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Glycoprotein and carbohydrate binding protein expression in the placenta in early pregnancy loss

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ABSTRACT

Glycoproteins expressed at the fetal–maternal interface have been shown to exert immunomodulating effects. Glycodelin, hCG and transferrin have been used in *in vitro* experiments as ligands to block E-selectin-mediated cell adhesion. We found that glycodelin is a strong inhibitor of the E-selectin-mediated cell adhesion with a 10^3-fold increase in potency compared to the monovalent tetrasaccharide sialyl Lewis X. HCG with distinct carbohydrate expression is also an effective selectin antagonist, whereas the potency of transferrin is low. This could indicate a possible role of glycodelin, hCG and transferrin in preventing leukocyte adhesion to the fetal trophoblast. In decidual tissue of abortion patients, glycodelin expression was significantly reduced compared to normal gestation. These results were confirmed by in situ hybridization. Moreover, glycodelin expression in endometrial cells *in vitro* could be stimulated by addition of hCG. Because hCG is down-regulated in women with abortion, we speculate that hCG could be one of the factors regulating glycodelin expression. Galectins are structurally related proteins with the ability to bind β-galactosides through a conserved carbohydrate recognition domain. Galectin-1 (gal-1) expression in the syncytiotrophoblast is down-regulated in early pregnancy loss. Gal-1 recognizes the Thomsen–Friedenreich disaccharide (Galβ1-3GalNAc-) on the syncytiotrophoblast and extravillous trophoblast. Gal-1 also inhibited trophoblast cell proliferation but did not induce apoptosis in BeWo cells. Ligation of Gal-1 on trophoblast cells may have regulatory effects on trophoblast cell differentiation. Decreased expression of Gal-1 may partly explain disturbed trophoblast differentiation during early placentation leading to early pregnancy loss.

1. Introduction

Complex carbohydrate structures on glycoproteins have an important role in different processes of cell–cell interaction (Varki, 1993). N- and O-glycans play a primary role in interaction with complementary carbohydrate recognition proteins called lectins. In so-called N-glycans, carbohydrate structures are connected via the amino acid asparagine (Asn) to the peptide backbone (Fig. 1a). O-Glycans are connected to serine (Ser) or threonine (Thr) residues in the protein (Fig. 1b). N-Glycans in addition demand a special peptide sequence consisting of Asn-X-Ser/Thr, where X can be any amino acid except proline (Pro) (Roth, 2002). N- and O-linked oligosaccharide chains of glycoproteins are known to play important roles during inflammatory processes, lymphocyte homing, and the initial stage of

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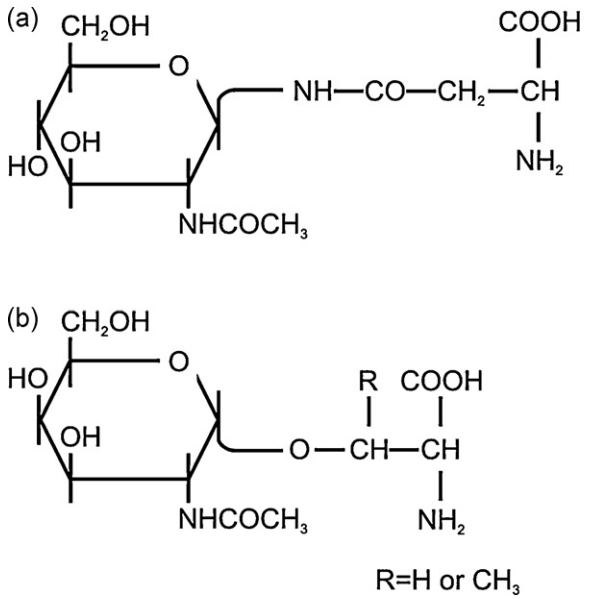

Fig. 1. (a) N-Glycosidic bound carbohydrates are connected to the peptide backbone via: GlcNAc-Asn. (b) O-Glycosidic bound carbohydrates are connected to the peptide backbone via: GalNAc-Ser/Thr.

gamete binding (Oehninger et al., 1998). In particular, Lewis antigens belong to a group of carbohydrate antigens that mediate cellular adhesion through interaction with selectins (Stahn et al., 1998, 2001). The Thomsen-Friedenreich (TF) antigen (or, more precisely, epitope Galβ1-3GalNAcα-O-) on the other hand has been known for a long time as a carcinoma-associated antigen (Cao et al., 1996). It binds galectins (Fautsch et al., 2003; Jeschke et al., 2006). Galectins (gal), are members of the mammalian β-galactoside-binding proteins which preferentially recognize Galβ1-4GlcNAc sequences of several cell surface oligosaccharides (Walzel et al., 2000).

This review describes the biological relevance of the glycosylation of glycodelin as the main product of the decidua in the first trimester of pregnancy, as well as hCG as the main glycoprotein hormone of the trophoblast throughout gestation. In addition to the Lewis-selectin system, the TF-galectin interaction is described as an additional possibility for cell–cell adhesion processes.

2. Interaction of glycodelin with the maternal immune system

Glycodelin was previously known as placental protein 14 (PP14). It is immunologically indistinguishable from chorionic 2-microglobulin (CAG-2) and placental specific 2-microglobulin (PAMG-2), and is expressed in the glandular epithelium of both the endometrium (Julkunen et al., 1986) and decidua (Toth et al., 2008). Glycodelin suppresses the cytolytic capacity of natural killer cells (NK cells) *in vitro* (Okamoto et al., 1991).

Glycodelin has a molecular weight of 28 kDa with two identical subunits held together by non-covalent bonds and a carbohydrate content of 17.5% with a unique carbohydrate configuration. Glycodelin isolated from amniotic fluid (glycodelin A) contains fucosylated LacdiNAc structures that are very unusual for mammals (Dell et al., 1995). Julkunen et al. (1988) confirmed the findings of homology between glycodelin A and β-lactoglobulin by deducing its complete amino acid sequence. A similar glycoprotein, glycodelin S, is found in seminal plasma, but it is differentially glycosylated from glycodelin A (Koistinen et al., 1996).

Glycodelin is a major reproductive glycoprotein with several functions in cell recognition and differentiation (Seppala et al., 2001) and is mainly synthesized in secretory endometrial glands (Seppala et al., 1988), gestational decidua (Bell and Bohn, 1986), seminal vesicles (Julkunen et al., 1984), the ovary (Tornehave et al., 1989) and in megakaryocytic/erythroid precursors of the bone marrow under physiological conditions (Kamarainen et al., 1994). Furthermore, glycodelin is also expressed in a variety of carcinomas including endometrial, cervical, mammary and ovarian tumours, although the precise role of this glycoprotein remains unknown (Jeschke et al., 2009a; Scholz et al., 2009).

There is substantial evidence that glycodelin A may be a mediator for immunomodulatory and immunosuppressive effects in several human tissues. Glycodelin A suppresses the release of interleukin-2 (IL-2) and interleukin-2 receptor (IL-2R) from stimulated lymphocytes (Pockley and Bolton, 1989). It also inhibits the activity of NK cells and suppresses both the allogenic mixed lymphocyte reaction and lymphocyte responsiveness to phytohemagglutinin (Bolton et al., 1987). The cytotoxic activity of NK cells is inhibited by glycodelin A in the concentration range of 1–50 μg/ml (Okamoto et al., 1991). This immunosuppressive effects of glycodelin might be depended on the blocking of E-selectin-mediated cell adhesion (Dell et al., 1995). The fucosylated LacdiNAc structures are able to bind E-selectin more effectively than sialylated Lewis X antigens.

Recently, it was demonstrated that both glycodelin A and serum glycodelin are very efficient inhibitors of the E-selectin-mediated cell adhesion *in vitro*, suggesting an important role in inhibition of selectin-mediated cell–cell adhesion (Jeschke et al., 2003b). Furthermore, we were able to demonstrate that glycodelin is a potent modulator of dendritic cell maturation. Dendritic cells are central regulators of the adaptive and innate immune response and express a regulatory receptor (DC-SIGN) that has the strongest binding affinity towards fucosylated LacdiNAc structures that are specific for glycodelin (Scholz et al., 2008). In addition, former investigations (Tomczak et al., 1996) suggested a relationship between serum levels of glycodelin and threatened abortion. Serum glycodelin levels were significantly lower in patients with threatened abortion at 10–20 weeks of gestation than in normal pregnancies.

3. Glycosylation and immunosuppressive effects of human serum and amniotic fluid hCG

Serum levels of human chorionic gonadotropin (hCG) rise exponentially after conception, with a doubling time of around 16 h in the phase of maximum rise (Bergh and Navot, 1992). HCG is a glycoprotein hormone that consists