Detection of pregnancy specific β₁-glycoprotein in formalin-fixed tissues

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SUMMARY Using an enzyme-bridge immunoperoxidase method, pregnancy specific β₁-glycoprotein (PSβG) has been demonstrated in the cytoplasm of the trophoblast in several formalin-fixed tissues, namely, implantation sites of ovum, normal placentae, hydatidiform moles, invasive moles, and choriocarcinomata of uterus and testis. It is suggested that this technique may prove helpful in the detection of choriocarcinomatous elements in malignant tumours.

We have recently shown (Horne et al., 1976), by a variety of techniques that the trophoblast produces pregnancy specific β₁-glycoprotein (PSβG), first described by Bohn (1972) and referred to by him as SP₁. Electron microscopic studies of fixed first trimester placentae clearly demonstrated that, at least in vivo, the syncytiotrophoblast and not the cytotrophoblast is the source of this protein (Horne et al., 1976). Subsequent to these observations we found that it was possible to demonstrate the presence of PSβG in formalin-fixed paraffin-embedded normal placentae using an established enzyme-bridge immunoperoxidase technique (Mason et al., 1969; Streefkerk, 1972).

In this paper we present evidence for the distribution of PSβG in normal placentae, implantation sites of ovum, hydatidiform moles, invasive moles, and choriocarcinomata, including cases of choriocarcinoma arising in malignant teratoma of testis.

Material and methods

Paraffin-embedded blocks of placental tissue and trophoblastic tumours which had been stored for up to eight years were obtained from our own departmental files. Unstained sections of trophoblastic tumours (10 cases) were also kindly provided by Professor W. W. Park, University Department of Pathology, Dundee from the files of the Registry for Diseases of the Trophoblast.

In addition to 10 normal placentae (12-41 weeks' gestation), the following specimens were examined: implantation site of ovum (1-3 weeks' gestation)—3 cases; hydatidiform mole—5 cases; invasive mole—4 cases; choriocarcinoma of uterus—5 cases; and malignant teratoma of testis—5 cases.

ENZYME-BRIDGE IMMUNOPEROXIDASE METHOD

Sections (5 μm) were cut from paraffin embedded blocks of tissue which had been fixed in neutral buffered formalin. After taking the sections to water, partial blocking of the endogenous peroxidase activity was achieved by treating the sections with 0.3% hydrogen peroxide/methanol/0.74% HCl for 30 minutes at room temperature (Streefkerk, 1972; Weir et al., 1974). Thereafter the staining procedure was essentially that of Mason et al. (1969).

Test sections were incubated with a 1/40 dilution of rabbit antihuman β₁ SP₁-glycoprotein antiserum (Behringwerke AG). Following titration this dilution gave optimal staining with minimal background reactivity. The rabbit antiserum was shown to be monospecific by immunoelectrophoresis with undiluted pregnancy plasma. Control sections were incubated with 5 mM Tris/saline buffer, pH 7.6, or with normal rabbit serum in place of the specific antiserum. There was no difference between the use of buffer or normal rabbit serum in controls other than a slight increase in the non-specific staining of connective tissue when the normal rabbit serum was used.

Marked diminution but not complete loss of the positive staining was obtained by absorption of the rabbit antiserum with a freeze-dried purified PSβG (kindly provided by Dr Bohn, Behringwerke AG).
Figs 1-5  Sections stained using the enzyme-bridge immunoperoxidase technique described in the text. The test section is shown on the left and the control on the right.

Fig 1  Implanted ovum (gestational age 2-3 weeks) showing positive staining in syncytiotrophoblast (arrowed) (× 440).

Fig 2  Normal placenta showing positive staining of trophoblast (× 440).
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Fig 3  Hydatidiform mole showing dense staining of syncytiotrophoblast ($\times$ 180).

Fig 4  Invasive mole with positive staining of large trophoblast cells (arrowed) ($\times$ 440).
Results

Of three cases of implantation of ovum, two, morphologically aged 2 to 3 weeks, showed evidence of PSβG in the cytoplasm of the syncytiotrophoblast layer in 'test' but not 'control' sections (Fig. 1). The implanted ovum which was negative for PSβG appeared to be less than 2 weeks old. All normal placentae examined contained PSβG in the syncytiotrophoblast (Fig. 2) but not in the cytotrophoblast. Similarly, PSβG was detected in the syncytiotrophoblast of all hydatidiform moles (Fig. 3) and invasive moles (Fig. 4). The degree of staining of the syncytiotrophoblast in the choriocarcinoma of uterus was more variable than that observed in the normal placentae and hydatidiform moles. Figure 5 clearly shows that only the syncytiotrophoblast in this choriocarcinoma contains PSβG, the cytotrophoblast being negative for this protein.

Of the five cases of malignant teratoma of testis, two were known to contain choriocarcinomatous elements. Although, in these cases, the primary tumours were not available for study, metastases from each were examined for the presence of PSβG. These tumours, a metastasis in stomach wall and a metastasis in lung, both contained tumour cells which stained positively for PSβG.

Discussion

Pregnancy specific β1-glycoprotein (PSβG) was present in the syncytiotrophoblast of all normal placentae (12-41 weeks gestational age) in agreement with the findings of Bohn (1972) and Towler et al (1976) that this protein is present in maternal serum throughout the second and third trimesters of pregnancy. The demonstration of PSβG in the syncytiotrophoblast of implanted ova with a gestational age of 2-3 weeks clearly indicates that this protein is in fact synthesised very early in the first trimester of pregnancy. By using a sufficiently sensitive assay technique such as radioimmunoassay, it may therefore be possible to detect PSβG in the peripheral blood a few weeks after conception and so provide an alternative diagnostic test for pregnancy.

Our detection of PSβG in the syncytiotrophoblast elements of placentae and trophoblastic tumours is in keeping with our previous observation (Horne et al., 1976) that this protein is synthesised in vivo by the syncytiotrophoblast. Although PSβG may be a specific marker for the trophoblast, it is important to note that the other 'pregnancy-specific' proteins, placental lactogen (hPL) and chorionic gonadotrophin (hCG), are produced by a small percentage...
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of non-trophoblastic tumours (Weintraub and Rosen, 1971; Braunstein et al, 1973). Clearly, therefore, a much larger group of malignant tumours should be screened for evidence of PS$\beta$G production.

If PS$\beta$G is shown to be a truly specific marker for trophoblast, staining for this protein may well be useful in examining tumours thought to contain choriocarcinomatous elements. Such a use is exemplified by our demonstration of trophoblast in metastases arising from malignant teratomata of testis, the primary tumours being unavailable for study.

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