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Peritoneal ectopic lesions from women with endometriosis show abnormalities in progesterone-dependent glycan expression

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Abstract

Examination of 12 paired peritoneal ectopic and eutopic endometria for histochemical binding of *Dolichos biflorus* agglutinin, normally found in the mid–late secretory part of the cycle, showed a failure of lectin binding in 9 of 11 secretory-phase lesions although the eutopic specimens generally stained normally. This failure of glycan expression in the secretory phase may result from various anomalies, including an inability to respond to progesterone, possibly due to a lack of, or to nonfunctional, progesterone receptors, suggesting that an ectopic environment may produce changes in tissue cell biology and hormonal responsiveness compared with that of eutopic endometrium.

Our recent study (1) on the ultrastructure of ectopic lesions from a cohort of women with endometriosis showed many abnormalities, including indications of a failure to exhibit the normal process of differentiation in the secretory half of the cycle. This was manifested by an absence of glycogen deposition in basal vacuoles and of both giant mitochondria and nucleolar channel systems in the early–midsecretory phase of the cycle. There was also considerable heterogeneity in the morphology of the glands and cystic structures observed in the electron microscope. We have now investigated whether these ultrastructural changes are accompanied by a parallel failure to show biochemical differentiation, because we have also previously shown that normal endometrial tissue undergoes a progesterone-dependent biosynthesis of glycans bound by *Dolichos biflorus* lectin (2,3).

The study group originally comprised 26 women with visually and biopsy-proven endometriosis who had undergone laparoscopic excision of endometriotic deposits and endometrial curettage, as previously described (1); however, only 12 biopsies proved to contain glandular structures in the area of tissue selected for this part of the study. Menstrual cycles (28–30 days) were regular according to the history taken at the time of the outpatient appointment, and normal ovulatory pattern was confirmed by day 21 serum progesterone. The stage of endometriosis at the time of laparoscopy was determined according to the revised American Society for Reproductive Medicine scoring system (4); all were red lesions. Eutopic tissue in each case was also taken, processed, and stained simultaneously with identical protocols.

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The study was approved by the local Research Ethics Committee (ref. no. 06/Q1407/173) as well as by the Universities of Manchester and Padua. All women gave written informed consent to participate in the study.

Tissues (Table 1) were obtained from the Department of Obstetrics and Gynecology, University of Padua, Italy. All chemicals and reagents were obtained from Sigma (U.K. or Italy) unless otherwise specified. Tissue was fixed in half-strength Karnovsky fixative (2% [w/v] paraformaldehyde and 2.5% [v/v] glutaraldehyde in 0.1 mol/L phosphate buffer, pH 7.2) for 24 to 48 hours and then rinsed in buffer and transported to the U.K. Tissues were diced into 1-mm-thick slices before further processing into TAAB epoxy resin (TAAB Laboratories Equipment, Aldermaston, U.K.). For lectin histochemistry, sections were cut, mounted on APES-coated slides (5) and stained with the lectin *Dolichos biflorus* agglutinin (DBA) as previously described (3,6). Sections were assessed using a semiquantitative ranking system of analysis where staining intensity was allocated a grade from – (negative) to ++++(intense staining).

It was found that almost all of the secretory-phase ectopic specimens failed to bind DBA, which recognises the N-acetyl galactosamine sequences (7) such as GalNAc α 1,3(LFuc α 1,2)Gal β 1,3/4GlcNAc β 1- and which are normally expressed in the eutopic endometrium during the mid-late secretory phase of the cycle (2,3). The only exceptions were one case (PL020) with weak to moderate surface staining of parts of the cyst lining in a day 22 lesion, and another case (PL021) with areas of strong surface staining on a day 17 lesion which at the ultrastructural level had been shown to have a thick, clearly abnormal glycocalyx in places (1), suggesting aberrant glycosyl transferase activity, possibly resulting from abnormal distribution or function of progesterone receptors. In the lesions, DBA-positive secretions were never seen. In contrast, the DBA staining intensity in the eutopic endometrium from the same cases generally appeared normal, or even increased, with staining of both the apical glandular tissue and the secretions in the early-midsecretory phase. In the mid-late secretory phase, however, abnormalities appeared, as in PL020, coincident with a morphologic failure of differentiation, as seen in both the light and electron microscope (data not presented), with narrow tubular glands and the presence of mitotic figures. An ectopic environment thus produces changes in tissue cell biology and hormonal responsiveness compared with that of eutopic endometrium.

This absence of stain in the ectopic lesions may indicate a mesothelial origin of some glands, which was suggested also by the ultrastructural appearance of some of the lesions (1). However, progesterone resistance, together with a deficiency in 17 β -hydroxysteroid dehydrogenase type 2 in glandular epithelial cell, has also been put forward to explain some of the phenomena observed here (8,9), because endometriotic lesions have been shown to contain no progesterone receptors of isoform B and very low levels of isoform A (9). There are reports of aromatase activity, which catalyses the conversion of C₁₉ steroids to estrogens, in endometriotic lesions (10–12), and such an increase in endogenous estrogen biosynthesis may be one of the factors that maintain the growth of endometriotic lesions, mediated by estrogen receptor α -positive stromal cells (13), which have been described in peritoneal lesions (14). There has been some controversy regarding the distribution of progesterone receptors A and B in endometriosis, which have been reported to be both low (15) and unusually high (16) in endometriotic tissue. Nisolle and Donnez (17) reviewed these findings in 1997 and concluded that a persistence of progesterone receptors in both the lesion epithelium and the stroma may explain the inefficacy of medical therapy. Currently, we are engaged in mapping the distribution of progesterone receptors in formalin-fixed wax-embedded ectopic lesions to elucidate their role in the etiology of this disease.

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Details of ectopic biopsies and DBA staining ranging from negative (–) to intense (++++), with equivalent eutopic endometrium for control purposes.

TABLE 1

Sample.	Stage	Day of cycle	DBA staining of lesions	DBA in eutopic endometrium	
				Gland surface	Secretion
PL003	II	9	–	–	–
PL001	I	15	–	++	+
PL017	III	15	–	+ (patchy)	L
PL005	III	17	–	–/+	–/+
PL027	III	17	–	++++	++++
PL021	I	17	+++ (surface)	++ ^a	+
PL012	II	19	–	+++ (patchy)	–
PL028	III	20	–	+++	++
PL031	III	21	–	+++	+++
PL020	II	22	++ (surface)	–/+ ^a (patchy)	–
PL026	III	24	–	++++	+++
PL022	IV	27	–	–	–

Note: DBA = *Dolichos biflorus* agglutinin; patchy = staining restricted to occasional foci on the apical surface of glands.

^aTubular glands and mitoses present.