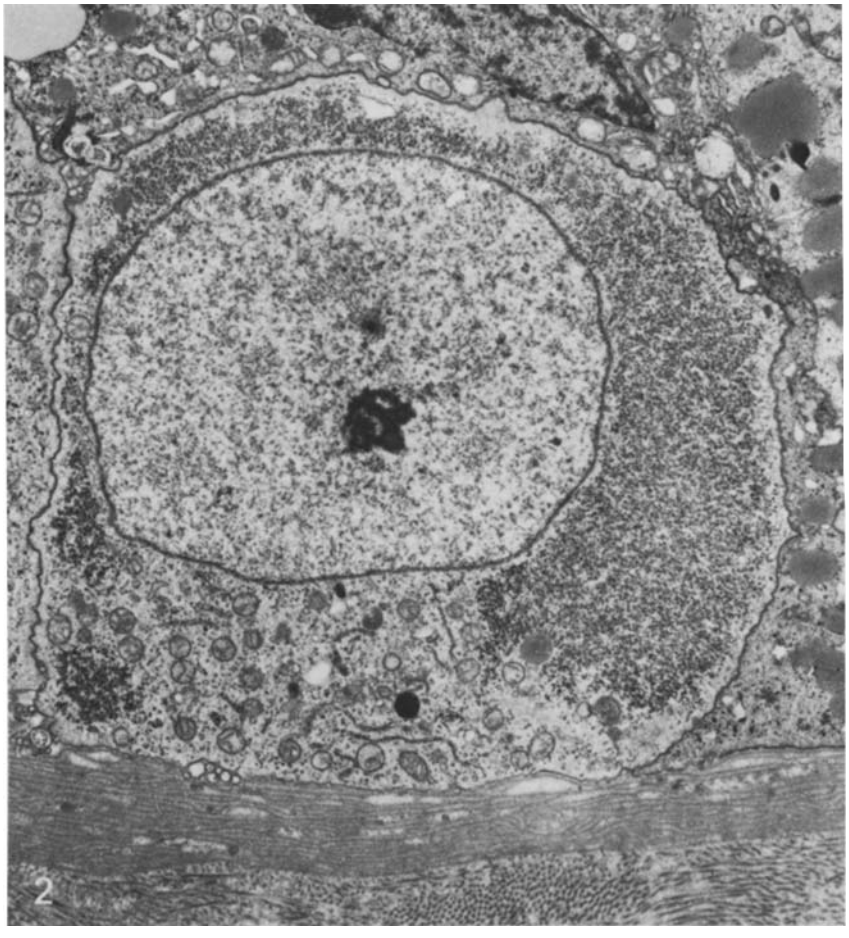

Fig. 2. AGC with high content of glycogen and polarization of the cell organelles, surrounded by Sertoli cells with intracytoplasmic lipid-like inclusions. × 6700

large. They consist of broad, in part rete-type rods and occasionally enclose a pars amorpha. Nuclear inclusions are frequently found as lipid-like masses, electron-dense areas without membrane or large cisterns filled with flaky material (Fig. 3c). Only in the AGC of biopsy specimens are some of these inclusions absent. Microtubular formations are rare. Alterations of the nuclear membrane such as duplications (strips) and eversion blebs (according to Rowley et al. 1971) occur in all diagnostic groups. Occasionally, loss of the inner nuclear membrane and foldings of the outer membrane can be demonstrated (Fig. 3c).

The most striking feature in the *cytoplasm* of the AGC is the high content of glycogen, though it may vary greatly (Fig. 2). The glycogen granules are usually concentrated in an area free of cell organelles. The dense cytoplasm exhibits numerous ribosomes and polymorphous cisterns of endoplasmic reticulum of different sizes.

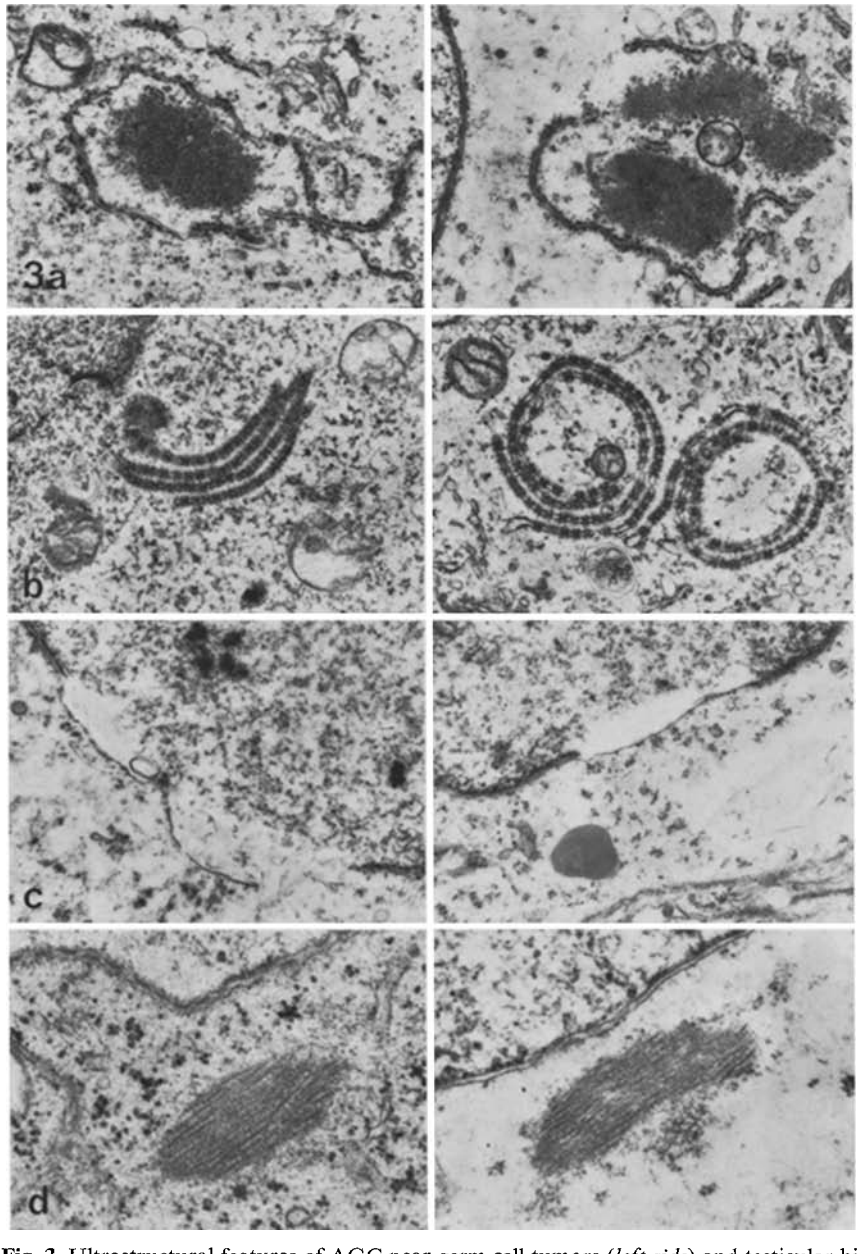

Fig. 3. Ultrastructural features of AGC near germ cell tumors (*left side*) and testicular biopsies (*right side*): **a** Nuage-like formation in close relation with endoplasmic reticulum ×14400. **b** Annulated lamellae ×11000. **c** Intranuclear cisternae with loss of the inner nuclear membrane left: ×11500; right: ×16100. **d** "Cristalloid"-like structure near nuclei ×26500

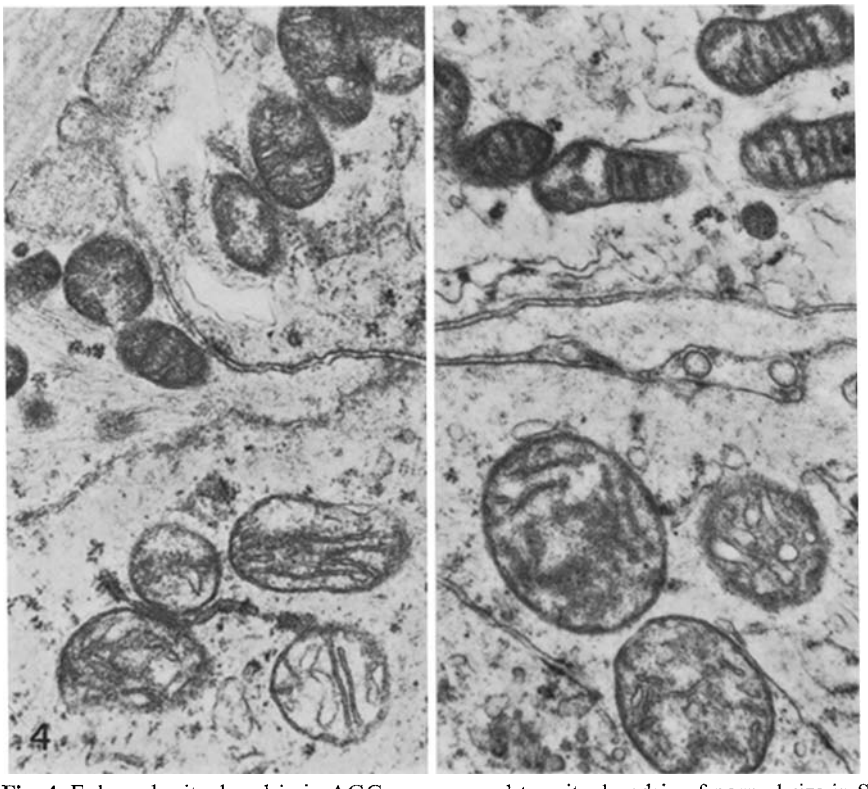

Fig. 4. Enlarged mitochondria in AGC as compared to mitochondria of normal size in Sertoli cells in the upper part of the picture. *Left side:* AGC near germ cell tumor; *right side:* AGC in testicular biopsy × 21 500

With a few exceptions, all the large *mitochondria* appear isolated in the cytoplasm, intermitochondrial cement-like material is hardly ever present (Fig. 4). In several cells, the mitochondria show vacuolar dilatation of the cisternae. Direct contact of the mitochondria with the rough endoplasmic reticulum is quite common. However, such contacts with the nuclear membrane are exceptional. Protrusions of mitochondria are present in all four diagnostic groups.

Annulated lamellae of various sizes (Fig. 3b) and generally multiple myelin-type structures (Fig. 5) are a regular feature in AGC. Other frequent findings are nuage-like alterations, namely electron-dense areas of finely granular material closely related to rough endoplasmic reticulum and lipid-like inclusions (Fig. 3a). Microtubular elements are only occasionally present. Many cells show a zone consisting of fine filaments interweaving in all directions on the inner side of the cell membrane. Golgi bodies are scanty. Equivalents of cristalloids up to 3 μm in size (Nagano 1969; Sohval et al. 1971) are occasionally found (Fig. 3d). Constructed as rodlike formations out of numerous elements parallel to the longitudinal axis, they are embedded in a finely dense material.

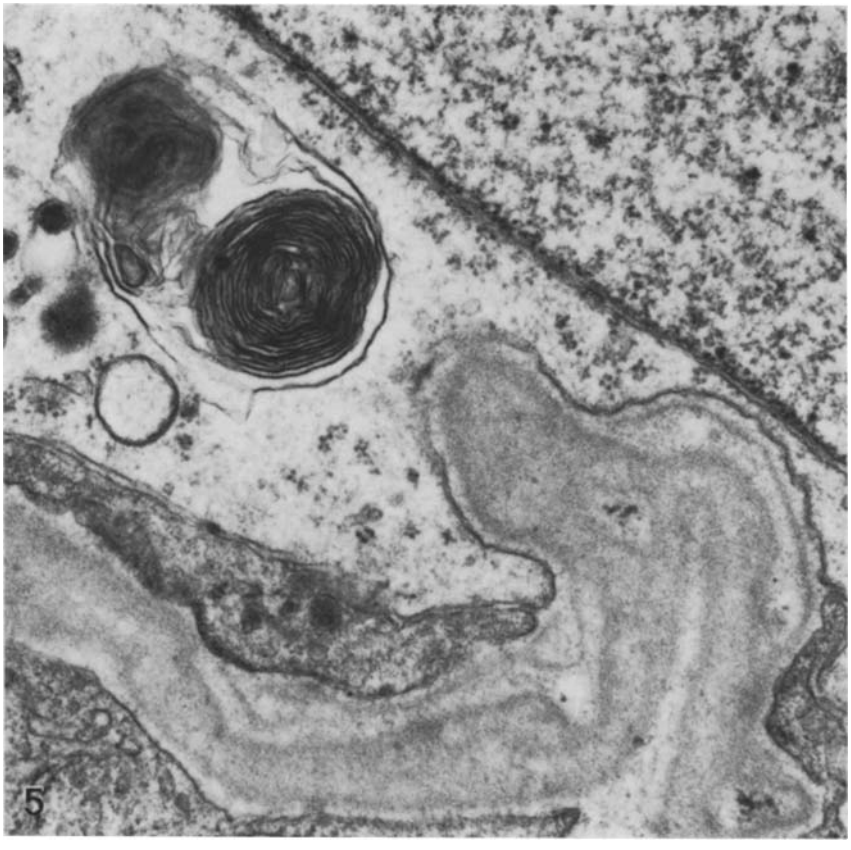

Fig. 5. Intracytoplasmic myelin-like formation in AGC. Protrusion of the thickened basement into the AGC. ×27600

Microvillous cell protrusions of the *cell membrane* are lacking. Occasional desmosome-like structures are irregular in form and vary greatly in electron density. No definite intercellular bridges can be identified.

The *basement membrane* appears multilayered in all the seminiferous tubules with AGC; in addition, the tunica propria shows broadening of its collagenous layers. In the basement membrane of all four groups electron-dense, occasionally illdefined polymorphous deposits including tubular structures of varying diameter can be recognized (Fig. 6).

In conclusion, the AGC in all four histological groups (seminomas, NSGCT, combined tumors as well as testicular biopsies in male infertility) are very similar in appearance. Neither the nuclei nor the cytoplasm show any constantly recurring and pathognomonic alterations which distinguish the AGC of the four groups from each other.

2. Immunohistochemical investigations on AGC

All AGC examined in the vicinity of seminomas, NSGCT, combined tumors, as well as in biopsy specimens are negative for α-FP and β-HCG. On the

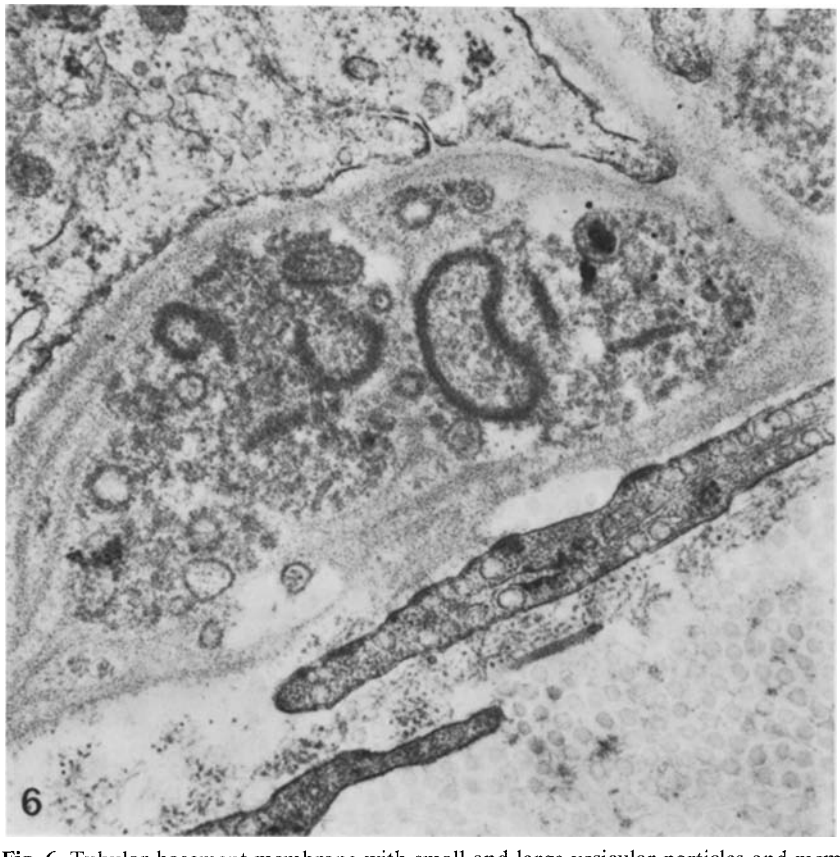

Fig. 6. Tubular basement membrane with small and large vesicular particles and membranous profiles. ×44100

other hand, a positive reaction with antiferritin was found in 6 cases: 2 seminomas and 4 NSGCT. In all these cases, however, only very few tubules contained positively reacting AGC. No definite qualitative or quantitative immunohistochemical differences could be found between AGC in seminomas and NSGCT.

Discussion

In the few ultrastructural studies on AGC, such elements have been described in the testicular tissue surrounding seminomas (Schütte et al. 1981), embryonal carcinomas (Nielson et al. 1974), and combined embryonal carcinoma and seminoma (Akhtar and Sidiki 1978). Besides, AGC have been examined electron microscopically in testicular biopsies and orchiectomy specimens of patients with impaired fertility (Albrechtsen et al. 1982; Nielsen et al. 1974; Schütte et al. 1981). In all these studies, some morphological characteristics of the AGC have been found regularly such as the broad contact of these cells with the basement membrane, their consistently large nuclei of varying size, the number and size of the nucleoli and the distribu-

tion of cell organelles. According to Nielsen et al. (1974), who compared AGC in testicular biopsies with AGC near an embryonal carcinoma, lack of intermitochondrial electrondense material, intracytoplasmic annulated lamellae, microtubules and lipid-like inclusions are common findings. Schütte et al. (1981) emphasize the presence of a peripheral margin with numerous microfilaments as described in primordial germ cells (Nielsen et al. 1974; Wartenberg et al. 1971). In addition, so-called nuages, dense-cored vesicles (Albrechtsen et al. 1982), abundant glycogen (Akhtar and Sidiki 1979; Nielsen et al. 1974) and absence of intercellular bridges as well as desmosomes are cited. Except for the finding of heterochromatin by Akhtar and Sidiki (1979) which could not be confirmed by Nielsen et al. (1974), no divergent structures of AGC are described in the literature.

In our four diagnostic groups, we were able to illustrate all the typical findings described above. The AGC, varying in size from 15 to 20 μm exhibit a broad-based contact with the basement membrane, a mostly centrally placed nucleus, polarization of the cell organelles, and large amounts of glycogen. The round to slightly ovoid nuclei show a dense chromatin with inhomogenous distribution and multiple, large nucleoli. Sometimes nuclear inclusions such as electron-dense areas, myelin-like and lipid-like formations are present. Changes of the nuclear membrane are frequent: duplications, infoldings or loss of the inner membrane. The intermitochondrial electrondense material is missing and the remarkably large mitochondria display protrusions. Annulated lamellae, microtubules and structures resembling so-called nuages (Söderström 1981) are common findings, whereas myelin-like formations, lipid droplets and cristalloids are infrequent. In the cytoplasm a small margin next to the cell membrane contains microfilaments. Intercellular bridges are lacking, while some desmosome-like connections between AGC are demonstrable. Usually the basement membrane appears multilayered and depositions of electron-dense granules and tubular formations can be recognized in every diagnostic group. On electron microscopy no pathognomonic structures could be found, which might enable distinction of the AGCs of the four diagnostic groups.

In recent years, a number of tumor-associated antigens (so-called tumor markers) have been determined in the serum of patients suffering from germ cell tumors of the testis. In AGC, Jacobsen et al. (1981) found positive staining reactions for ferritin while alpha-feto-protein (α-FP) as well as beta-human-chorionic-gonadotropin (β-HCG) could not be clearly demonstrated. In all our 15 cases, the AGC were negative for anti-α-FP and anti-β-HCG. In 6 cases, the reaction with anti-ferritin was positive. However, this positive reaction concerns cases of various diagnostic groups including patients with seminomas as well as patients with NSGCT.

Thus AGC of the different diagnostic groups (I–IV) are not distinguishable with electron microscopic or immunohistochemical methods. No predictions can be made with regard to the tumor type, seminoma or NSGCT into which these AGC might develop. It is also impossible to differentiate between AGC of testes with already existing germ cell tumors and AGC in tumor-free testes. Therefore, the problem as to whether these cells corre-

spond to a reactive alteration, a precancerous lesion, a carcinoma in situ or even to the still unknown stem cells of all germ cell tumors of the testis is still unsolved. Further investigations including studies on cell cultures may provide more information on the exact nature of these cells.

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