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Selective immunolabeling of early gestational cytotrophoblast and its neoplastic counterpart by the monoclonal antibody Ber-EP4

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Summary. This report presents preliminary observations on the distribution of an epithelial surface antigen in human trophoblastic cells studied with the monoclonal antibody Ber-EP4 by means of an immunoperoxidase technique. Three cases of gestational choriocarcinoma and 20 cases of both villous and extravillous trophoblast, including one complete hydatidiform mole and one exaggerated placental site, were examined with a panel of monoclonal antibodies against epithelial antigen (Ber-EP4), cytokeratin (AE_1 and AE_3), vimentin (VIM), carcinoembryonic antigen (CEA) and epithelial membrane antigen (EMA), as well as with polyclonal antibodies against the beta subunit of human chorionic gonadotropin (hCG), human placental lactogen (hPL) and placental alkaline phosphatase (PlAP). While, as anticipated, all currently defined trophoblastic cell lines expressed low molecular weight cytokeratin only first trimester cytotrophoblastic cells and their neoplastic counterpart were found to possess the epithelial antigen detected by Ber-EP4. This distinctive property of the cytotrophoblast declined rapidly after eight weeks of gestational age but persisted in second trimester molar tissue and was prominently displayed in choriocarcinoma. By identifying the presence of cytotrophoblastic cell lines in malignancy more reliably than can be achieved with conventional panels of hCG, hPL and PlAP, Ber-EP4 has potential diagnostic utility in gestational trophoblastic disease. Our findings also suggest that the monoclonal anti-CEA antibody may be helpful in distinguishing between syncytiotrophoblastic cells and non-reactive multinucleate cells of the intermediate trophoblast.

Key words: Ber-EP4, Epithelial surface antigen, Cytotrophoblast, Syncytiotrophoblast, Intermediate trophoblast

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Introduction

The monoclonal antibody Ber-EP4 recognizes a partially formol resistant epitope on the protein moiety of two glycoproteins of 34 and 39 kD which have been found to be present on the surface and in the cytoplasm of all epithelial cells except the apical cells of stratified squamous epithelia, adult hepatocytes, and gastric parietal cells (Latza et al., 1990). It has been reported to be of particular use in the differential diagnosis of malignant mesothelioma versus adenocarcinoma (Sheibani et al., 1991). Todate, few discrepant results have been recorded (Gaffey et al., 1992). We have used Ber-EP4 for over two years as part of a screening panel for epithelial neoplasia and have observed no instances of «false» positive immunostaining as well as very few examples of fully non-reactive epithelial tumors.

In a recent case of gestational choriocarcinoma screened for epithelial markers we noted that only columns of presumptive cytotrophoblastic cells exhibited membranous staining with Ber-EP4 although all tumor cells reacted as epithelial cells for cytokeratin. This study was undertaken to determine if Ber-EP4 immunoreactivity was an exclusive property of cytotrophoblastic cells that would distinguish them from intermediate and syncytial trophoblastic cells in both normal pregnancy and gestational trophoblastic disease.

Materials and methods

Selection of cases

Twenty cases of control trophoblast and three cases of gestational choriocarcinoma were chosen from the surgical pathology files at Harlem Hospital Center. The control group comprised fifteen cases of termination of pregnancy by curettage and three cases of tubal pregnancy which were selected so that each week of gestational age between six and fourteen weeks was represented by two samples with adequate amounts of

both chorionic villi and placental site. Two second trimester cases of more advanced gestational age (about 18 weeks) were added to this group: one complete hydatidiform mole from a 17 year old primigravida and one exaggerated placental site from a 27 year old G3 P2 woman in whom the initial differential diagnosis was placental site trophoblastic tumor (PSTT).

The case of gestational choriocarcinoma that prompted this study was that of a 34 year old G4 P2 patient with a history of molar pregnancy at age 28; she had been lost to follow up until age 32 and was then repeatedly explored for suspected incomplete abortion and elevated hCG levels, with negative findings on curettage and eventual hysterectomy. She was again lost to follow up but sixteen months later an exploratory laparotomy under emergency conditions revealed extensive intraperitoneal malignancy with massive involvement of liver and intestines. The gestational choriocarcinomas of the other two cases occurred in the uterus of a 36 year old G3 P2 and that of a 37 year old G1 PO woman, respectively.

Immunohistochemistry

All tissue samples from 17 uterine curettings, 3 fallopian tubes, 2 hysterectomy specimens and one segment of small intestine had been routinely fixed in buffered formalin and embedded in paraffin. Sections were cut at 5 micron thickness and were mounted on silane-coated slides. The labeled streptavidin-biotin (LSAB) method was used with comercial kits (DAKO, Carpinteria, CA). The source and working dilutions of the primary antibodies have been listed in Table 1. Sections to be processed for AE_1, AE_3, Ber-EP4, CEA and PlAP were pre-digested for 10 minutes at room temperature with 0.05% pronase E (SIGMA, St. Louis, MO) in tris-hydrochloride buffer, pH 7.6. Endogenous peroxidase was quenched with 3% hydrogen peroxide in methanol. Incubations with primary antibodies were carried out at 4 °C overnight. Primary antibodies were substituted with non-immune serum in negative controls. Known positive controls were used only for AE_3 (skin), and for CEA and EMA (adenocarcinoma of colon). Internal controls for the remaining antibodies were adequate in most tissues. Positive immunostaining was visualized with diaminobenzidine and a light nuclear counterstain was obtained with 1% methyl green in citrate buffer, pH 5.3.

Results

The patterns of immunoreactivity of the basic cell types of choriocarcinoma and gestational trophoblast have been compared in Table 2. The only

Table 1. Immunohistochemical reagents used in the study of trophoblastic cells.

ANTIBODY	SOURCE	DILUTION	ENZYME
AE_1-cytokeratin, LMW (40, 48, 50, 56.5 kD), clone AE_1	BioGenex Laboratories	1:200	P
AE_3-cytokeratin, HMW (52, 54, 58, 65-67 kD), clone AE_3	BioGenex Laboratories	1:200	P
Ber-EP4-epithelial antigen, clone Ber-EP4	DAKO Corporation	1:150	P
VIM-vimetntin, clone V9	DAKO Corporation	1:20	
CEA-carcinoembryonic antigen, clone A5B7	DAKO Corporation	1:30	P
EMA-epithelial membrane antigen, clone E29	DAKO Corporation	1:100	
hCG-human ß-chorionic gonadotropin, rabbit anti-human	DAKO Corporation	1:16,000	
hPL-human placental lactogen, rabbit anti-human	DAKO Corporation	1:400	
PlAP-placental alkaline phosphatase, rabbit anti-human	DAKO Corporation	1:200	P
LSAB-labeled streptavidin-biotin kit	DAKO Corporation	PD	

LMW: low molecular weight; HMW: high molecular weight; P: sections pre-digested with pronase; PD: pre-diluted by manufacturer

Table 2. Distribution of Ber-EP4 immunoreactivity in comparison with that of other markers in gestational trophoblast (6-14 weeks) and gestational choriocarcinoma.

	Ber-EP4	AE_1	AE_3	VIM	CEA	EMA	hCG	hPL	PlAP	COMMENT
Gestational trophoblast (18 cases)										
CT 6-10 weeks	++	+++	-	-	-	-	-	-	±	Ber-EP4 patchy after 8 weeks
11-14 weeks	-	+++	-	-	-	-	-	-	±	Ber-EP4 negative beyond 10 weeks
ST	-	+++	F+	-	+++	±	+++	+++	++	
IT villous	-	+++	-	-	-	-	±	±	±	
extravillous	-	+++	-	-	-	-	±	+++	++	
Gestational choriocarcinoma (3 cases)										
CT	++	+++	-	-	-	-	++	F+	+	
ST	-	+++	F+	-	F++	F+	+++	F++	++	
IT	-	+++	F+	-	-	F+	++	F++	++	Tentatively identified

CT: cytotrophoblast; ST: syncytial trophoblast; IT: intermediate trophoblast; -: negative; +: weak; ++: moderate; +++: intense immunostaining; ±: mostly negative staining; F: focal immunostaining in individual cells.

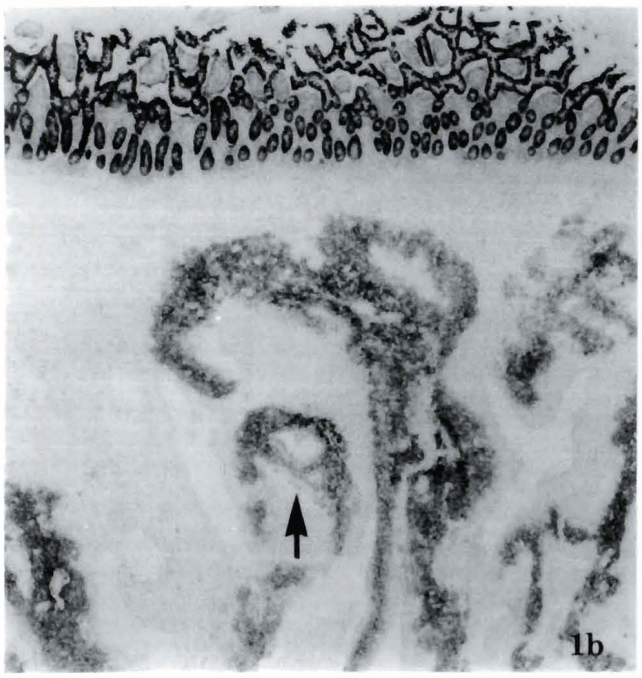
properties equally shared by all cell types of both the neoplastic and physiologic trophoblast were the diffuse cytoplasmic presence of low molecular weight cytokeratin and the absence of vimentin.

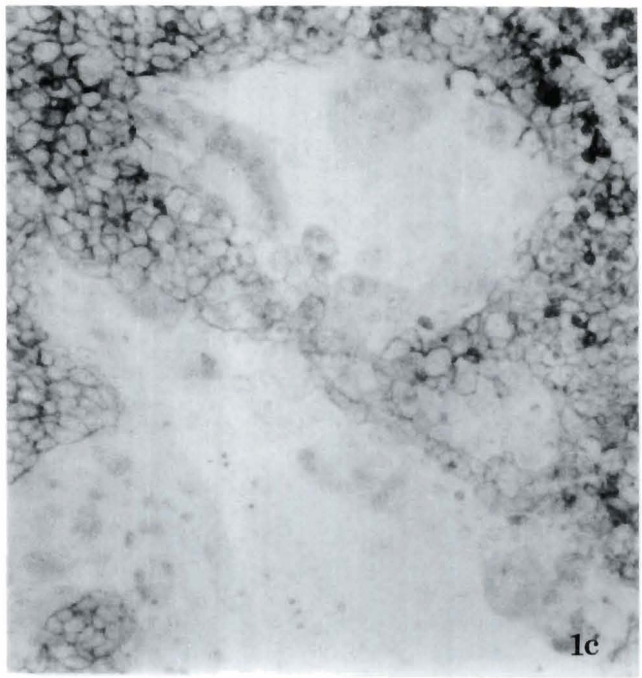
Choriocarcinoma

Staining for low molecular weight cytokeratin served as an excellent marker of scattered infiltrating choriocarcinoma cells in the periphery of the tumor (Fig. 1a). In all the three tumors Ber-EP4 singled out cohesive masses of mononucleate cells in delicate patterns of membranous staining (Figs 1b,c). While these were occasionally the smallest cells that were morphologically consistent with cytotrophoblastic equivalents they were often as large as adjacent non-reactive cells and were indistinguishable from intermediate trophoblast in stains for hCG. The remaining antibodies produced less discriminatory results. Multinucleate giant cells stained consistently for CEA, beta-hCG and hPL, variably for PlAP, and only focally and weakly for high molecular weight cytokeratin and EMA. Significantly, mononucleate cells of both cytotrophoblastic and intermediate morphology immunostained to a variable degree for beta-hCG and PlAP in all cases, and for hPL in two cases of choriocarcinoma.

Control trophoblast

Immunostaining for low molecular weight cytokeratin was clearly unrelated to gestational age and was of similar intensity in cytotrophoblastic (CT) and intermediate trophoblastic (IT) cells though seemingly less pronounced in multinucleate syncytiotrophoblastic





Figs. 1. Gestational choriocarcinoma in wall of small bowel. Intact mucosa serves as internal control for epithelial markers AE_1 and Ber-EP4. Immmunoperoxidase, counterstained with methyl green. **a**. Uniform expression of cytokeratin by choriocarcinoma. Individual infiltrating cells are clearly labeled in periphery of tumor area. x 25. **b.** Same segment of choriocarcinoma immunostained with Ber-EP4: only cohesive columns of mononuclear cells are labeled. Area marked with arrow is shown at higher magnification in c. x 25. **c.** Delicate labeling by Ber-EP4 has a characteristic cell membrane localization; the smallest mononucleate cells show also cytoplasmic staining. x 200

(ST) cells (Fig. 2). Ber-EP4 labeled exclusively the surface of CT cells of up to 10 weeks gestational age. The transition between reactive CT and non-reactive villous IT cells was abrupt (Fig. 3). This membranous immunostaining of the CT had a smooth linear character in the early weeks, became granular and less uniform at 8 weeks, increasingly discontinuous and spotty during the 9th and 10th weeks, and altogether negative in the last two weeks of the first trimester.

In contrast to choriocarcinoma, gestational CT cells were distinctly non-reactive to antibodies against beta-hCG, hPL and CEA. Strong cytoplasmic staining for CEA, hCG and hPL was characteristic of both villous and extravillous ST cells during the first trimester but declined somewhat during the second trimester. Significantly, the complete hydatidiform mole was the only second trimester tissue with intact Ber-EP4 immunoreactivity of CT cells (Fig. 4).

The distribution of hPL in IT cells varied not only in relation to gestational age but also in relation to site. Villous IT cells showed mostly negative or weak focal staining whereas extravillous IT cells in placental sites stained strongly and uniformly during the early weeks and less consistently during the second trimester. A

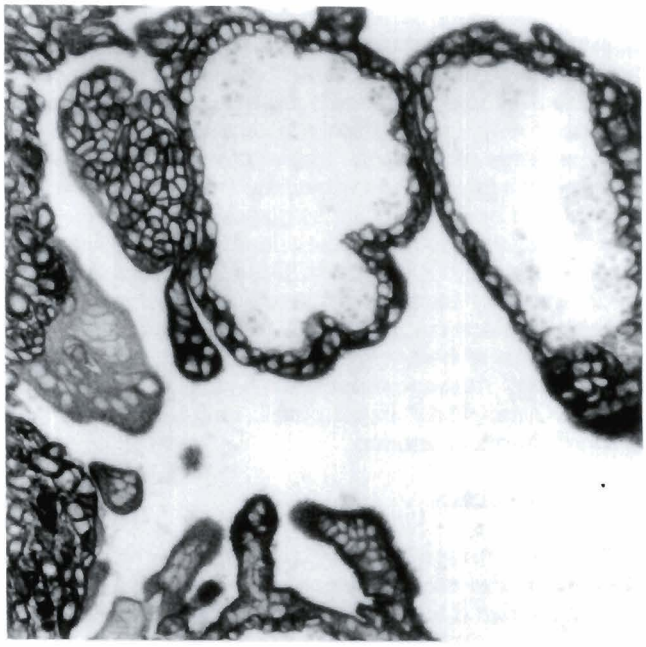


Fig. 2. Uniform localization of LMW cytokeratin in 8 weeks villous trophoblast, slightly less pronounced in multinucleate ST cells. Immunoperoxidase, counterstained with methyl green. x 200

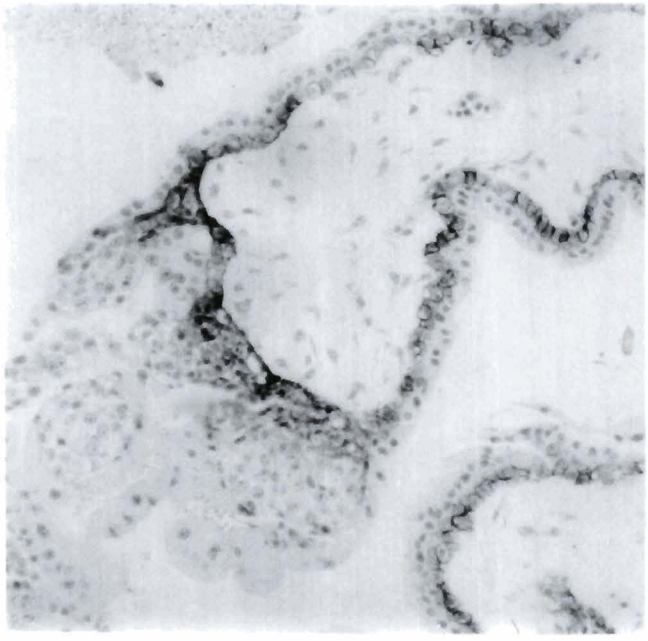


Fig. 3. Example of selective immunolocalization of Ber-EP4 antigen in CT cells at 8 weeks gestational age. Note abrupt transition between CT and villous IT cells. Immunoperoxidase, counterstained with methyl green. x 200

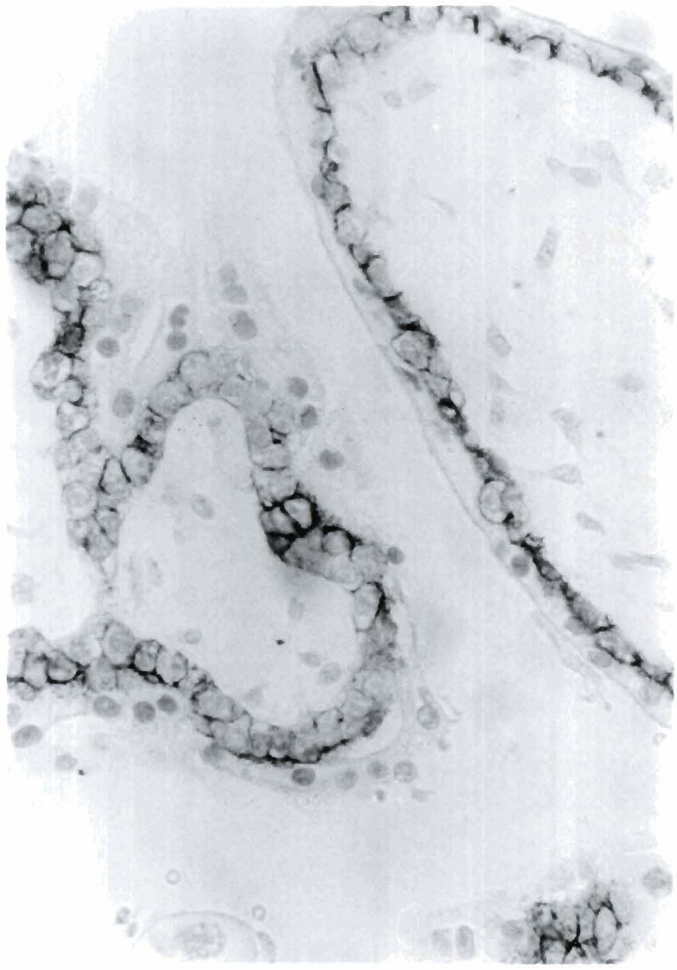


Fig. 4. Complete hydatidiform mole at 18 weeks gestational age immunostained with Ber-EP4: cell surface antigen in CT cells was found to be preserved at level of early first trimester pregnancy. Immunoperoxidase, counterstained with methyl green. x 400

heterogeneous mosaic pattern of hPL staining was particularly evident in the case of the second trimester exaggerated placental site reaction. Unlike ST cells, multinucleate IT cells did not stain for CEA. Both villous and extravillous IT cells were invariably negative for Ber-EP4 and CEA but reacted focally for beta-hCG and PlAP.

Discussion

Trophoblastic cells are currently classified into three cytohistologic variants: cytotrophoblastic, syncytiotrophoblastic, and intermediate. All three variants express low molecular weight cytokeratin (Sasagawa et al., 1986) but their functional immunophenotype varies substantially according to anatomic distribution and gestational age. The variations in the distribution of beta-hCG, hPL and PlAP among ST and IT cells throughout gestation have been described in detail by Kurman et al. (1984a). CT cells have been defined as small stem cells usually exhibiting clear cytoplasm and distinct cell borders. They have proliferative activity but lack hormone synthesis (Silverberg and Kurman, 1992). ST cells are terminally differentiated multinucleated cells which have abundant eosinophilic cytoplasm and synthesize the various placental steroid and protein hormones (Kurman, 1991). IT cells have been characterized only recently as the third distinctive variant (Kurman et al., 1984b). They are mostly mononucleate but larger than CT cells and may be spindle shaped or quite pleomorphic, including binucleate, trinucleate and multinucleate forms. Emanating in solid columns from CT cells of what are to become anchoring villi, they spread to form the extensive trophoblastic infiltrate of the placental site where they typically react with antibodies against hPL but rarely with beta-hCG (Kurman et al., 1984a). Proliferations of IT cells may produce two kinds of pseudotumoral lesions known as exaggerated placental site reaction and placental site nodule, respectively (Young et al., 1988). They may also give rise to a rare true neoplasm designated placental site trophoblastic tumor (PSTT) (Young and Scully, 1984) which in 10 to 15 per cent of cases so far studied has behaved as a malignancy indistinguishable from choriocarcinoma (Young et al., 1988).

The basic panel of antibodies against hCG, hPL and PlAP, with or without the addition of epithelial or other markers, has played a pivotal role in delineating the immunoprofiles of PSTT (Kurman et al., 1984a; Young et al., 1988; Kurman, 1991), those of complete and partial hydatidiform moles (Brescia et al., 1987), and those of germ cell and trophoblastic neoplasms (Niehans et al., 1988). Immunostaining for cytokeratin has facilitated the identification of IT cells within non-reactive decidual or mesenchymal tissue in the differential diagnosis of uterine versus ectopic pregnancy when only limited endometrial specimens were available (O'Connor and Kurman, 1988).

The addition of the monoclonal antibody Ber-EP4 to our panel has demonstrated that the epithelial antigen it recognizes is exclusively present on primitive CT cells but not on IT or ST cells and that, significantly, this distinctive property remains intact in neoplastic CT equivalents. Although CT cells have well-defined light microscopic features and are easy to recognize in the villous trophoblast because of their lack of staining for hCG and hPL their distinction from IT cells may be extremely difficult if not impossible in extravillous location and neoplasia. It is clear from our observations that neoplastic CT cells may be quite large and morphologically indistinguishable from neoplastic IT cells. On the other hand, they may show the same degree of immunostaining as IT cells for placental hormones and may also have an indistinguishable immunoprofile. The fact that CT cells in choriocarcinoma may stain positively with anti-hCG antibodies has been stressed by Kurman et al. (1984a). Hence, Ber-EP4 seems to be a unique marker of an important subset of trophoblastic stem cells that play a crucial role in trophoblastic neoplasia. The extravillous occurrence of CT cells has been traditionally regarded as pathognomomic of choriocarcinoma although the correct interpretation with conventional methods has been open to error.

We believe that Ber-EP4 may also have applications in the differential diagnosis of complete versus partial hydatidiform moles, and in that of PSTT and choriocarcinoma. While the orderly admixture of CT, IT and ST cells in choriocarcinoma recapitulates the pattern of differentiation of pre-villous trophoblast, PSTT is believed to represent a neoplastic transformation of extravillous IT at a later stage of trophoblastic maturation (Kurman, 1991). The ultrastructural findings by Duncan and Mazur (1989) who were unable to identify any primitive CT components in a case of PSTT appear to confirm the existence of an entity that is entirely composed of neoplastic IT cells. The question of whether CT and ST cells may be a minor component of PSTT (Silverberg et al., 1992) and whether intermediate forms between PSTT and choriocarcinoma do exist (Kurman, 1991) remains to be settled by further studies.

Finally, we wish to point out that the monoclonal anti-CEA antibody may be a useful discriminant between ST cells and non-reactive multinucleate IT cells of the placental site.

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