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Non-seminomatous Germ Cell Tumors of the Testis. Analysis of CEA Production in Primary Tumors and in Retroperitoneal Lymph Node Metastases after PVB Chemotherapy*†

A. J. H. SUURMEIJER,‡ J. W. OOSTERHUIS,‡ J. MARRINK,§ TH. OCKHUIZEN,§ D. TH. SLEIJFER,‖ H. SCHRAFFORDT KOOPS¶ and G. J. FLEUREN‡

‡*Department of Pathology, University of Groningen, Oostersingel 63, 9713 EZ Groningen, The Netherlands and §Laboratory of Immunochemistry and Departments of ‖Medical and ¶Surgical Oncology, University Hospital of Groningen, The Netherlands*

Abstract—*In the present investigation we compared CEA immunoperoxidase staining in testicular tumors (before PVB chemotherapy) and retroperitoneal tumors (after PVB chemotherapy) with CEA levels in the cyst fluid of retroperitoneal mature teratoma and in the patients' serum. CEA had no value as a serum tumor marker since serum CEA elevations were not associated with tumor activity. Only one elevated CEA level after chemotherapy was associated with bleomycin pneumonitis. Despite normal serum levels, CEA was localized immunohistochemically in yolk sac tumor and mature teratoma in the primary tumors and in retroperitoneal mature teratoma following PVB chemotherapy. The presence of CEA in cells lining cystic mature teratoma was associated with high CEA levels in the cyst fluid.*

INTRODUCTION

ALPHAFETOPROTEIN (AFP) and/or human chorionic gonadotropin (HCG) serve as tumor markers in 60–90% of patients with advanced stage non-seminomatous germ cell tumors (NSGCT) of the testis [1–7].

Negative AFP and HCG determinations are the result of the cellular heterogeneity of NSGCT. These tumors are composed of different histological elements with differences in AFP and HCG production [8–10]. Residual mature teratoma after chemotherapy, for instance, cannot be detected with AFP or HCG assays [10]. The value of carcinoembryonic antigen (CEA) as a tumor marker for NSGCT and its histological correlation have not yet been adequately assessed [11]. In most studies CEA has no value as tumor marker in NSGCT patients, because elevated serum levels are seldom found and changes in CEA concentrations do not correlate with tumor activity [5, 12]. In some other studies elevated CEA serum levels were found in relation to teratocarcinoma with differentiated structures [13]. Furthermore, immunohistochemical studies have shown the presence of CEA in teratomatous elements of NSGCT [14].

These results prompted us to investigate CEA production in NSGCT of the testis and in retroperitoneal mature teratoma after combination chemotherapy, comparing CEA tissue localization with serum CEA values and with CEA levels in the cyst fluid of mature teratoma after chemotherapy.

MATERIALS AND METHODS

Patients

Twenty-nine patients with bulky non-resectable stage II and III NSGCT of the testis, who had been treated with combination chemotherapy consisting of *cis*-platinum, vinblastine and bleomycin (PVB) [15, 16] at the University Hospital of

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Groningen between August 1977 and November 1981, were included in this study. The surgicopathological tumor stage was determined as described by Samuels *et al.* [17]. All but five patients underwent orchidectomy in referring hospitals. After PVB chemotherapy a retroperitoneal lymph node dissection (RLND) was performed in our hospital in order to assess response to chemotherapy and to remove residual tumor.

Formalin-fixed, paraffin-embedded tissue blocks of the orchidectomy and RLND specimens as well as serum samples after orchidectomy (before PVB chemotherapy) and at the time of the RLND (after PVB chemotherapy) were available.

Histological evaluation and tissue preparation

The testicular tumors were classified by listing their different histological components: seminoma (SE), embryonal carcinoma (EM), choriocarcinoma (CH), yolk sac tumor or endodermal sinus tumor (YO), differentiated or mature teratoma (TD) and immature teratoma (TI), as proposed by Hajdu [18]. The RLND specimens were extensively sampled in our pathology department and scored for the presence of the above-mentioned components and also for lesions consisting of necrosis and fibrosis (NE/FI), apparently regressed retroperitoneal tumor tissue. Histologically and cytologically benign mature teratoma occurring after chemotherapy was subdivided in elements with differentiation at tissue level (cuboidal, cylindrical, mucinous and squamous epithelium, smooth muscle, cartilage etc.) and differentiation at organoid level (gut-like or bronchus-like cavities).

All available blocks of orchidectomy and RLND specimens were serially cut. H & E staining and immunohistochemical staining was performed on sections from the same tissue blocks. RLND specimens containing necrosis and fibrosis only were excluded from immunohistochemical methods. Cyst fluid samples were available for CEA measurement in 5 out of 15 cases of residual retroperitoneal mature teratoma after PVB chemotherapy.

Immunohistochemical procedures

CEA localization at the light-microscopic level was studied in sections of formalin-fixed tumor tissue using a modification of the indirect immunoperoxidase technique as described by Sternberger *et al.* [19]. This method has been described previously [20]. The rabbit anti-CEA serum was purchased from DAKO, Copenhagen, Denmark (code A115).

Cross reactivity of the antiserum against CEA with non-specific cross-reacting antigen (NCA), biliary glycoprotein I (BGP_1), NCA_2, the gastric-CEA-like antigen and ABO blood group antigens was eliminated after absorption with granulocytes, homogenized human liver and gastric tissue and AB erythrocytes. The absorbed antiserum to CEA has been extensively tested for immunohistochemistry by Nap *et al.* [21]. CEA staining in normal colon and in a well-differentiated adenocarcinoma of the colon served as positive controls. Sections of normal lung, spleen, liver and stomach were used as negative tissue controls, and anti-CEA serum absorbed with purified CEA (Hoffman La Roche) also served as a negative control. Ultrastructural localization of CEA was studied in a freshly obtained testicular tumor, not included in this patient series. This testicular tumor contained mature teratoma with areas of differentiation at tissue level, namely cysts lined by cuboidal, cylindrical and mucinous epithelium.

Tissue pieces (1 mm^3) from the cyst wall were fixed in 2% glutaraldehyde in phosphate buffer for 2 hr and washed in phosphate-buffered saline for 20 min. Subsequently immunostaining for CEA was achieved using the indirect peroxidase technique (pre-embedding technique).

The tissue pieces were post-fixed in 2% osmium tetroxide and embedded in Epon. Ultrathin sections were examined with a Philips 300 electron microscope.

CEA measurement

CEA concentrations in the patients' serum and in cyst fluid were measured using a commercial enzyme immunoassay (Abbott Laboratories). The sensitivity of this CEA assay is 0.5 ng/ml of patient specimen. Eighty-eight percent of a population of 1020 smokers and non-smokers had a CEA level below 2.5 ng/ml, while 98% had a level below 5 ng/ml (results from Abbott Laboratories). Five nanograms per milliliter was chosen as the upper limit of normal.

Cross-reactivity with NCA was low. NCA standard preparations with a concentration range of 2500–20,000 ng/ml (kindly donated by Dr. B. Wahren, Karolinska Institute, Stockholm) resulted in CEA values of 0.6–2.4 ng/ml, thus resulting in a cross-reactivity of 0.1–0.3 ‰.

RESULTS

The histology and CEA immunohistochemical findings of the 29 NSGCTs of the testis and the treated retroperitoneal tumors, as well as their relation to the serum CEA values before and after PVB chemotherapy and CEA values in the cyst fluid of mature teratoma after chemotherapy, are summarized in Table 1.

Table 1. Histology and CEA immunohistology of testicular and retroperitoneal tumors in association with CEA values in patients' serum and mature teratoma cyst fluid

Patient No.	Histology of testis tumor	Before PVB chemotherapy s-CEA	t-CEA	Histology of RLND tumor	After PVB chemotherapy s-CEA	c-CEA	t-CEA
1	EM/TD/TI	1.65	+ (TD)	NE/FI	2.85		
2	EM/TD/TI/SE	0.9	+ (TD)	TD	1.8	n.d	-
3	EM/TD/TI/SE	2.1	-	TD	1.2	756	+
4	EM/TD/TI/CH	1.5	-	TD	1.8	n.d	+
5	EM/TD/TI	1.2	-	TD	1.05	n.d	-
6	EM/SE	3.0	-	NE/FI	22		
7	EM/YO	3.6	+ (YO)	NE/FI	4.2		
8	EM/CH	1.8	-	EM	2.4	n.d	-
9	TD	1.2	+ (TD)	TD	1.2	n.d	-
10	EM/TI	0.75	-	NE/FI	0.9		
11	EM/TD/SE	2.1	-	NE/FI	3.0		
12	EM/TD/YO	3.0	-	TD	2.1	4272	+
13	EM/TI	1.8	-	NE/FI	2.4		
14	EM/TD/TI	0.9	-	TD	0.6	n.d	+
15	EM/TD/TI/CH	1.95	-	TD	1.65	n.d	-
16	CH/TD	1.5	-	TD	1.95	4030	+
17	EM	1.8	-	NE/FI	2.7		
18	EM/TD/SE	3.45	-	NE/FI	4.35		
19	EM	0.9	-	TD	0.9	n.d	+
20	EM/TD/TI	1.35	+ (TD)	NE/FI	1.65		
21	EM/TD/TI/YO	1.5	+ (YO)	NE/FI	1.5		
22	EM/TD/TI	1.65	-	NE/FI	0.9		
23	EM	0.75	-	NE/FI	1.65		
24	EM/SE	1.8	-	NE/FI	2.4		
25	EM/TD/CH	0.6	+ (TD)	TD	1.2	n.d	+
26	EM	1.8	-	TD	3.9	n.d	-
27	TD	0.8	+ (TD)	TD	5.85	5909	+
28	TD/SE	2.4	-	TD	1.4	92	-
29	EM/TD/TI	1.0	+ (TD)	TD	1.7	n.d	+

n.d = not determined; s-CEA = serum CEA: c-CEA = cyst fluid CEA; t-CEA = tissue CEA.

Histology

The classification of the 29 testicular tumors by listing their different histological components resulted in 23 mixed-type and 6 pure-type tumors. Embryonal carcinoma was seen in 25 tumors, mature teratoma in 19 tumors, immature teratoma in 13 tumors, choriocarcinoma in 5 tumors, yolk sac tumor in 3 tumors and seminoma in 7 tumors (Table 2).

Sampling the RLND specimens after PVB chemotherapy resulted in 15 tumors with mature teratoma, 1 tumor with remnants of embryonal carcinoma and 13 tumors with regressed tumor tissue (necrosis and fibrosis only) (Table 3).

Immunohistochemical findings

The results of immunoperoxidase staining in the different histological components of the 29 testicular tumors are presented in Table 2. Positive staining for CEA was seen in 9 testicular tumors (31%), namely in 7 out of 19 mature teratoma components (37%) and in 2 out of 3 yolk sac tumor components (67%).

In mature teratoma, CEA staining was prominent along the luminal surface of the cysts lined by cuboidal or cylindrical cells (Fig. 1). Sometimes slight CEA staining was found in the cytoplasm of these cells. CEA was also localized focally along the apical surface of some organoid gut-like cavities.

Table 2. CEA tissue localization in the different histological components of the testicular tumors

Component	No.	CEA tissue positivity
EM	25	0
TD	19	7 (37%)
TI	13	0
CH	5	0
YO	3	2 (67%)
SE	7	0

Table 3. CEA tissue localization in retroperitoneal tumors

Component	No.	CEA tissue positivity
TD	15	9 (60%)
EM	1	0
NE/FI	13	n.d

n.d = not determined.

In yolk sac tumor, intracytoplasmic CEA staining was demonstrated in few cells, in solid or microcystic areas (Fig. 2). No CEA staining was seen in embryonal carcinoma, immature teratoma, choriocarcinoma or seminoma.

The results of immunoperoxidase staining for CEA in the 29 retroperitoneal specimens are presented in Table 3. CEA staining was seen in 9 out of 15 mature teratoma components (60%), mainly along the luminal surface of cysts lined by cuboidal or cylindrical epithelium and along the luminal surface of gut-like cavities (Fig. 3).

The tumor with remnants of embryonal carcinoma was negative for CEA.

In general, the CEA distribution pattern in mature teratoma of retroperitoneal tumors resembled the staining pattern in testicular mature teratoma. Ultrastructurally CEA was uniformly present along the microvilli of cuboidal and cylindrical mucus-producing cells lining cystic mature teratoma but not along the basolateral surfaces of these cells. CEA was also seen in the mucus of some of these cells (Fig. 4).

Comparison of CEA in serum, tissue and cyst fluid

Before PVB chemotherapy all 29 patients had normal serum CEA values, but CEA staining was observed in mature teratoma components (7 cases) and in yolk sac tumor components (2 cases) of the testicular tumors (Tables 1 and 2). After PVB chemotherapy elevated serum CEA values were measured in two patients (7%). One patient (No. 6 in Table 1), who died of a bleomycin pneumonitis, had rising CEA levels up to 22 ng/ml. Autopsy of this patient revealed retroperitoneal and lung deposits consisting of necrosis and fibrosis only. The other patient (No. 27 in Table 1) had a slight CEA elevation (5.85 ng/ml) together with positive CEA staining in mature teratoma and a cyst fluid CEA concentration of 5909 ng/ml.

Eight more cases with CEA staining in mature teratoma after chemotherapy were accompanied by normal CEA values (Table 1).

Cyst fluid CEA values of 756, 4030, 4272 and 5909 ng/ml were all associated with CEA staining in cystic mature teratoma with intestinal differentiation (patients 3, 16, 12 and 27 in Table 1). The cyst fluid CEA value of 92 ng/ml (patient 28 in Table 1) was accompanied by negative CEA staining. The cyst fluid CEA values of 92, 756, 4030 and 4272 ng/ml were associated with normal serum CEA values.

DISCUSSION

In about 50% of NSGCT patients metastatic deposits consist exclusively of mature teratoma after combination chemotherapy [22]. Until now no serum tumor marker has been available for the detection of residual mature teratoma. Few reports mention the possible value of CEA in these cases [12–14]. However, in our patient material CEA had no value as a serum tumor marker before or after chemotherapy since CEA values were normal in all but one patient. This is in accordance with the findings of Bosl *et al.* [5]. The raised CEA value was associated with bleomycin pneumonitis and not with residual tumor after chemotherapy.

Despite normal CEA serum levels, positive CEA immunoperoxidase staining was seen in yolk sac tumor in testicular tumors and in mature teratoma in testicular and retroperitoneal tumors after chemotherapy. Moreover, CEA staining in residual retroperitoneal mature teratoma after chemotherapy was associated with high CEA concentrations in the cyst fluid of this component. The occurrence of high intracystic CEA levels with normal serum CEA values has also been reported for benign cystic teratoma of the ovary [23]. A clear relation was found between CEA staining in residual retroperitoneal mature teratoma and CEA concentrations in the cyst fluid of this component: negative CEA staining was related to a cyst fluid CEA concentration of 92 ng/ml, whereas concentrations above 756 ng/ml were associated with CEA positivity in structures with intestinal differentiation.

Apparently, CEA diffusion into the bloodstream is too scant when this glycoprotein is produced in cystic mature teratoma. This is imaginable since CEA is localized light-microscopically and ultrastructurally along the luminal surface and in the mucus of cells lining the cyst walls.

Our results suggest the existence of a diffusion barrier and question whether any mature teratoma substances can serve as a serum tumor marker at all. The localization of CEA in teratomatous elements suggestive of intestinal differentiation has also been reported by Heyderman [14].

Apart from the staining in mature teratoma with intestinal differentiation, we also found staining in cuboidal and cylindrical cells in mature teratoma (differentiation at tissue level) in testicular and retroperitoneal tumors and staining in few yolk sac tumor cells in testicular tumors.

During embryogenesis the wall of the human yolk sac consists of an inner lining of endodermal epithelium continuous with that of the midgut [24]. So, analogously to AFP expression in yolk sac tumor and in less highly differentiated cystic mature teratoma [10], the expression of CEA in both well-differentiated and less highly differentiated teratomatous structures as well as in yolk

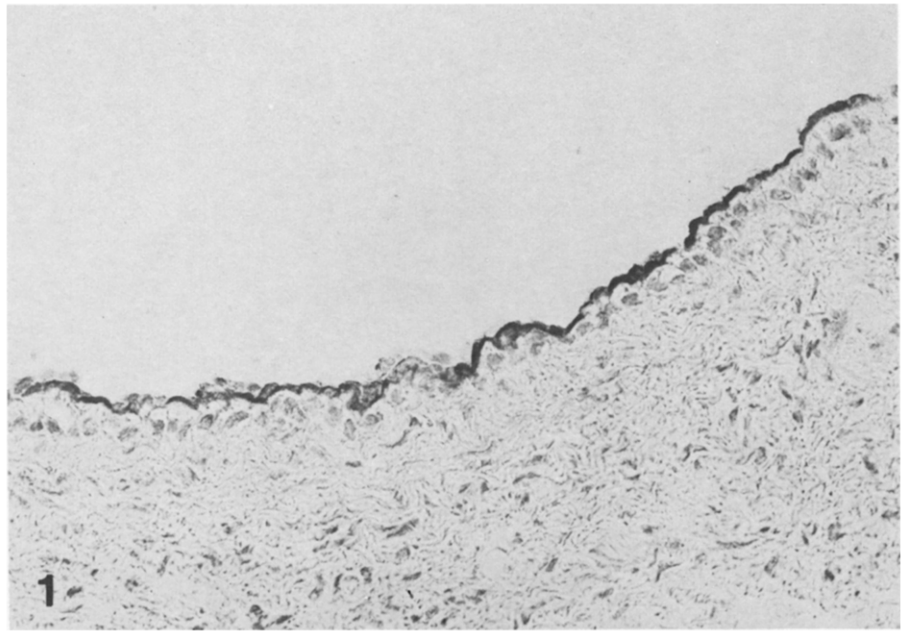

Fig. 1. CEA staining along the luminal surface of cuboidal cells lining cystic mature teratoma (immunoperoxidase, ×350).

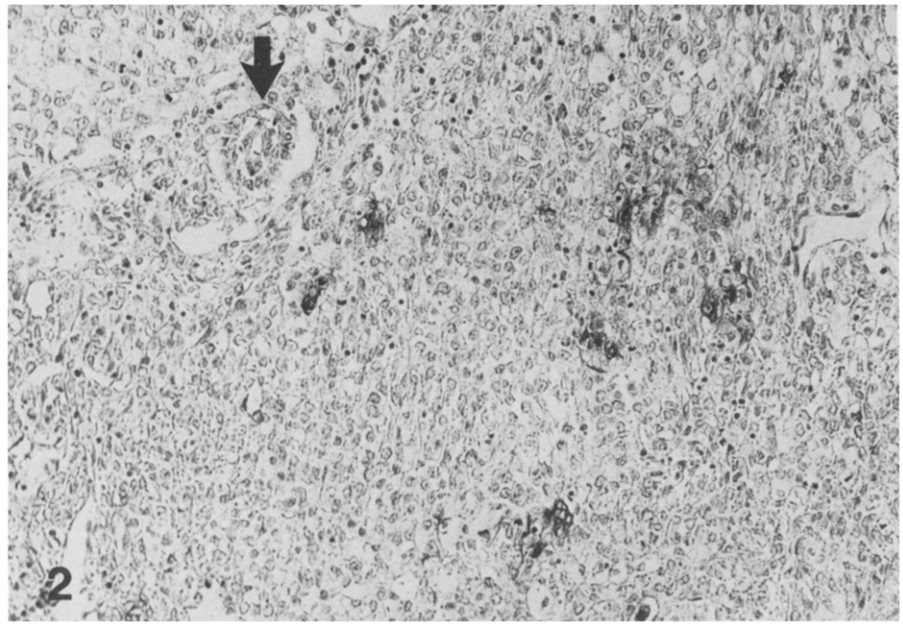

Fig. 2. CEA staining in a few cells in solid yolk sac tumor adjacent to a Schiller-Duval body (arrow) (immunoperoxidase, ×140).

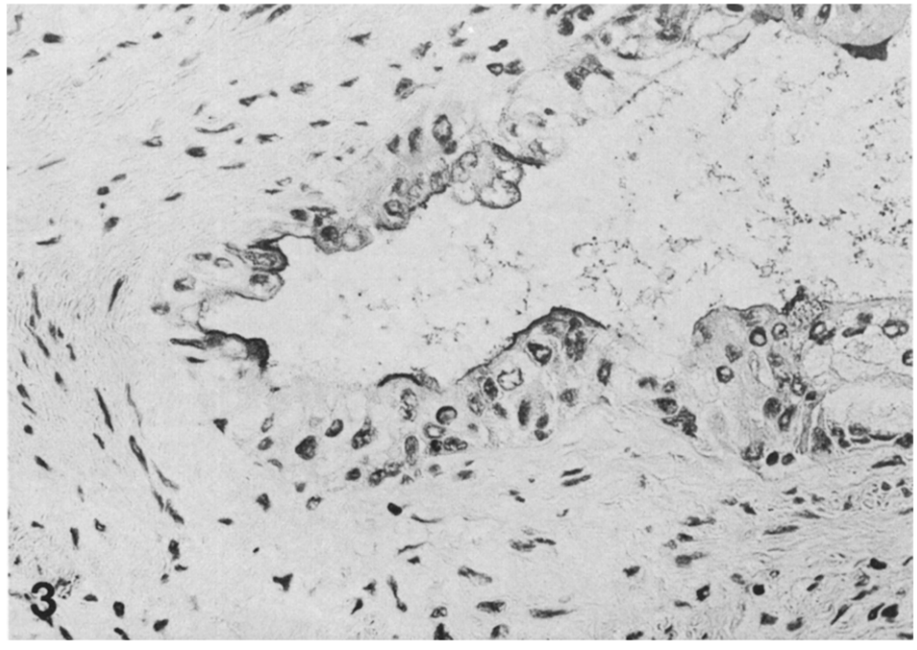

Fig. 3. CEA staining in mature teratoma with intestinal-like differentiation after chemotherapy (immunoperoxidase, ×350).

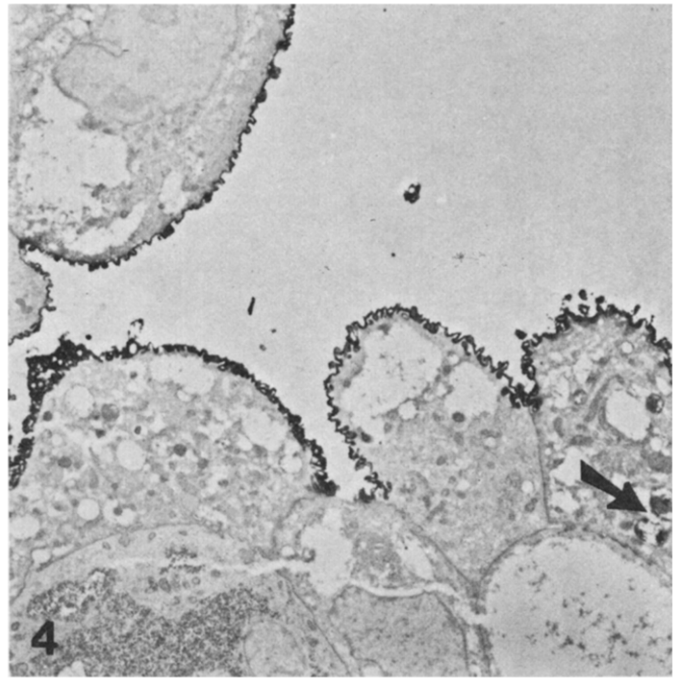

Fig. 4. Ultrastructural CEA localization along the luminal surface and in the mucus (arrow) of cylindrical cells lining cystic mature teratoma of the testis (immunoperoxidase, ×4500).

sac tumor is probably related to endodermal derivation and differentiation of these components.

However, unlike AFP, the production of CEA by yolk sac tumor must be minimal, since serum CEA levels were normal and CEA staining was only seen in a few cells. The presence of CEA on the microvilli and in the mucus of fully differentiated columnar cells in the normal colon [25] is similar to the distribution of CEA in mature teratoma with intestinal differentiation. This provides additional evidence for the mature, differentiated character of this component.

Highly organized structures with intestinal differentiation are more often found in residual retroperitoneal tumors after chemotherapy than in testicular tumors. This is reflected in the higher percentage of CEA staining in residual retroperitoneal tumors as compared to testicular tumors (60 and 37% respectively).

In conclusion, CEA staining was observed in endoderm-derived yolk sac tumor and teratomatous elements, but associated with normal serum levels. High CEA concentrations were measured in the cyst fluid of mature teratoma. Apparently, CEA diffusion in the bloodstream is too scant when this marker is produced in cystic mature teratoma. Our results question whether any substance produced by cystic mature teratoma can serve as a tumor marker at all.

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