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Functional changes in Hofbauer cell glycobiology during human pregnancy

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ABSTRACT

Introduction: This study examines the glucose metabolism and glycosylation of villous macrophages (Hofbauer cells) over the course of pregnancy.
Materials and methods: Sections of placentae from 6 weeks to term were stained with antibodies to α-amylase, glycogen synthase, glycogen phosphorylase and glucose transporters 1 and 3 (GLUT-1 and GLUT-3) while a panel of 24 lectins was applied to resin sections from 4 weeks onwards. Hofbauer cells were identified by the binding of anti-CD 163 antibody.
Results: Little stored glycogen could be demonstrated by *Bandeiraea simplicifolia*-II agglutinin binding and, by immunocytochemistry, low levels of glycogen synthase were located within the cells, though glycogen phosphorylase expression, an enzyme releasing glucose from glycogen chains, was intense. Glucose transporter-3 but not −1 was present in the cells as has been found in other types of macrophage. Lectin histochemistry showed that many classes of glycan were present in the cells, both N and O-linked, though simple fucose residues could not be demonstrated. Glycan profiles were obtained for Hofbauer cell plasma membranes, cytosol, lysosomes and small granules. With some lectins, the intensity of binding diminished after the second trimester. Morphological changes also occurred over the course of pregnancy.
Discussion: Hofbauer cells have properties commensurate with their phagocytic activity with numerous lysosomal vacuoles and heavily glycosylated plasma membranes and granules, most evident in the first half of pregnancy. Their carbohydrate metabolism appears to rely on glucose mobilisation rather than storage as glycogen, reflecting their peripatetic mode of existence.

1. Introduction

We recently showed how, in the first trimester of pregnancy, glycogen was broken down and secreted from endometrial glands into histiotrophe and then transported to the villous placental trophoblast where it was ingested and stored again as glycogen, mainly in the cytotrophoblast cells [1]. Here we examine villous Hofbauer cells [2,3], or placental macrophages, with similar techniques in order to establish whether they have a similar pattern of glycogen uptake and usage as trophoblast, as well as investigating their glycan profile. Hofbauer cells are crucial to the growth and development of the human placenta and they are particularly evident during the first and second trimester of pregnancy [4].

Although they have a unique phenotypic profile, they share many features with other tissue macrophages including expression of Fc receptors and phagocytosis, growth following stimulation by colony-stimulating factor-1, and expression of various macrophage markers [5]. They can exhibit both M1 (classically activated or pro-inflammatory) and M2 (alternatively activated or anti-inflammatory and wound healing) characteristics depending on the local microenvironment and can switch from one to the other, while recruitment from monocyte subpopulations may also have an effect [5]. Numerous functions have been ascribed to this cell apart from phagocytosis, including fetal defence, regulation of stromal water, cytokine secretion, removal and remodelling of stromal components [4–6]. They play a role in early placental vasculogenesis [7] as well as expressing factors that stimulate both angiogenesis and hCG and hPL secretion by trophoblasts [8] and play a pivotal role in the synthesis of important mediators involved in the establishment and maintenance of pregnancy, parturition,

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lactation, local immune reactions and maternal–fetal tolerance [5]. These functions contrast strongly with those of the trophoblast and we therefore hypothesise that this will be reflected in a difference in their metabolic profile, glycogen uptake and usage and in their glycosylation. We have used immuno- and lectin histochemistry to examine these features in order to extend our knowledge of their biology and function, as well as referring to an archive of electron micrographs prepared by one of us (CJPJ) from previous studies [4,9,10] in order to evaluate glycogen deposition within cells and changes in cell morphology over the course of pregnancy.

2. Materials and methods

2.1. Immunohistochemistry

Wax-embedded samples were used as in our previous study [1], comprising placentae with gestational ages of 8, 9, 10, 12, 14, 15, 16, 18, 19 weeks and two term specimens. These were obtained with informed consent from women attending St Mary's Hospital, Manchester (NRES 08/H1010/55). Samples were fixed in buffered formalin and treated as previously described [1]. Tissue sections 5 μm thick were immunostained as before, using primary antibodies to α-amylase (1:1600) and glycogen synthase (1:100, both from Cell Signaling Technology, Danvers, MA, USA) and glycogen phosphorylase PYGL (1:750, Sigma Aldrich, Poole, UK), with biotinylated secondary antibodies (2.5 μg/ml) followed by 5 μg/ml of avidin-peroxidase in TBS after washing. Sites of antibody binding were revealed with diaminobenzidine/hydrogen peroxide (SIGMAFAST ™ Sigma Aldrich, Poole UK) and counterstained with Harris' haematoxylin. Sections were also stained with anti CD163 antibody (AbD Serotec 1: 100), with 2.5 μg/ml biotinylated goat anti-mouse secondary antibody; adjacent sections were stained with the antibody to glycogen phosphorylase PYGL (1:750). Antibodies to GLUT-1 (1:3000, a gift from FBP Wooding) and GLUT-3 (1:300, Biorbyt, Cambridge, UK) were also applied to the specimens, with 2.5 μg/ml anti-swine secondary antibodies. Sections of human pancreas were used as positive controls for α-amylase and human liver for the glycogen synthase and phosphorylase antibodies. Substitution of appropriate concentrations of non-immunised rabbit IgG for the antibody was used as a negative control.

2.2. Lectin histochemistry

The same cohort of samples from our previous study of trophoblast glycosylation [1] was used again for examination of Hofbauer cell lectin staining, comprising placentae with gestational ages of 4, 6, 7, 8, 14 and 18 weeks, and four at term. They were processed into epoxy resin as before and semithin sections 0.75 μm were cut and stained with a panel of 24 lectins as previously described [11]. Sections stained with AHA, ECA, SBA, SNA-1, MAA, PAA and WGA were treated with 0.1 units/ml neuraminidase (0.1 units/ml, type VI from *Clostridium perfringens*, Sigma) for 2 h at 37 °C before incubation in the lectin to cleave off terminal sialic acid. No trypsinisation was carried out with sections stained with BSA-II; as a control for BSA–II binding to glycogen, 30 min incubation in 1% aqueous amylase (Sigma) at 37 °C was used to predigest glycogen. Comments on the results of pretreatment with neuraminidase are restricted to those which showed a clear effect. Some sections were counterstained in Harris' haematoxylin for 30 s before washing and mounting. A semiquantitative ranking system was used to assess the results of the lectin histochemistry in which staining intensity was allocated a grade from 0 (negative) to 4 (intense) staining. Controls were carried out as before [11]. For lectin binding specificities, readers are referred to Table 2.

Table 1
Immunostaining of Hofbauer cells. (0: negative, 1: weak, 2: moderate, 3: strong, 4: intense).

Gest age (wk)	GLUT-1	GLUT-3	PYGL	Glyc synth	CD163
8	0	1–2	4	2	4
9	0	1–2	1–2	1–2	3–4
10	0	1–2	4	1–2	4
12	0	1–2	3	1–2	3
14	0	1–2	4	1–2	4
15	0	1–2	4	1–2	4
16	0	1–2	4	1–2	4
18	0	1–2	4	1–2	4
19	0	1–2	4	1–2	4
TERM	0	0–1	4	0	4
TERM	0	0–1	4	0	4

2.3. Ultrastructural evaluation

Electron micrographs from previous studies of first, second, and normal full term placentae [4,9,10] were examined specifically to identify glycogen deposits within Hofbauer cells and associated fibroblasts as well as to note features indicative of macrophage function and changes over the course of pregnancy. The Gomori method for the demonstration of acid phosphatase at the ultrastructural level has been previously described [12].

3. Results

3.1. Immunohistochemistry

As shown in Table 1, Hofbauer cells in all the specimens examined stained intensely with antibody to glycogen phosphorylase PYGL (Fig. 1a) though the α-amylase antibody showed no binding to the cells (Fig. 1b); human pancreas showed activity, however, at the same antibody concentration (Fig. 1b insert). Glycogen synthase expression was less apparent, being only weak to moderate in intensity (Fig. 1c) and was at similar levels in many fibroblasts. At term, there was staining of circulating leucocytes in fetal blood vessels but binding to Hofbauer cells was not detectable though sections of liver bound glycogen synthase antibody well (Fig. 1c insert). Serial sections stained with CD163 and compared with glycogen phosphorylase (Fig. 2a,b) confirmed that the deeply stained cells were, indeed, Hofbauer cells and not other stromal cells. GLUT-1 was not detected on the Hofbauer cells though evident on both the syncytio- and cytotrophoblast apical surfaces and endothelial cells in all specimens (Fig. 2c). Hofbauer cells did, however, express GLUT-3 as did many other components of the villus (Fig. 2d). The IgG negative control showed no staining of any tissue component (Fig. 2e).

3.2. Lectin histochemistry

The main findings have been summarised in Table 2. In early pregnancy, Hofbauer cells were abundant but nearer term their number diminished markedly in most cases [4] and they were not easy to locate. After lectin staining, the placental macrophages were recognized morphologically by their intracellular vacuoles, the walls of which were heavily glycosylated, as well large granules found within these cells and the surface membrane (Fig. 3a). Heavy staining was seen with CONA, PSA, e-PHA, ALA, MPA, DSA, STA, LEA, WFA, SNA-1, MAA and AHA after neuraminidase, and ECA, PAA and WGA - these last three both with and without neuraminidase, but apart from binding by DSA, STA and SNA-1 there tended to be a diminution in cytoplasmic staining intensity over the course of