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Hyperplasia and hypertrophy of Leydig cells associated with testicular germ cell tumours containing syncytiotrophoblastic giant cells *

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Summary. In patients with seminomas and non-seminomatous germ cell tumours of the testis syncytiotrophoblastic giant cells (STGC) are largely responsible for elevated levels of chorionic gonadotropin. In searching for these cells, the question arises whether hyperplasia and/or hypertrophy of Leydig cells in the adjacent testicular tissue is a relevant finding. To elucidate this problem we analysed the tumour-free testicular tissue of 20 seminomas and 20 combined seminomatous and non-seminomatous germ cell tumours with or without immunohistochemically demonstrable STGC morphometrically. Extension of Leydig cell areas and the surface areas of Leydig cells per tubule are increased significantly in seminomas and combined tumours when STGC are present. There is also an apparent increase in the number of Leydig cells per tubule in seminomas and combined germ cell tumours with STGC. The difference, however, is not significant statistically in the group of seminomas. Hypertrophy and/or hyperplasia of Leydig cells must be considered as a relevant finding in germ cell tumours with STGC. It is most easily recorded by evaluating the surface area of Leydig cells per test area or per tubule.

Key words. Testicular germ cell tumours – Syncytiotrophoblastic giant cells – Human chorionic gonadotropin – Leydig cells

Introduction

Elevated serum titres of human chorionic gonadotropin (hCG) are a typical finding in patients with choriocarcinoma or other germ cell tumours of the testis containing choriocarcinomatous elements. However, embryonal carcinomas, teratomas and seminomas without typical choriocarcinomatous foci can also produce elevated titres of hCG (Heydermann and Neville 1976; Javadpour et al. 1978a, b) and generally such tumours include syncytiotrophoblastic giant cells (STGC). STGC, therefore, appear to be essential for the elevated levels of hCG. In fact, Kurman et al. (1977) were able to demonstrate the presence of β-hCG in STGC alone or in syncytiotrophoblastic components of choriocarcinomas in 12 out of 13 testicular tumours of patients with elevated serum hCG, using immunohistochemistry. STGC were most frequently associated with embryonal carcinomas, rarely with yolk sac tumours or seminomas. In seminomas, STGC by themselves do not seem to worsen the prognosis (Javadpour 1984; von Hochstetter et al. 1985; Rüther et al. 1987). Of both prognostic and therapeutic importance is the presence (or absence) of non-seminomatous, especially choriocarcinomatous foci. In the search for STGC the question arises whether hyperplasia and or hypertrophy of the Leydig cells is a relevant variable.

Mark and Hedinger (1965) were able to demonstrate pronounced, sometimes nodular hyperplasia of Leydig cells in the remaining tumour-free testicular tissue of 6 patients with germ cell tumours, 4 of which included trophoblastic (choriocarcinomatous) foci. Two patients had other signs of gonadotropin hyperactivity. In 1984, Asa et al. reported the case of a patient with an embryonal carcinoma, elevated serum titres of β-hCG and α-fetoprotein, and pronounced Leydig cell hyperplasia and hypertrophy. In order to elucidate the diagnostic value of such Leydig cell hypertrophy or hyperplasia as an indicator of the presence of STGC in otherwise pure seminomas or in combined germ cell tumors we analysed the tumour-free testicular tissue of 20 seminomas and 20 combined seminomatous and non-seminomatous germ cell tumours with or without β-hCG producing STGC morphometrically.

* Dedicated to Prof. N. Papacharalampous, Athens, on the occasion of his 70th anniversary

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Materials and methods

The investigations are based on 40 testicular germ cell tumours from the collection of the Institute of Pathology, University of Zürich, Switzerland. Only tumours in which enough tumour-free testicular tissue was at our disposition were included. The tumours were grouped as follows: group I, Pure seminomas without β-hCG-positive STGC (10 cases); group II, Seminomas with β-hCG-positive STGC (10 cases); group III, Combined seminomatous and non-seminomatous germ cell tumours without β-hCG-positive STGC or choriocarcinomatous foci (9 cases); group IV, Combined seminomatous and non-seminomatous germ cell tumours with β-hCG-positive STGC (11 cases).

Specimens were fixed in 4% buffered formalin, embedded in paraffin and stained with haematoxylin and eosin. STGC were investigated immunocytochemically with anti-β-hCG by the indirect immunoperoxidase method with monoclonal antibodies for formaldehyde-fixed paraffin sections: monoclonal anti-β-hCG (Bio-Science, Emmenbrücke, Switzerland) and peroxidase conjugated swine anti-goat IgG antibody (Tago, Burlingame, Calif.).

In all 40 tumours the number and surface area of the Leydig cells and of the seminiferous tubules were determined, as was the entire interstitial surface area (Sargent and McDonald 1948; Clegg 1961). For morphometry, a square grid was used with a side length of 660 μm and 100 single fields. In each case 20 test areas were determined with the point-to-count method at an enlargement of ×80. Due to the infiltration of the testicular tissue by the tumour it was generally not possible to evaluate more than 20 tumour-free test areas. To evaluate the number of tubules, only those lying completely inside the test area were considered. Areas with artefactual shrinking of tissue were not included. The statistical significance of the mean values of the four groups was compared using the Wilcoxon test.

Results

The results of our investigation are summarized in Table 1 and Fig. 1. With respect to the difference between seminomas with or without β-hCG-positive SCTG (groups I and II) there is a significant increase of the percentage surface area of Leydig cells per test area in the group of seminomas with β-hCG-positive STGC (Table 1; Fig. 1a, 3). For the percentage surface area of Leydig cells in a given percentage surface area of interstitial tissue we found a statistically significant increase (Table 1). However, regarding the number of Leydig cells per test area the difference is not significant statistically, though the results demonstrated in Table 1 and Fig. 1b and the microphotographs of Figs. 2 and 3 give the impression of a pronounced increase. Referring to the tubules, the surface areas of the Leydig cells again show a statistically significant difference in favour of tumours with STGC (Table 1, Fig. 1c). But, once more, groups I and II do not differ significantly in the number of Leydig cells per tubule (Table 1, Fig. 1d). From these results the enlarged surface area appears to be the consequence of hypertrophy rather than of hyperplasia of the Leydig cells. In seminomas with STGC the average surface area of a Leydig cell measures 411 ± 108 μm²; in those without STGC it is 290 ± 58 μm².

In groups III and IV (seminomas combined with non-seminomatous germ cell tumours with or without STGC) the percentage surface areas of the Leydig cells in the adjacent tumour-free testicular tissue per test area

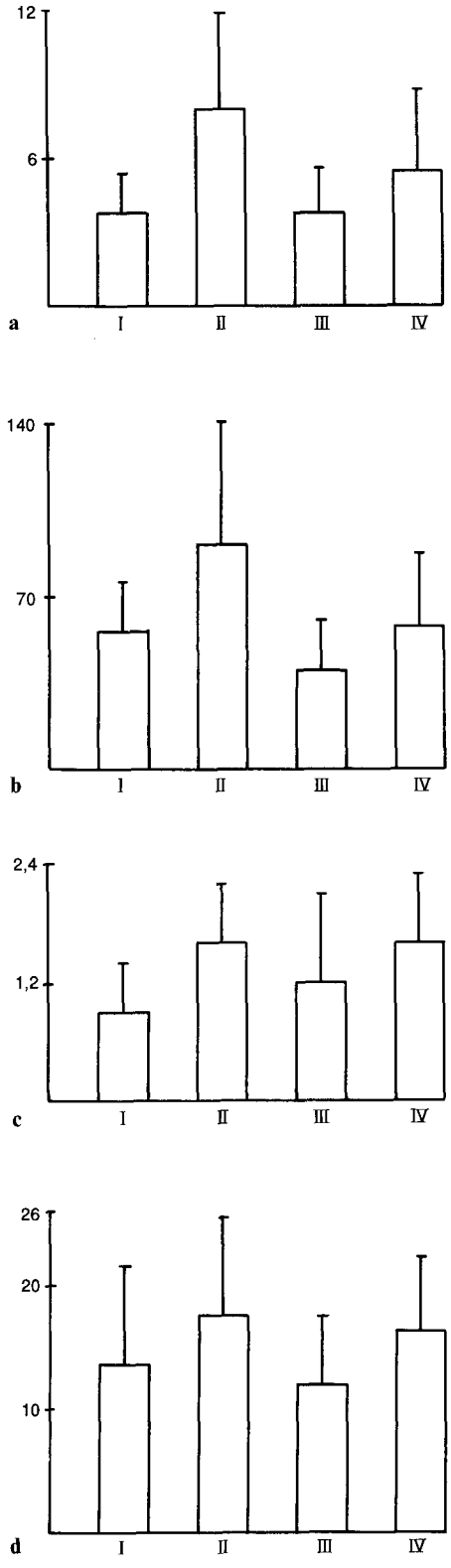

Fig. 1a–d. Percentage surface area and number of Leydig cells per test area or per tubule. **a** Percentage surface area of Leydig cells per test area; **b** number of Leydig cells per test area; **c** percentage surface area of Leydig cells per tubule; **d** number of Leydig cells per tubule. Group I, Pure seminomas; group II, seminomas with β-human chorionic gonadotropin (hCG)-positive syncytiotrophoblastic giant cells (STGC); group III, combined germ cell tumours without β-hCG-positive STGC; group IV, combined germ cell tumours with β-hCG-positive STGC

Table 1. Percentage surface area and number of Leydig cells per test area or tubule in the remaining tumour-free testicular tissue of seminomas and combined germ cell tumours with or without β-hCG-positive STGC: group I, pure seminomas; group II, seminomas with STGC; group III, combined germ cell tumours without STGC; group IV, combined germ cell tumours with STGC

	% SLC	LC/TA	% SLC/T	LC/T	%SLC/%I
Group I	3.8±1.6	56.5±19.8	0.9±0.5	13.6±7.8	0.09±0.04
Group II	8±3.9	91.3±52.3	1.6±0.6	17.7±8	0.16±0.08
Significance	0.005	none	0.001	none	0.005
Group III	3.8±1.8	41±20.5	1.2±0.9	12.2±5.7	0.09±0.05
Group IV	5.5±3.3	57.9±28.8	1.6±0.7	16.5±5.8	0.11±0.06
Significance	0.025	0.005	0.01	0.01	none

hCG, Human chorionic gonadotropin; STGC, syncytiotrophoblastic giant cells; % SLC, percentage surface area of Leydig cells per test area; LC/TA, number of Leydig cells per test area; % SLC/T, percentage surface area of Leydig cells per tubule; LC/T, number of Leydig cells per tubule; % SLC/% I, percentage surface area of Leydig cells per percent surface area of total interstitial tissue

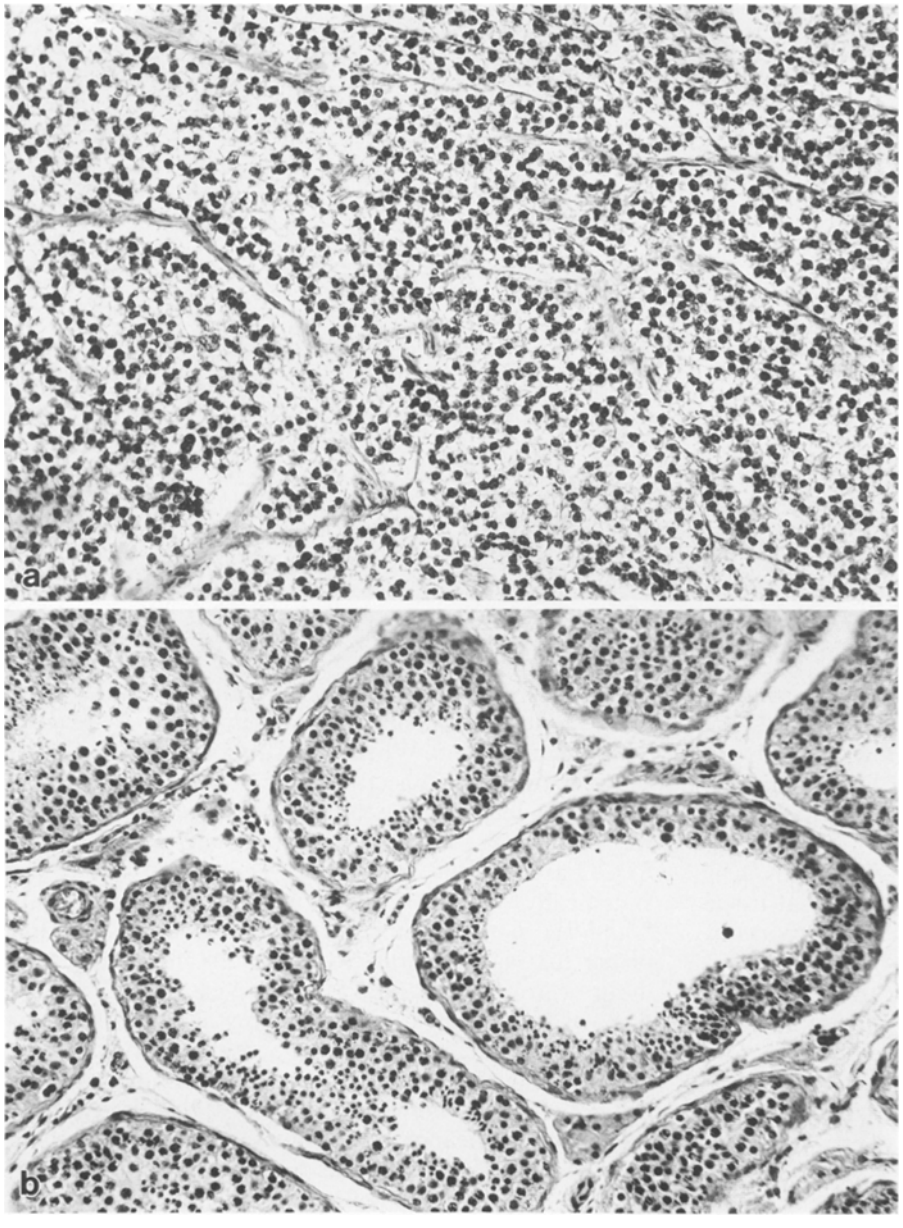

Fig. 2. Pure seminoma without β-hCG-positive STGC. [25-year-old man, haematoxylin and eosin (H&E), ×130]: **a** tumour, **b** tumour-free testicular tissue

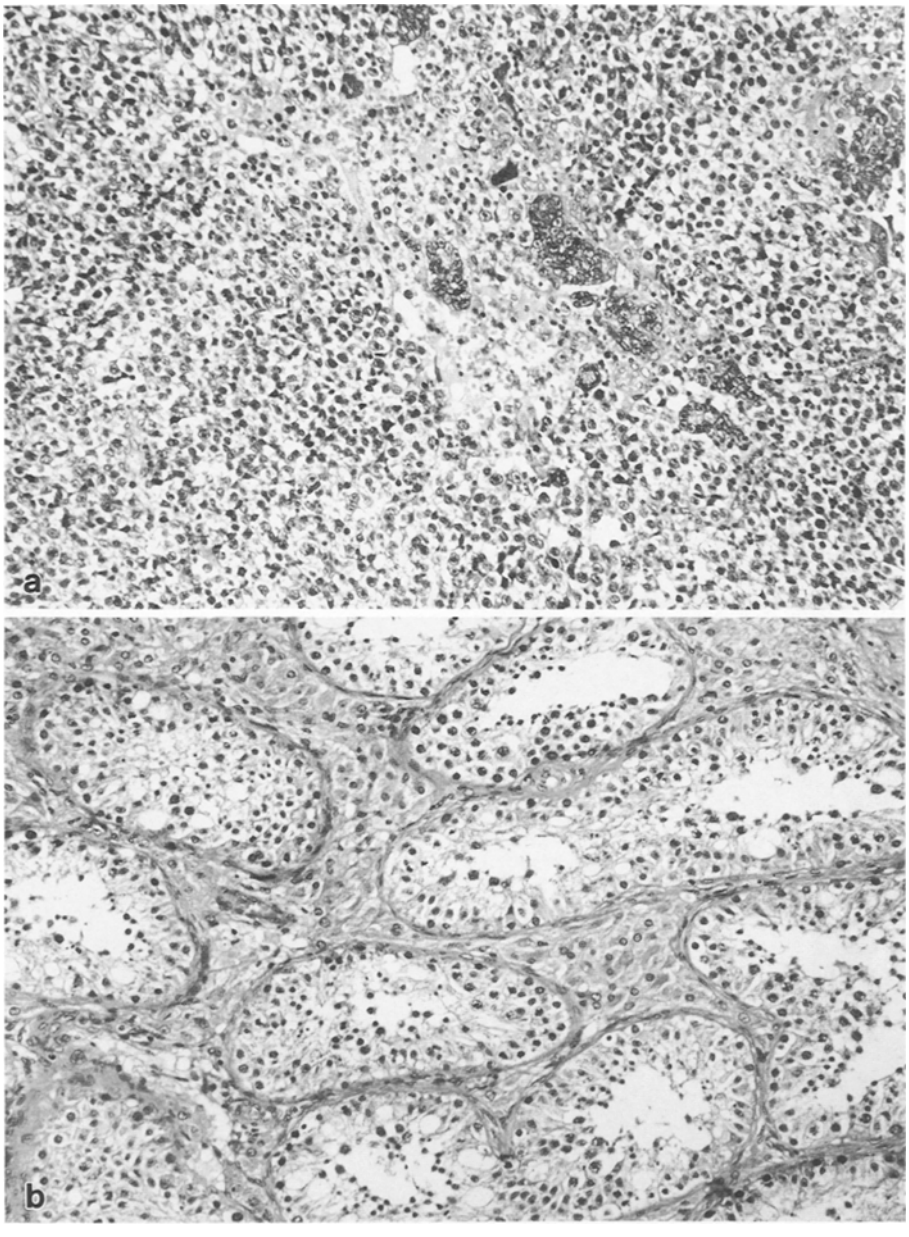

Fig. 3. Seminoma with β-hCG-positive STGC (32-year-old man, H&E, ×130): **a** tumour, **b** tumour-free testicular tissue: hypertrophy and hyperplasia of Leydig cells

or per tubule again differ significantly, as in groups I and II (Table 1; Fig. 1a, c). In contrast to groups I and II, tumours of group IV with STCG show not only an increase of the percentage surface area of Leydig cells but also a significant increase of the number of these cells per test area or per tubule (Table 1; Fig. 1b, d). The ratio of the percentage surface area of Leydig cells for a given percentage surface area of interstitial tissue shows a slight but statistically insignificant increase in favour of the combined tumours with STGC (Table 1).

No statistical differences are demonstrable between the different groups as to number and surface areas of the tubules, or the surface areas of the "non-Leydig cell" interstitial tissue.

Discussion

The findings presented show that the extension of the Leydig cell areas in the remaining tumour-free testicular tissue of seminomas or of such tumours combined with other germ cell elements increases if β-hCG-positive STGC are present. Where the surface areas of Leydig cells per tubule is concerned, there is also a statistically significant increase in seminomas (groups I and II) as well as in combined tumours (groups III and IV) when STGC are present. Interestingly, we could not find a statistically significant difference in seminomas (groups I and II) in the number of Leydig cells per test area or per tubule in contrast to the results in combined tumours (groups III and IV). However, as Fig. 1b and d shows, the mean values concerning the number of Leydig cells per test area or per tubule in seminomas with STGC seem to be higher than in seminomas without STGC, although the wide standard deviations preclude statistical significance. Therefore, the increase of the surface areas of the Leydig cells may be due to an enlargement of single cells or an increase of their number. As shown above, the enlargement of single cells seems to

prevail in pure seminomas, while increase in cell number occurs in seminomas combined with other germ cell tumour elements.

Similar differences in hypertrophy or hyperplasia of the Leydig cells have been recorded in men and animals under treatment with hCG. In patients with hypogonadotropic hypogonadism, treatment with hCG produces hyperplasia and differentiation of Leydig cells (Bartter et al. 1952). Maddock and Nelson (1952) noticed a similar increase in the number and staining of Leydig cells in three adult patients treated with hCG for impotence without signs of hypogonadism. In oligospermic patients De Kretser (1967) demonstrated by electron microscopy a functional activation of the Leydig cells under treatment with hCG and Chemes et al. (1985) reported differentiation of their precursors. In healthy adults Heller and Leach (1971) found an increase in cellular and nuclear size of Leydig cells.

In adult rats and monkeys treated with hCG the number of Leydig cells increases according to Schoen and Samuels (1965), Christensen and Peacock (1980), Lamano-Carvalho et al. (1987) and Teerds et al. (1988, 1989a). The same has been described to occur in normal or hypophysectomized immature rats (Chemes et al. 1976; Kuopio et al. 1989; Teerds et al. 1989b) and guinea pigs (Merkow et al. 1968). However, according to Hodgson and De Kretser (1984), Bergh (1987), Andreis et al. (1989), Teerds et al. (1988, 1989a) and Leon et al. (1990) hypertrophy and differentiation of Leydig cells are more prominent than hyperplasia, which is easily demonstrable by electron microscopy even in isolated cells of mice, rats and humans (Schwarz and Merker 1965; Simpson et al. 1987; Bilinska 1989). Summarizing these different results on the behaviour of Leydig cells of man and animals under treatment with hCG, there is no doubt that Leydig cells are activated by this treatment with hypertrophy generally preceding hyperplasia.

As shown by Mark and Hedinger (1965) and Asa et al. (1984) similar activation of the Leydig cells can be demonstrated in the tumour-free testicular tissue of patients with testicular germ cell tumours producing hCG. According to the results of our investigation hypertrophy or hyperplasia of the Leydig cells can most easily be recorded by evaluating the surface area of Leydig cells per test area or per tubule. Both combined germ cell tumours of the testis secreting hCG and apparently pure seminomas give rise to such a Leydig cell activation provided they contain β-hCG-producing STGC. Schütte et al. (1981) and Lauke et al. (1989) noted slight hyperplasia of Leydig cells adjacent to early and predominantly intratubular germ cell tumours. Lauke et al. (1989) even found Leydig cells in the process of mitosis. This Leydig cell hyperplasia, however, was local and topographically related to the tumour spread, and not diffuse as in the tumour-free testicular tissue of germ cell tumours with STGC.

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