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Glycodelin: A Major Lipocalin Protein of the Reproductive Axis with Diverse Actions in Cell Recognition and Differentiation

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Glycodelin is a glycoprotein that belongs to the lipocalin superfamily. Depending on glycosylation, glycodelin appears in various isoforms. In the uterus, glycodelin-A is the major progesterone-regulated glycoprotein secreted into uterine luminal cavity by secretory/decidualized endometrial glands. The other tissues expressing glycodelin include fallopian tubes, ovary, breast, seminal vesicle, bone marrow, and eccrine glands. Glycodelin-A potently and dose-dependently inhibits human sperm-egg binding, whereas differently glycosylated glycodelin-S from seminal plasma has no such effect. Absence of contraceptive glycodelin-A in the uterus during periovulatory midcycle is consistent with an open "fertile window." Glycodelin induced by local or systemic administration of progestogens may potentially reduce the fertilizing capacity of sperm in any phase of the menstrual cycle. Glycodelin also has immunosuppressive activity. Its high concentration at the fetomaternal interface may contribute to protection of the embryonic semiallograft. Besides being an epithelial differentiation marker, glycodelin appears to play a role in glandular morphogenesis, as transfection of glycodelin cDNA into a glycodelin-negative breast cancer cells resulted in formation of gland-like structures, restricted proliferation, and induction of other epithelial markers. These various properties, as well as the chemistry, biology, and clinical aspects of glycodelin, continue to be areas of active investigation reviewed in this communication. (*Endocrine Reviews* 23: 401–430, 2002)

Abbreviations: CHO, Chinese hamster ovary; COH, controlled ovarian hyperstimulation; ER, estrogen receptor; Gp, glycodelin peptide; hCG, human chorionic gonadotropin; hMG, human menopausal gonadotropin; HRT, hormone replacement treatment; HUVECs, human umbilical cord vein endothelial cells; IGFBP-1, IGF-binding protein-1; IUD, intrauterine device; IVF, *in vitro* fertilization; IVF-ET, IVF and embryo transfer; LGL, large granular lymphocytes; LPA, lysophosphatidic acid; MPA, medroxyprogesterone acetate; NETA, norethisterone acetate; NK, natural killer; PAEP, progesterone-associated endometrial protein; α2-PEG, pregnancy-associated endometrial α2-globulin; PEP, progestagen-dependent endometrial protein; PP14, placental protein 14; PR, progesterone receptor; PRA, progesterone receptor A; PRB, progesterone receptor B; PRE, putative glucocorticoid/progesterone response element; Sp1, promoter-specific transcription factor-1; TPA, tetradecanoylphorbol acetate; VEGF, vascular endothelial factor.

I. Introduction

IN THE 1970s and 1980s, several investigators identified and/or isolated a protein in the human placenta, amniotic fluid, pregnancy decidua, and seminal plasma and, independently of each other, gave it various names according to the site of origin or physicochemical characteristics (see Table 1). Thus, names such as placental α2-globulin (1), chorionic α2-microglobulin (2), α-uterine protein (3), placental protein 14 (PP14) (4), progestogen-dependent endometrial protein (PEP) (5), pregnancy-associated endometrial α2-globulin (α2-PEG) (6, 7), human β-lactoglobulin homolog (8–11), and progesterone-associated endometrial protein (PAEP) (12) were suggested. The name PP14 was given on the basis of its purification from the human placenta that also contained the fetal membranes, the amnion, and the chorion (4). Parts of decidua are attached to the chorion, contaminating placental tissue, and this was found to be the source of the placental PP14 (13). In immunological tests, PP14 was found to be related to α2-PEG (14), PEP (15), and α2-chorionic α2-microglobulin (16). PEP, in turn, was found to be immunologically related to α2-uterine protein (17). The N-terminal amino acid sequence was first reported for PP14 (8, 9), disclosing similarity with β-lactoglobulins from various species. The same sequence was reported for α2-PEG (10). The full primary structure of PP14 was first resolved from a decidual cDNA library (18), and essentially the same structure, with some splicing variants, was later confirmed for α2-PEG (19). Because PP14 was found to be synthesized in tissues other than endometrium and its molecular mass was not 14 kDa, the name PP14 appeared to be a misnomer.

Remarkable differences were found in the glycosylation of this glycoprotein produced in various tissues. Therefore, replacement of the misnomer PP14 by "glycodelin" was suggested by those researchers who had pioneered research on its primary structure, oligosaccharide structure, and sites of synthesis (16, 18, 20–23). The name glycodelin was also agreed upon by those investigators who had originally introduced the term PP14, PAEP, and some of the other names (4, 11, 12, 16, 24), which are likely to refer to the same protein. Therefore, in this review, the word glycodelin is used for all these proteins, and the names related to the original papers are given in Table 1 and identified in the references made to the original work throughout the text.

II. The Glycodelin and Lipocalin Genes

The glycodelin gene is 5.05 kb long and is divided into seven exons (Ref. 38; see Fig. 1). The size of the exons (or of their coding sequences) varies between 16 and 139 bp. The introns are also of a limited size (269–1371 bp). An ATACATAA sequence (considered as an equivalent of a TATA box) is localized 29 bases upstream from the cap site. Promoter-specific transcription factor-1 (Sp1)-like binding sites (GGCGGG) are present at positions −56 and −67 and in reverse orientation at −207 and −243. Four putative glucocorticoid/progesterone response elements (PREs) are found at positions −1799, −1071, −745, and −302 in the gene promoter, and two additional putative PREs are present at +1912 and +1965. Transcription is initiated 48 bp upstream from the first coding ATG of the glycodelin signal sequence.

A 400-bp sequence starting at position −2260 shows 96% homology to a sequence present in exon 4 and intron 4. This duplication corresponds to the right arm of an Alu element lying on the complementary strand. Considering the number of mutations present in this duplication and the rate of sequence evolution, it was estimated that the duplication occurred 15 million years ago (38).

Use of somatic hybrid cells and of *in situ* hybridization allowed assignment of the glycodelin gene to chromosome 9q34 (39). This localization in the region of the ABO blood group locus was consistent with the observation made in bovines of a linkage between β-lactoglobulins and the J blood group (homologous to the human blood group in this species). Indeed, sequencing of glycodelin cDNA showed 70% homology with the coding sequence of ovine β-lactoglobulin (9, 18). At the chromosomal level, these similarities extended to the exon-intron organization: both genes contain seven exons, and in all cases the exons encode the same protein domains. The Human Genome Organization's Gene Nomenclature Committee has decided that the official symbol for the glycodelin gene is *PAEP* (12).

After these initial observations, the DNA similarities were extended to a family of proteins referred to as lipocalins (48–51). About 17 of these proteins, called kernel lipocalins, share 3 conserved sequence motifs. Most are secretory proteins. Several of these serve transport functions, binding hydrophobic ligands. Although the remaining lipocalins display low sequence similarity, they share a similar ligand-binding cleft, comprised of eight antiparallel β-strands. The vertebrate lipocalin genes are characterized in most cases by a seven-exon/six-intron structure. Furthermore, most lipocalin genes are clustered at bands 33 and 34 on the long arm of human chromosome 9. In *Mus musculus*, these genes form clusters on syntenic regions of chromosomes 2 and 4. Lipocalins thus form a protein superfamily which, despite quite divergent primary sequence structure, shares a highly conserved folding conformation (49).

III. Physicochemical Properties

A. mRNA

Glycodelin is encoded by a 900-bp mRNA, which is highly similar to those of β-lactoglobulins of various species and other lipocalins. The highest similarity is in exon 2, which encodes the amino acid sequence involved in retinoic acid binding by β-lactoglobulin. Single nucleotide polymorphism (39) and *Hin*fI restriction enzyme polymorphism have been described, with a 5% frequency for allele A1 and 95% frequency for allele A2, in Finland (12). The latter has not been characterized any further to assess whether or not it represents a single nucleotide polymorphism.

Several splicing variants of glycodelin mRNA have been found in the female and male reproductive tracts (19, 45) and

TABLE 1. Original studies on various glycodelin-related proteins

Protein	Site	Author(s) (Ref.)
Placental α2-globulin	Placenta, chorion	Petrunin *et al.,* 1976 (1)
Chorionic α2-microglobulin	Chorion, sperm, endometrium, ovarian and uterine tumors	Petrunin *et al.,* 1980 (2)
α-Uterine protein	Uterus, amniotic fluid decidua	Sutcliffe *et al.,* 1978 (25)
		Sutcliffe *et al.,* 1980 (26)
		Horne *et al.,* 1982 (3)
Placenta-specific α2-microglobulin	Human fetus, seminal vesicle, placenta amniotic fluid	Tatarinov *et al.,* 1980 (24)
		Petrunin *et al.,* 1977 (27)
		Petrunin *et al.,* 1978 (28)
PEP	Endometrium	Joshi and colleagues, 1980 (5, 29)
	Nonpregnancy serum	Joshi and colleagues, 1980 and 1982 (29, 30)
PP14	Placenta, amniotic fluid, seminal plasma	Bohn *et al.,* 1982 (4)
	Pregnancy serum	Bolton *et al.,* 1983 (31)
	Seminal plasma, seminal vesicles amniotic fluid, decidua	Julkunen *et al.,* 1984 (32)
	Pregnancy serum	Julkunen *et al.,* 1985 (33)
	Seminal plasma	Bolton *et al.,* 1986 (34)
	Nonpregnant serum	Julkunen *et al.,* 1986 (35)
	Secretory endometrium	Julkunen *et al.,* 1986 (36)
	Decidua (mRNA)	Julkunen *et al.,* 1988 (18)
	cDNA sequence	Julkunen *et al.,* 1988 (18)
	Secretory endometrium (mRNA)	Julkunen *et al.,* 1990 (37)
	Gene structure	Vaisse *et al.,* 1990 (38)
	Chromosomal localization	Van Cong *et al.,* 1991 (39)
	Ovarian tumors	Riittinen, 1992 (40)
Pregnancy-associated endometrial α2-globulin	Endometrium, decidua	Bell *et al.,* 1985 (6)
	Amniotic fluid	Bell *et al.,* 1986 (41)
	Seminal plasma	Bell and Patel, 1987 (42)
	cDNA sequence decidua (mRNA)	Garde *et al.,* 1991 (19)
β-Lactoglobulin homolog	Amniotic fluid	Huhtala and colleagues, 1986 and 1987 (8, 9)
	Decidua	Bell *et al.,* 1987 (10)
	Serum	Seppälä *et al.,* 1987 (11)
PAEP	Polymorphism	Kämäräinen *et al.,* 1991 (12)
	Bone marrow (mRNA)	Kämäräinen *et al.,* 1994 (22)
Glycodelin-A	Amniotic fluid, oligosaccharide structure	Dell *et al.,* 1995 (20)
Glycodelin-S	Seminal plasma	Koistinen *et al.,* 1996 (43)
	Oligosaccharide structure	Morris *et al.,* 1996 (44)
Glycodelin	Ovary, ovarian tumors (mRNA)	Kämäräinen *et al.,* 1996 (21)
	Seminal vesicle (mRNA)	Koistinen *et al.,* 1997 (45)
	Various glands	Kämäräinen *et al.,* 1997 (23)
	Synovial sarcoma	Kämäräinen *et al.,* 1993 (46)
	Breast, breast cancer	Kämäräinen *et al.,* 1999 (47)

in hematopoietic cells of the megakaryocytic lineage (52). Some of the variants lack the coding sequences of the glycosylation sites and/or Thr-Asp-Tyr sequence, which is usually found in proteins of the lipocalin family (53).

B. Protein

Glycodelin is secreted from the decidua into amniotic fluid, which is an excellent source for purification (54, 55). In SDS-PAGE, the amniotic fluid glycodelin has a molecular mass of 28 kDa, and when studied by gel filtration, glycodelin is reported to behave as a homodimeric complex with a molecular mass of 50–60 kDa (4, 5, 7, 56). Based on molecular cloning and sequencing of its cDNA (18), glycodelin contains 180 amino acids, 18 of which correspond to the putative signal peptide. The predicted molecular mass of the mature polypeptide is 18,787 Da. The 162-residue sequence of glycodelin has extensive similarity to β-lactoglobulins of various species. There are four cystein residues (positions 66, 106, 119, and 160) responsible for intramolecular disulfide bridges in β-lactoglobulins, and they all are conserved in glycodelin. β-Lactoglobulins contain no carbohydrate, whereas glycodelin has three potential N-linked glycosylation sites, at Asn 28, Asn 63, and Asn 85 (18).

An immunoreactive form of glycodelin was detected in seminal plasma two decades ago (2, 4, 32, 43). In isoelectric focusing, the isoelectric point (pI) of seminal plasma glycodelin (5.2–5.4) is higher than that of amniotic fluid glycodelin (4.6–4.9). Its molecular mass differed slightly from that of amniotic fluid glycodelin-A (43). Whereas the molecular weight and isoelectric points of these two glycodelin isoforms are different (32, 43), they have identical tryptic peptide profiles and immunoreactivity, and their primary protein structure was the same. Seminal plasma also contains

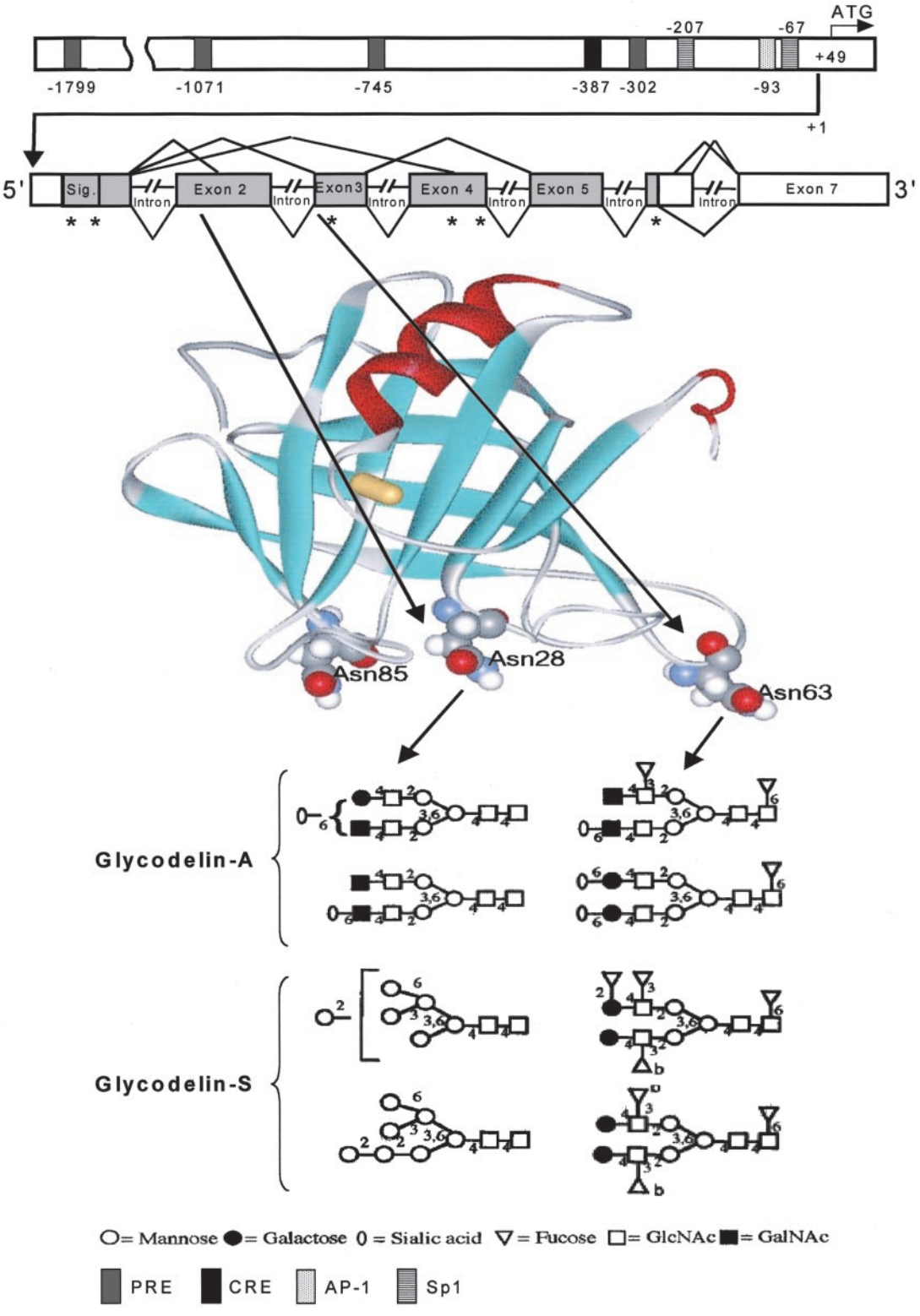

FIG. 1. Structure of glycodelin. Promoter region of the glycodelin gene (PAEP) is shown in the *upper part*. Numbering is relative to the transcriptional start site (the first base of exon 1 is number 1). ATG represents the translation-initiating codon. Sequences in the human glycodelin promoter with high degrees of homology to consensus *cis*-elements corresponding to PREs, cAMP responsive elements (CRE), activator protein-1 elements (AP-1), and Sp1 are indicated in the figure. Some of these sites have been mapped functionally by deletion or mutation of the wild-type promoter. [Adapted from C. Vaisse *et al.: DNA Cell Biol* 9:401–413, 1990 (38); R. N. Taylor and colleagues: *Am J Obstet Gynecol* 182:841–847, 2000 (68), and *J Clin Endocrinol Metab* 83:4006–4012, 1998 (69); and J. Gao *et al.: Mol Cell Endocrinol* 176:97–102, 2001 (92).] Splicing pattern of the glycodelin gene is shown *under the diagram of promoter region*. [Adapted from J. Garde *et al.: Proc Natl Acad Sci USA* 88:2456–2460, 1991 (19); C. Vaisse *et al.: DNA Cell Biol* 9:401–413, 1990 (38); and H. Koistinen *et al.: Lab Invest* 76:683–690, 1997 (45).] Exons

smaller immunoreactive forms of the glycodelin protein. Some of them are probably posttranslational cleavage products (43, 45).

C. Folding

Differentially glycosylated glycodelin isoforms share similar thermodynamic parameters of reversible denaturation, suggesting that native folding of these isoforms is not influenced by the differences in glycosylation. The tertiary structure of glycodelin was predicted using an automated Swiss-Model service that extrapolates the conformation of a target sequence from the known three-dimensional structure of related family members. The Swiss-Model-deduced tertiary structure of glycodelin was found to be similar to that of bovine β-lactoglobulin and other lipocalins (57). The circular dichroism spectrum of glycodelin was also similar to that of β-lactoglobulin (57–59). Notably, an important difference between the structures of β-lactoglobulin and glycodelin is in their glycosylation. Despite these structural similarities, the amino acid sequence of β-lactoglobulin does not contain any of the glycosylation sites present in glycodelin.

D. Glycosylation

Glycodelin contains 17.5% carbohydrate (4). The observed charge differences in the isoelectric points of amniotic fluid glycodelin-A and seminal plasma glycodelin-S suggested differences in glycosylation, because after enzymatic deglycosylation and desialylation, these two glycodelin isoforms were indistinguishable from each other on SDS-PAGE and isoelectric focusing (43). The difference in glycosylation was confirmed by lectin binding studies showing that, unlike amniotic fluid glycodelin-A, seminal plasma glycodelin-S does not react with lectins from *Wisteria floribunda* or *Sambucus nigra*. These lectins react with GalNAc and NeuAcα2–6GalNAc oligosaccharide sequences, respectively, present in glycodelin-A but absent from seminal plasma glycodelin-S.

The molecular weights of the glycodelin isoforms isolated from pregnancy serum, midterm and term amniotic fluid, first trimester decidua and term decidua, and secretory endometrium have been found to be identical (R. Koistinen, H. Koistinen, and M. Seppälä, unpublished observation). The glycodelin isoforms from all these female sources have similar isoelectric points, identical immunoreactivity, and they all react with *W. floribunda* and *S. nigra* lectins, indicating the presence of GalNAc and NeuAcα2–6GalNAc oligosaccharide sequences. This suggests that all these glycodelin isoforms in female reproductive tract and serum are similarly glycosylated and different from male glycodelin-S.

In the Swiss-Model-deduced tertiary structure of glycodelin, the glycans are located in a way that would allow them to form a clustered saccharide patch (60), *i.e.*, carbohydrates from more than one glycosylation site could form a cluster. Because the folding patterns of glycodelin-A and glycodelin-S appear to be identical, these glycoproteins provide an excellent model to study the effect of differential glycosylation on the conformational stability and function of a given glycoprotein.

Of the three putative glycosylation sites at Asn 28, Asn 63, and Asn 85, only the first two are glycosylated both in glycodelin-A and glycodelin-S (20, 44). The definitive carbohydrate structure analyses of glycodelin-A and glycodelin-S by fast atom bombardment and electrospray mass spectrometry showed that these two glycodelin isoforms are glycosylated in a completely different fashion. These remarkable differences provide evidence for gender- and tissue-specific glycosylation that may play an important role in reproduction.

1. Amniotic fluid glycodelin (glycodelin-A). The major nonreducing epitopes in the complex-type glycans are Galβ1–4GlcNAc (lacNAc), GalNAcβ1–4GlcNAc (lacdiNAc), NeuAcα2–6Galβ1–4GlcNAc (sialylated lacNAc), NeuAcα2–6GalNAcβ1–4GlcNAc (sialylated lacdiNAc), Galβ1–4(Fucα1–3)GlcNAc (blood group Lewis^x), and GalNAcβ1-(Fucα1–3)GlcNAc (lacdiNAc analog of Lewis^x) (Ref. 20 and Fig. 1). Oligosaccharides bearing sialylated lacNAc or lacdiNAc antennae at their terminal ends have been reported to manifest immunosuppressive effects by specifically blocking adhesive and activation-related events mediated by CD22, the human B cell receptor (61), and a biantennary N-linked oligosaccharide bearing Lewis^x has been reported to inhibit E-selectin-mediated adhesion (62). Because the latter fucosylated epitope is also expressed on glycodelin-A, it has been postulated (20) that the immunosuppressive effect of glycodelin is mediated via blocking of the selectin-like binding sites by this carbohydrate sequence. However, monocyte binding by glycodelin does not require glycosylation (63, 64).

2. Seminal plasma glycodelin-S. Analysis of the N-glycans of glycodelin-S by mass spectrometry revealed that the major difference from glycodelin-A is that glycodelin-S contains no sialylated glycans (44). Moreover, the glycans in glycodelin-S are unusually fucose rich, and the major complex-type structures are biantennary glycans with Lewis^x and Fucα1–2Galβ1–4(Fucα1–3)GlcNAc (Lewis^y)-type antennae. Lewis^y epitope is considered to be relatively rare in other human glycoproteins, although it has been observed in other seminal plasma-associated proteins. Interestingly, Lewis^y epitope has been associated with cancer and programmed cell death (65). Glycosylation in glycodelin-S is highly site specific, because the site at Asn-28 contains only high mannose structures, whereas Asn-63 carries only complex-type glycans.

E. Recombinant glycodelin

Glycodelin has been produced in *Pichia pastoris* and *Escherichia coli* (63, 66). Cells from Chinese hamster ovary (CHO)

encoding the mature protein are presented as *gray boxes*, 5′ and 3′ untranslated regions of exons as *white boxes*, and introns as *lines*. Part of exon 1 encoding the signal peptide (Sig.) is also shown. Splicing pattern presented *under* the figure represents the major glycodelin transcript. Codons encoding the Asn-28 and Asn-63 glycosylation sites are marked with *arrows*. Cystein residues are marked with *asterisks*. Swiss-Model-deduced tertiary structure of the glycodelin monomer is shown in the *middle*. [Adapted from H. Koistinen *et al.: FEBS Lett* 450:158–162, 1999 (57).] The S-S bridge is shown as a *cylinder*, and the atoms of asparagines of potential glycosylation sites (Asn-28, Asn-63, and Asn-85) are shown as *balls* (nitrogen as *blue* and oxygen as *red*). Representative examples of the major glycans in glycodelin-A and glycodelin-S are shown at the *bottom*. [Reproduced with permission from A. Dell *et al.: J Biol Chem* 270:24116–24126, 1995 (20), and H. R. Morris *et al.: J Biol Chem* 271:32159–32167, 1996 (44)].

and human embryonic kidney (HEK293) have been used to produce glycosylated recombinant glycodelin (67). Analyses by lectin immunoassays and fast atom bombardment mass spectrometry have shown that recombinant glycodelin from the CHO cells is devoid of any lacdiNAc-based complex-type oligosaccharide chains present in glycodelin-A (20), and most of the N-glycans in the CHO cell product are lacNAc-based complex-type glycans. Contrary to the CHO cells, the human HEK293 cells produce recombinant glycodelin that contains the same carbohydrate structures as in native glycodelin-A. This is possibly based on the activity of β1-GalNAc-transferase enzyme present in the HEK293 cells but hardly detectable in the CHO cells (67). Cultured in high glucose-containing media, the human HEK293 cells are particularly suitable for the production of the A-type recombinant glycodelin, as lowering of the glucose concentration and the addition of glucosamine results in higher relative amounts of oligomannosidic-type glycans and complex glycans with truncated antennae (67). Like glycodelin-A, recombinant glycodelin from the HEK293 cells reacts strongly with the *W. floribunda* lectin, whereas recombinant glycodelin from the CHO cells reacts only weakly, if at all.

IV. Temporal and Spatial Expression

A. Uterus

1. Endometrium and uterine flushings. Explants of human secretory endometrium and human pregnancy decidua synthesize glycodelin (13, 36), and monolayer cultures from early pregnancy decidua release glycodelin in tissue culture (70). Immunohistochemical staining has localized glycodelin to secretory endometrial glands (Fig. 2) and to the glandular epithelium of the decidua spongiosa (71–73). Except for the first days of menstrual cycle when glycodelin remains in basal glands, proliferative endometrium contains no detectable glycodelin. Studies by various groups (29, 71, 72) have shown that human endometrium contains no detectable glycodelin during the periovulatory midcycle. In a more recent study (74) employing accurate timing of ovulation, the first appearance of glycodelin in endometrium was observed as early as d 16 (LH +3) of the cycle. In most studies, the protein has been reported to appear in endometrial glands 4–5 d after ovulation, first in some glands, then gradually increasing so that 10 d after ovulation all the glands are strongly positive (72). This corresponds to the measured glycodelin concentration in endometrial tissue, increasing toward the end of an ovulatory cycle (36).

Interestingly, in the rhesus monkey, immunodetectable glycodelin shows marked similarity with that of human endometrium during the natural menstrual cycle (75). In the baboon, glycodelin is immunolocalized to endometrial mid functionalis and basal glands between d 10 and 12 post ovulation and increases markedly up to 18–25 d of pregnancy (76). As in the human, the decrease in glycodelin in baboon endometrium is associated with glandular regression during the first third of pregnancy. Glycodelin mRNA has been found in nearly all organs of the rat reproductive tract, both male and female (77) In the rat, one of the glycodelin fragments has been reported to be completely identical with exon 1 of the human glycodelin. Similar results have been obtained for pigs, cows, dogs, and mice (77). In the rat endometrium, glycodelin expression has the same pattern as in the human, localizing to epithelial cells (77). In animal species, studies on glycosylation patterns of glycodelin homologs are not available yet, and no glycodelin-A/-S distinction similar to that observed in the human has been reported. Interestingly, structural analysis of the oligosaccharides derived from schistosomes and filarial worms has revealed substantial amounts of N-linked oligosaccharides with fucosylated lacdiNAc antennae, the same rare sequences associated with glycodelin-A (20).

During human pregnancy, the glycodelin concentration in decidualized endometrium and amniotic fluid is highest at 10–18 wk (see Fig. 3; Refs. 33 and 41). Conclusive evidence for synthesis by the endometrium comes from the demonstration of glycodelin mRNA in secretory/decidualized endometrium, as well as from the studies on incorporation of labeled precursor amino acids into immunoreactive glycodelin in cultured endometrium explants (18, 36, 37) and in isolated endometrial epithelial cells (69, 78). Similar studies on the placenta have given negative results (18, 36).

Early work localized glycodelin in glandular epithelium and suggested that this protein is secreted into the uterine lumen (3). Therefore, it was not surprising to find high concentrations of glycodelin in uterine luminal fluid and uterine flushings in the secretory phase of the menstrual cycle (79, 80). As in endometrial tissues, in uterine flushings the glycodelin level is not detectable in the proliferative phase or early secretory phase. Glycodelin appears 6 d after the LH surge, and its level rises with a short doubling time (6.6–14.6 h) in the midsecretory phase (81). In late secretory phase, the glycodelin concentration in uterine flushings is over 100-fold higher than that in the corresponding serum (Table 2).

2. Uterine cervix. Experiments employing immunohistochemical staining have demonstrated glycodelin in 79% of archival paraffin-embedded sections of squamous cervical epithelia. Both normal and neoplastic specimens sometimes express glycodelin, whereas normal glandular epithelia have been found negative (83). Considering the immunosuppressive properties of glycodelin, these results are of interest with regard to the association of human papilloma virus infections and malignant transformation in the uterine cervix.

B. Fallopian tubes

Given that the fallopian tubes and the uterus are of Müllerian-tract origin, it is not surprising that glycodelin is present in fallopian tubes. The first study (82) addressed glycodelin concentration and localization at various phases of the menstrual cycle. Although no difference was observed in the glycodelin concentration between isthmic and ampullar parts of the tubes, cyclical variation in the glycodelin concentration was found in the fimbrial part, the concentration being higher in the secretory than in the proliferative phase of the cycle. A subsequent study (37) using *in situ* hybridization substantiated glycodelin synthesis in the fallopian tubal epithelium. Other investigators (84, 85) have confirmed these observations by demonstrating release of

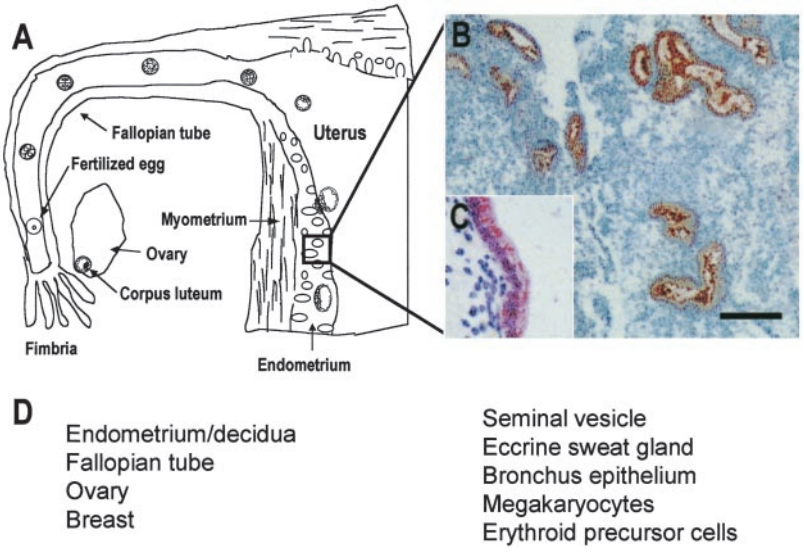

FIG. 2. Tissues synthesizing glycodelins. A, Ovary, fallopian tube, and endometrial glands. B, Localization of glycodelin in secretory endometrium by immunohistochemical staining. C, *In situ* hybridization of glycodelin mRNA in secretory endometrium. D, Tissues and cells in which glycodelin synthesis has been demonstrated.

glycodelin from cells prepared from the mucosal layer, grown in monolayer from fimbrial, proximal ampullary, and distal ampullary regions of the human fallopian tube. Remarkable inter-individual variations have been observed, and there is no difference in the glycodelin production by the cells prepared from the proximal ampullary and distal ampullary parts of the tube from the same patient. However, cells from the fimbrial region appear to be more responsive to steroid stimulation of glycodelin secretion compared with the cells prepared from either the proximal or the distal ampullary regions (84), explaining the temporal changes observed in the first study (82).

C. Ovary

Glycodelin has been identified by immunoperoxidase staining in the normal human ovary (21). In the follicular phase, glycodelin is localized to areas of stromal cell condensation in ovarian cortex, theca interna, and the granulosa. In the luteal phase, glycodelin is stained in theca interna of the corpus luteum and luteinized granulosa cells, and also in the corpus albicans and Leydig cells of the ovarian hilus. However, these staining results were not correlated with the presence of glycodelin mRNA. In the rat ovary, glycodelin mRNA is restricted to granulosa cells (77).

The biological role of ovarian glycodelin is only beginning to be uncovered. In human folliculogenesis, glycodelin protein becomes detectable in granulosa and thecal cells in late secondary follicles. There is glycodelin in follicular fluid. However, the concentrations in follicular fluid and granulosa cells are low compared with those in amniotic fluid and decidualized endometrium. Importantly, only the luteinized granulosa cells, but not the cumulus cells, express glycodelin mRNA (86). The more restricted expression of the mRNA compared with protein results from glycodelin uptake by the cumulus cells, shown by experiments involving radiolabeled glycodelin. As glycodelin appears to bind on the acrosome area of the sperm (86), the role of cumulus cells in removing this glycodelin is an interesting possibility that is currently being investigated. Granulosa cells from ovarian disorders such as the polycystic ovary syndrome remain to be studied with respect to glycodelin synthesis.

D. Seminal plasma and seminal vesicles

The first identification of immunoreactive glycodelin in seminal plasma dates back to the 1970s (1, 2, 27) and is confirmed in many subsequent studies (4, 32, 34, 42, 45). The levels in vasectomized and nonvasectomized men are similar (32). Northern blot, *in situ* hybridization, and RT-PCR analyses showed that glycodelin mRNA is expressed in seminal vesicles and ampulla of the vas deferens, not in the testis, epididymis, or the prostate. Likewise, immunohistochemical staining and *in situ* hybridization have localized glycodelin to the epithelial cells and lumen of glands in the seminal vesicles and to the ampullary part of the vas deferens (45).

E. Hematopoietic cells

Contrary to mature red blood cells, erythroid precursors of human bone marrow cells contain immunoreactive glycodelin (22). Whereas untreated K562 leukemia cells do not contain glycodelin, treatment with tetradecanoylphorbol acetate (TPA), a differentiation stimulus, can induce strong expression of glycodelin mRNA and release of the protein from these cells (22, 52).

Two differentially spliced isoforms of glycodelin have been identified in conditioned medium of TPA-stimulated K562 cells (52). Both isoforms have also been detected by RT-PCR in two human megakaryocytic cell lines and in normal human megakaryocytes and platelets. The finding of hematopoietic glycodelin within the megakaryocytic

TABLE 2. Glycodelin concentrations in reproductive tissues, fluids, and peripheral serum

Source	Glycodelin concentration	Author(s) (Ref.)
Endometrium		
Mid-proliferative	<0.1 mg/g protein	Julkunen *et al.*, 1986 (36)
Mid-secretory	7.8 mg/g protein	Julkunen *et al.*, 1986 (36)
Late secretory	23 mg/g protein	Julkunen *et al.*, 1986 (36)
Decidua		
9 wk	160 mg/g protein	Julkunen *et al.*, 1985 (33)
40 wk	0.8 mg/g protein	Julkunen *et al.*, 1985 (33)
Fallopian tube		
Proliferative phase	4.3 μg/g protein	Julkunen *et al.*, 1986 (82)
Secretory phase	16 μg/g protein	
Uterine flushing		
Proliferative phase	Not detectable	
Early secretory phase	Not detectable	
Mid-secretory phase	12 mg/liter	Li *et al.*, 1993 (80)
Amniotic fluid		
12 wk	13 mg/liter	Julkunen *et al.*, 1985 (33)
16 wk	125 mg/liter	Julkunen *et al.*, 1985 (33)
40 wk	1 mg/liter	Julkunen *et al.*, 1985 (33)
Seminal plasma	95 mg/liter	Julkunen *et al.*, 1984 (32)
Serum (men)	<20 μg/liter	
Serum (women)		
Mid-proliferative phase	<20 μg/liter	Julkunen *et al.*, 1986 (36)
Mid-luteal phase	35 μg/liter	Julkunen *et al.*, 1986 (36)
Late luteal phase	47 μg/liter	Julkunen *et al.*, 1986 (36)
Menstrual	74 μg/liter	Julkunen *et al.*, 1986 (36)
Pregnancy (12 wk)	1200 μg/liter	Julkunen *et al.*, 1985 (33)
Pregnancy (40 wk)	100 μg/liter	Julkunen *et al.*, 1985 (33)

lineage has been interpreted as an additional link between the coagulation, reproductive, and immune systems (52). Possible gender differences and regulation of glycodelin synthesis by progesterone in sites other than the female reproductive tract are obvious questions that remain to be elucidated.

F. Breast

The finding that glycodelin has significant sequence similarity with bovine β-lactoglobulin (8, 9, 18), a normal constituent of whey, suggested that glycodelin is produced in the breast. Immunohistochemistry, Northern blotting, and RT-PCR analyses have been used to study glycodelin expression in normal breast tissue. As expected, glycodelin has been identified in ductal and lobular epithelium of normal breast tissue, and also in morphologically normal parts of the breast removed from breast cancer patients (47). The presence of glycodelin in normal breast tissue leaves open questions about the significance of glycodelin in breast cancer (see *Section VII.I.3*).

G. Other tissues

Immunohistochemical staining has been employed to study glycodelin in fetal, and adult nonreproductive tissues. No significant staining has been noted in any tissue in the fetus (87). In archival adult tissues, both glycodelin and its mRNA have been found in glandular tissues, *e.g.*, in the lung and eccrine sweat glands (23). Glycodelin in these sites has not been characterized beyond immunoreactivity. Nevertheless, these findings demonstrate that none of the previously introduced tissue-specific names can correctly reflect all the sites of glycodelin synthesis.

V. Regulation of Synthesis

A. Estrogen

Glycodelin synthesis and secretion are spatially and temporally regulated (Figs. 2 and 3). Proliferative phase endometrium does not contain glycodelin, and estrogens given for hormone replacement treatment (HRT) do not increase the circulating glycodelin concentration (11, 88). However, studies have indicated an association between follicular-phase serum estradiol levels and luteal-phase serum glycodelin concentration, supporting an effect of estrogen priming (80, 88, 89). More recently, a significant positive correlation has been found between serum estradiol concentration and endometrial glycodelin staining between d 12 and 24 in the natural cycle (73). However, transcriptional regulation of the glycodelin gene promoter expressed in HeLa cells is not estrogen dependent (69). The effect of estrogen on glycodelin transcription is not direct (68), but may be mediated by endometrial cell differentiation, *e.g.*, via up-regulation of progesterone receptors.

B. Progesterone, progestogens, and antiprogestins

Several PREs have been identified in the glycodelin gene (38). This and the temporal expression in endometrium suggest that the synthesis of this glycoprotein is progesterone regulated. Glycodelin production by endometrial epithelial cells is directly up-regulated 4- to 9-fold by progestogens *in vitro* (69).

Northern blotting, metabolic labeling, and fluorography have been used to assess glycodelin mRNA and protein synthesis in endometrial tissue and cells (68). Luciferase reporter constructs transfected into HeLa cells and endometrial adenocarcinoma cells (Ishikawa cells) have been used to

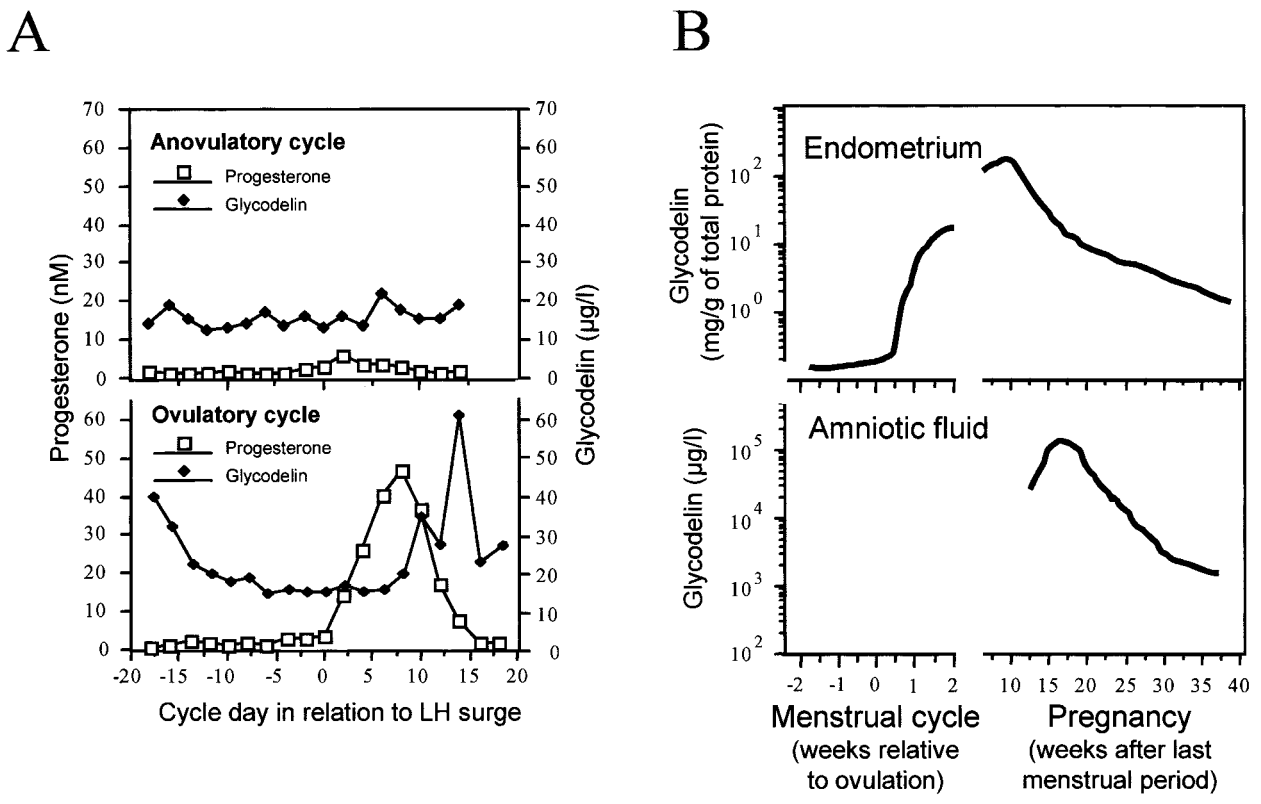

FIG. 3. Glycodelin concentrations in women. A, Serum with respect to ovulation and progesterone secretion. B, Endometrium and amniotic fluid.

determine whether progestogens could activate the glycodelin gene promoter. Progestogens stimulated glycodelin secretion in primary epithelial cell cultures. Glycodelin promoter-luciferase constructs expressing sequences 1100, 914, and 452 bp upstream of the transcriptional start site were sufficient to allow transactivation by promegestone (90), and transcriptional activity was dependent upon coexpression of progesterone receptors (PR). Here, progesterone receptor B (PRB), the predominant PR in secretory endometrial epithelium (91), was more stimulatory than progesterone receptor A (PRA) (68).

Basal glycodelin promoter activity has been localized to the region between −304 and +20 bp (92). This region contains three putative Sp1 binding sites. Mutation analysis at these sites has shown that two of them are active. In cells treated with medroxyprogesterone acetate, the promoter activity of glycodelin-luciferase construct increased 2.6- to 3-fold when cells were cotransfected with PRA or PRB. However, promoter activity was unchanged (90) or even slightly reduced (92) in cells treated with estradiol and cotransfected with estrogen receptor (ER) expression vector. Thus, whereas the effects of estrogen on glycodelin promoter function remain inconclusive, what is less controversial is that ligand-activated PRs stimulate glycodelin gene expression, mediated through the functional Sp1 sites. Curiously, this is not the first example of progestogen activation of an endometrial gene via Sp1 elements. In decidualized human endometrial stromal cells, progestogens increase the expression of Sp1 protein, which in turn appears to activate transcription of the tissue factor gene via overlapping Sp1 *cis*-elements in the gene promoter (93). Inhibitory interactions between PR complexes and Sp1 also have been observed and are postulated to reflect competition for the same transcriptional cofactors (94). On the basis of all the above findings, it can be concluded that glycodelin transcription, synthesis, and secretion by endometrial epithelial cells are stimulated by progesterone and progestogens.

The role of antiprogestins is intriguing. While having a stimulatory effect in some experimental settings *in vitro* (69) and a small, transient increase in serum levels after early pregnancy interruption (95), the antiprogestin mifepristone given to women with normal menstrual cycles in low daily doses brings about retarded endometrial histology and a significant decrease in endometrial expression of glycodelin (96).

In vivo studies also support the view that glycodelin secretion is associated with estrogen priming and progesterone action. Cyclical expression of glycodelin in endometrial tissue follows progesterone exposure (35, 36). After controlled ovarian hyperstimulation (COH) and natural cycle patients, endometrial glycodelin expression begins on cycle d 16 and increases as the luteal phase progresses (74). A significantly higher increase in glycodelin expression is found in COH cycles compared with natural cycles. From the onset, COH cycles show more glycodelin localization in a larger proportion of endometrial cells compared with natural cycles, and this increase is highly correlated with advancement of endometrial morphological dating (74). Increased expression of glycodelin in the endometria of COH cycles may be secondary to increased sex steroid and receptor levels.

A double-blind placebo-controlled study showed that micronized progesterone given over the luteal phase to women with unexplained infertility is accompanied by elevated serum glycodelin levels (97), and postmenopausal women taking estrogen-progestogen replacement treatment have elevated serum glycodelin levels at the end of progestogen treatment (11, 88). However, studies on explants from early pregnancy decidua have shown no increased glycodelin production when cultured in medium supplemented with progesterone (98). Here, prior *in vivo* exposure of decidual tissue to endogenous progesterone and human chorionic gonadotropin (hCG) may be confounding factors, so that high endogenous concentrations result in the relative insensitivity of decidual tissue to respond to the same exogenous hormones.

To determine whether subnormal glycodelin levels could be improved by progesterone treatment used to correct endometrial defects, correlation between serum glycodelin levels and histological maturation of endometrial biopsies taken during late luteal phase has been evaluated (99). Poor correlation has been found between serum glycodelin levels and histological maturation in endometrial biopsies, and there is no significant correlation between glycodelin levels and midluteal phase progesterone and estradiol. Moreover, no statistically significant differences in glycodelin values have been found depending on whether progesterone or any fertility drug is taken or not. However, such clinical observations cannot be taken as evidence against regulation of glycodelin secretion by progesterone because glycodelin secretion follows progesterone secretion by 3–4 d, explaining the lack of correlation between serum progesterone and glycodelin concentrations. Evidence shows that there is no rise in serum glycodelin concentration unless the serum progesterone rises first (35). Interestingly, in a normal ovulatory cycle, the administration of progestogens can actually decrease endogenous progesterone secretion (100), so the net effects of progestogens plus endogenous progesterone may not be as strong as expected. It is concluded that both progesterone and progestogens can be considered as regulators of glycodelin secretion in the uterus, whereas the effects of progesterone on glycodelin synthesis in sites other than the uterus remain to be elucidated.

C. Relaxin

In conception and nonconception cycles, profiles of serum relaxin and glycodelin concentrations are closely correlated, with the onset of relaxin secretion preceding that of glycodelin by 1–2 d (101). Relaxin is absent in the circulation of women without functional ovaries who become pregnant through donated embryo transfer (102, 103). Likewise, the glycodelin levels are also low or undetectable (104–106). These studies raise the possibility that relaxin may regulate glycodelin synthesis or secretion.

Published studies report discordant results on this aspect. Isolated human endometrial glandular epithelial cells have been cultured either with or without added porcine relaxin for up to 4 d (78). Cells incubated with relaxin increase the glycodelin production rate 2- to 6-fold and, as determined by solution hybridization/ribonuclease protection assay, the glycodelin mRNA concentrations increase 2- to 11-fold in cells incubated with relaxin, suggesting that relaxin activates glycodelin transcription. But this has not been found in all studies. Human relaxin has failed to stimulate *de novo* production of glycodelin and, in fact, relaxin has been found to repress progestogen-stimulated activation of the glycodelin promoter (68).

Two *in vivo* studies have also produced conflicting results. The first was a placebo-controlled study on the effect of intravaginal administration of human recombinant relaxin given for induction of labor at term pregnancy (107). Although there was a small increase in serum relaxin concentration, no difference was observed in serum glycodelin concentrations.

The second *in vivo* study using recombinant human relaxin injected to nonpregnant women for 28 d was also carried out in a double-blind and placebo-controlled fashion (101). Those women who demonstrated ovarian cyclicity showed sustained elevation of serum glycodelin levels during relaxin treatment, whereas those without ovarian cyclicity or placebo treated women showed no elevation. During relaxin administration, the elevation in glycodelin spanned over the whole menstrual cycle, including the periovulatory phase, when normally there is a nadir in serum glycodelin concentration. It is possible that, in a normal menstrual cycle, both luteal progesterone and relaxin are involved in the induction of endometrial glycodelin secretion. Progesterone may also stimulate endometrial relaxin synthesis (108, 109). The conflicting results of both *in vitro* and *in vivo* studies leave the conclusions on direct effects of relaxin on glycodelin synthesis and secretion open, because it is not known whether the increase in relaxin in any of the studies has been large enough to bring about a biological effect. Now that recombinant human relaxin is available, more clinical studies should become feasible to address the possible synergistic actions between progesterone and relaxin.

D. Human chorionic gonadotropin (hCG)

The patterns of the rise and the fall of circulating glycodelin and hCG concentrations are similar, the levels rising from implantation until pregnancy wk 10 and falling thereafter. Studies on explants of human secretory endometrium have failed to provide evidence for a stimulating role of hCG in endometrial glycodelin secretion (110), and similar results have been reported in studies on explants from early pregnancy decidua when cultured in medium supplemented with hCG (98). In baboons, between d 10 and 12 post ovulation, where the mid and apical regions of the endometrial glandular epithelium show a distinct punctate pattern, glycodelin increases between d 12 and 18 of pregnancy. The protein and mRNA expression was consistently higher in the deeper glands of the functionalis and basalis during early pregnancy. Exogenous hCG followed by estrogen and progesterone treatment in intact ovariectomized baboons upregulated glycodelin expression between d 18 and 25 post ovulation, whereas estrogen and progesterone treatment in the absence of exogenous hCG did not increase the glycodelin synthesis (111). This would suggest that hCG acts in concert with estrogen and progesterone in increasing the glycodelin secretion. Analysis of uterine flushings from

hCG-treated animals indicates that a minimum of 7 d of hCG treatment is required for glycodelin to be detectable in the uterine lumen.

E. Other

1. Clomiphene citrate. Clinical studies on women undergoing stimulation of follicular growth with clomiphene citrate, followed by preovulatory hCG administration, have shown decreasing serum glycodelin concentrations during the administration of clomiphene (112). Here, clomiphene was given during the follicular phase, during which proliferative endometrium does not contain glycodelin. A direct glycodelin synthesis-reducing effect of clomiphene has been demonstrated in normally cycling young women undergoing tubal ligation (113). Reduced synthesis of glycodelin was found in endometrium 7–9 d after the urinary LH surge. The reduction was greater after larger doses of clomiphene (100 and 150 mg), indicating that the antiestrogenic effect of clomiphene citrate is reflected as reduced endometrial glycodelin secretion.

2. Tamoxifen and mifepristone. Antiestrogen (tamoxifen) and antiprogesterone (mifepristone) appear to prolong the luteal phase when taken in combination (200 mg mifepristone and 40 mg tamoxifen) for 3 d starting on d LH +1 (114). The glycodelin levels were elevated when tamoxifen was given alone, but lower with combined treatment. Different modes of administration of these compounds show varying results. Thus, 5 mg mifepristone given once weekly did not inhibit ovulation, but it prolonged the follicular phase by 6–13 d and delayed endometrial development. This was associated with lower serum glycodelin levels (115). In another study (96), daily administration of 0.5 mg mifepristone significantly decreased endometrial expression of glycodelin. On the basis of these published studies, it is difficult to reconcile between the controversial findings with the two antiestrogens, clomiphene and tamoxifen, because the timing of drug intake relative to the normally occurring glycodelin secretion has been different.

As-yet-uncharacterized paracrine factors appear to play an important role in the regulation of glycodelin secretion from endometrial epithelial cells. Primary epithelial cells, but not stromal cells, secrete significant concentrations of glycodelin. However, when epithelial cells were placed in coculture with normal endometrial stromal cells embedded in a basement membrane extract (Matrigel, Collaborative Biomedical Products, Bedford, MA) substratum, glycodelin secretion by the epithelial cells was stimulated approximately 8-fold *in vitro* (116). As this effect was not observed in cocultures of endometrial stromal and epithelial cells grown on plastic, the data suggest that Matrigel can induce stromal factors to stimulate differentiation of the nearby epithelial cells. The factor(s) in Matrigel that can induce the epithelial cell-activating capacity of stromal cells have not yet been identified.

VI. Biological Activity

A. Effects on the immune system

1. Fetomaternal defense mechanisms. Implantation involves an intimate contact between cells from two genetically disparate organisms, the embryo (trophoblast) and the mother (decidua). Despite being a semiallograft, the human conceptus is not subjected to the laws of classical transplantation immunity. The major immune cells present at the implantation site are T cells, natural killer (NK) cells and macrophages. Macrophages and NK cells belong to the innate immune system—they require no prior exposure to antigen to react. Carbohydrate-lectin interactions are used by these cells to recognize foreign cells. B cells are absent or only few in number at the implantation site, confined to lymphoid aggregates in the basal layer (117, 118).

The first study (119) sparking research on immunosuppressive properties of glycodelin was based on observations that extracts of decidual tissue obtained from the first trimester of pregnancy showed potent immunosuppressive activity in mixed lymphocyte cultures (Table 3). This could be neutralized by treatment with a monoclonal antiglycodelin antibody. Purified glycodelin also exhibited *in vitro* immunosuppressive activity (119). Glycodelin-containing first-trimester decidual extract brings about a

TABLE 3. Various biological activities reported for glycodelin

Activity	Author(s) (Ref.)
Immune system	
Inhibition of thymidine uptake in mixed lymphocyte cultures and phytohemagglutinin-stimulated lymphocytes	Bolton *et al.,* 1987 (119) Pockley *et al.,* 1988 (120)
Decreases synthesis of IL-1 and IL-2 and release of soluble IL-2 receptors in mitogenically stimulated lymphocytes and mononuclear cell cultures	Pockley and Bolton, 1989 and 1990 (121, 122)
Inhibition of NK cell cytotoxicity	Okamoto *et al.,* 1991 (123)
Increases IL-6 production by epithelial cells from secretory endometrium	Laird *et al.,* 1994 (124)
Inhibition of T cell proliferation	Rachmilewitz *et al.,* 1999 (125)
Through binding to α2-macroglobulin potentiates the inhibitory effect on T cell proliferation	Riely *et al.,* 2000 (126)
Induction of T cell apoptosis	Mukhopadhyay *et al.,* 2001 (127)
Inhibition of chemotaxis of monocyte-like U937 cells	Vigne *et al.,* 2001 (64)
Differentiation	
Glycodelin is a marker of epithelial differentiation	Arnold and colleagues, 2001 and 2002 (116, 211)
Transfection of glycodelin cDNA induces epithelial differentiation in MCF-7 breast cancer cells	Kämäräinen *et al.,* 1997 (23)
Gamete adhesion	
Inhibition of sperm-egg binding	Oehninger *et al.,* 1995 (128)

dose-dependent suppression of the mitogenic response to phytohemagglutinin (120), inhibits the production of IL-2 from mitogenically stimulated lymphocytes, leads to a reduced IL-2 receptor release (121), and reduces IL-1 production from mitogenically stimulated mononuclear cell cultures (117).

The decidua of human pregnancy contains CD3−/CD56+ large granular lymphocytes (LGL)/NK cells. Their cytotoxicity is impaired by humoral factors (129). The K562 erythroleukemia cells are standard experimental target cells for human NK cells *in vitro*, but these cells become resistant to the NK cell-mediated lysis during induced differentiation (130). It is possible that the immunosuppressive glycodelin produced in these target cells in response to TPA-induced differentiation plays a role when the K562 cells become resistant to NK cell killing. Kinetic experiments have revealed that glycodelin-A inhibits NK cell activity (123). Here, manifestation of the suppression of NK cell cytotoxic activity by glycodelin-A requires 18 h of contact with the NK cells. In addition, glycodelin-A abrogates enhancement of IL-2-induced cytotoxicity, and the addition of glycodelin-A into IL-2-stimulated lymphocyte cultures markedly suppresses the proliferative response of LGL. Immunoblot analysis using antiphosphotyrosine antibodies has revealed that glycodelin-A inhibits IL-2-induced tyrosine phosphorylation. These results indicate that glycodelin-A suppresses the IL-2-induced functions of LGL, probably by inhibiting signal transduction after the binding of IL-2 to IL-2 receptors (123, 131). Nonglycosylated recombinant glycodelin produced in *E. coli* has been shown to bind to CD14+ monocyte lineage cells, but not to CD 20+ (B cell lineage) or CD3+ (T cell lineage) cells (63). Interestingly, immunopurified glycodelin from first-trimester amniotic fluid has been reported to function as a direct inhibitor of T cell proliferation (125), an inducer of T cell apoptosis (127), and purified glycodelin inhibits chemotaxis of monocyte-like U937 cells (64). Putative glycodelin receptors with a density of approximately 20,000/cell, a dissociation constant (K_d) of approximately 40 nM, and an apparent molecular mass of approximately 250 kDa have been described on monocytic cells (63, 64). These findings argue that glycosylation of glycodelin is not required to mediate glycodelin receptor binding or immunomodulatory effects on monocytes. This would be different from the actions glycodelin exerts on the male gametes (see *Interaction with the gametes*). Glycodelin has also been found to bind to α2-macroglubulin (126). Significantly, α2-macroglubulin potentiates the inhibitory effect of glycodelin in T cell proliferation assays.

Glycodelin dose-dependently increases IL-6 production by epithelial cells prepared from secretory endometrium, with stimulated levels reaching twice the basal values (124), although glycodelin is less effective than IL-1 at stimulating IL-6 production. These results show that IL-6 production by human endometrial epithelial cells is stimulated by other immunomodulatory peptides and suggest that glycodelin may be part of the network of peptides in the endometrium that influences embryo implantation.

B. Interaction with the gametes

It is now firmly established that complex carbohydrates play a role in cell adhesion processes, including sperm-egg binding (132–134). Data about human sperm-egg binding can be obtained using an *in vitro* system, known as the hemizona assay (135). The method involves microbisection of the human egg, resulting in the generation of two equally matched hemispheres of the zona pellucida (hemizonae). By comparing the binding of fertile sperm in the presence and absence of a test substance, it is possible to quantitate the contraceptive effect of the test substance using this internally controlled system, without bringing about fertilization and an embryo. The hemizona assay has been used to test a number of different oligosaccharides, polysaccharides, and glycoproteins for their ability to inhibit human sperm-egg binding (134, 136, 137).

Glycodelin-A is the first endogenous glycoprotein that was found to potently and dose-dependently inhibit binding of human sperm to the zona pellucida (128). This effect appears to result from interaction between glycodelin and the sperm rather than between glycodelin and the oocyte. The inhibitory activity of glycodelin-A on sperm-egg binding is virtually complete at the concentrations reported for uterine tissue and uterine flushings during the midluteal phase of a normal menstrual cycle (36, 80, 81). Data show that glycodelin-A mediates this biological activity via its unusual oligosaccharide sequences that are not present in glycodelin-S from seminal plasma (Fig. 4 and Ref. 44).

The importance of glycodelin glycosylation has been shown in comparative studies with human and hamster spermatozoa. Whereas nonglycosylated recombinant glycodelin increases sperm capacitation, glycosylated glycodelin inhibits capacitation and fertilization potential of human and hamster spermatozoa (138).

C. Endometrial receptivity

After fertilization in the fallopian tube, the zygote migrates to the uterus where it arrives around LH +4 and hatches. Implantation begins around d LH +5 with apposition of the hatched blastocyst to the luminal surface epithelium of the endometrium, followed by attachment, invasion, and anchorage of the trophoblast into the endometrial stroma (139). The limited period of uterine receptivity is estimated to span from d 18 to d 24 of a regular ovulatory cycle, *i.e.*, from LH +5 to LH +11 (140, 141).

According to most studies, significant endometrial glycodelin secretion begins 4–5 d after follicle aspiration or ovulation, at LH +5–6 (80, 142), *i.e.*, at the opening of the implantation window. Because glycodelin-A inhibits NK cell activity (123), monocytic cell chemotaxis (64), T cell proliferation (125), and induces T cell apoptosis (127) at the concentrations present in endometrial tissue and uterine fluid, it is likely that uterine glycodelin secretion plays an important role in the fetomaternal defense mechanisms (133). Interactions with the gametes on the one hand and with the immune cells on the other hand indicate that the recognition processes between immune cells and the gametes may have converged (20), whereas there appear to be differences with regards to the effects of glycosylation.

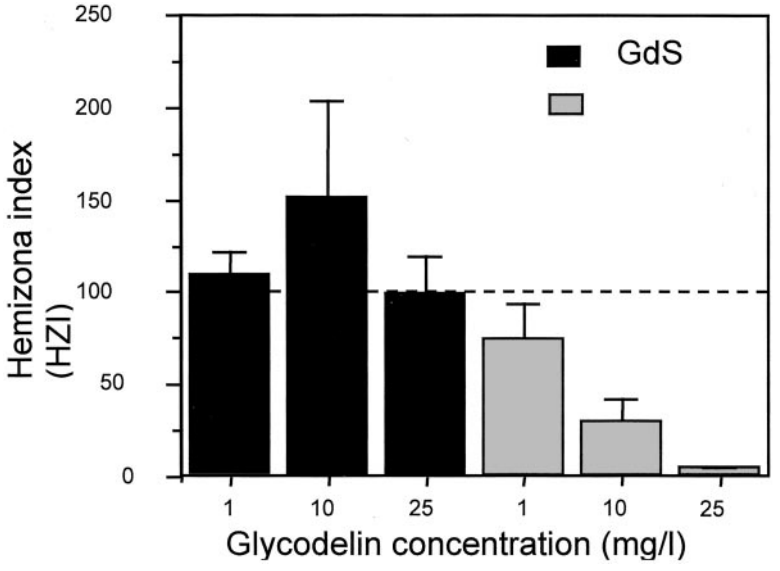

FIG. 4. Glycodelin-A inhibits human sperm-egg binding. [Reproduced with permission from H. R. Morris *et al.*: *J Biol Chem* 271:32159–32167, 1996 (44).] GdA, Glycodelin-A; GdS, glycodelin-S.

As compared with normal fertile women, patients with unexplained infertility have reduced concentrations of glycodelin in uterine flushings but not in plasma samples (143), suggesting that local glycodelin levels may play a role in uterine receptivity.

D. Glycodelin as a differentiation-related glandular morphogen

The glandular association of glycodelin expression is of particular interest because, besides in the endometrium, glycodelin has been found in glandular structures of many tissues including seminal vesicles, lobular and ductal epithelium of the breast, eccrine sweat glands, and parabronchial glands (Refs. 23 and 47 and Fig. 2). In view of its wide glandular expression, its role as a differentiation marker and in glandular morphogenesis has been addressed.

Under standard culture conditions, MCF-7 breast cancer cells do not express glycodelin. However, transfection of glycodelin cDNA into these cells causes dramatic changes in their growth behavior, with suppression of proliferation and formation of acinar structures (23). The transfected cells have lost their ability to grow on semisolid media because of apoptosis, and they exhibit up-regulated markers of organized epithelia, such as E-cadherin and cytokeratins 8 and 18. These observations suggest that glycodelin can induce epithelial differentiation. The other alterations in glycodelin cDNA-transfected cells include intracellular redistribution of β-catenin. Both the parental and the glycodelin-transfected cells express the β1 integrin subunit, whereas the parental MCF-7 cells do not express the collagen- and laminin-binding α2 integrin subunit. Interestingly, the α2 integrin appears on the surface of glycodelin-expressing cells, but the expression of another laminin-binding integrin, α6 subunit, is lost after glycodelin transfection. This is an unexpected finding, because the $\alpha 6\beta 1$ complex is a widely expressed laminin receptor in various glandular epithelia (144). Therefore, one laminin receptor appears to replace another after glycodelin expression. Taken together, the transfection of malignant cells with glycodelin cDNA can bring about changes resulting in less aggressive growth and more advanced differentiation, suggesting that glycodelin may play a role as a differentiation-related glandular morphogen.

E. Carrier functions

Glycodelin exhibits significant amino acid sequence similarity with β-lactoglobulins that bind retinoic acid and retinoids (8, 9, 18, 145). Also the folding patterns of glycodelin and bovine β-lactoglobulin are similar. However, glycodelin appears less stable than β-lactoglobulin against thermal denaturation (57), and glycodelin is a glycoprotein, whereas β-lactoglobulin is not.

Purified glycodelin-A has been used in retinoid binding experiments. Results by fluorescent quenching method show that, unlike β-lactoglobulin, glycodelin does not bind retinoic acid or retinol (57). The reasons for this include the possibility that, despite considerable similarity in the overall folding patterns between glycodelin and β-lactoglobulin, conformations of these proteins are different, as determined by their differences in the denaturation behavior (57).

VII. Clinical Perspectives

A. Circulating glycodelin levels and ovarian function

The first quantitative data on glycodelin in serum of normally menstruating women showed the highest levels during the late luteal phase, when progesterone levels were declining (30). This has been confirmed in many studies, suggesting that late-luteal-phase serum glycodelin determinations may provide quantitative information regarding functioning corpus luteum and endometrial responsiveness to progesterone (30, 146, 147). Importantly, the serum glycodelin concentration rises in ovulatory cycles only (35) but, indeed, there is no correlation between serum glycodelin and

progesterone levels. This is because the serum glycodelin level starts rising later than glycodelin becomes detectable in endometrial tissue and appears in uterine flushings. The glycodelin levels continue to rise until the onset of menstrual cycle over the period when progesterone levels decline. Despite the lack of a correlation in circulating levels, the association between glycodelin and progesterone is suggested by the observations that, in anovulatory cycles, both progesterone and glycodelin levels remain low throughout the menstrual cycle (see Fig. 3). As discussed below, the relationship between ovarian hormone production and local endometrial glycodelin synthesis shows better correlation than with peripheral serum levels.

In addition to the uterus, there are other tissues in which glycodelin synthesis has been demonstrated, and these may contribute to the circulating glycodelin pool. However, secretory and decidualized endometrium are by far the most important sources of the circulating glycodelin, demonstrated by the differences in levels between hysterectomized and nonhysterectomized postmenopausal women in response to HRT (11) and the marked elevation of serum glycodelin concentration during pregnancy (33), during which decidua and amniotic fluid have the highest concentrations (Table 2). Although their precise contribution remains to be investigated, in view of existing data, the contribution of nonreproductive tissues to serum glycodelin concentration is likely to be small.

B. Fertile window and contraceptive activity of the uterus

There is considerable variation in the timing of human fertilization during the menstrual cycle (148), mainly due to variation in the timing of ovulation. There is evidence that most fertilizations follow sexual encounters that have taken place during the 6 d that precede ovulation (149). The reasons for failure of fertilization during the postovulatory period are the lack of supernumerary ovulations and changes in the cervical mucus, hampering sperm motility. Other reasons may also exist, one of which may be glycodelin secretion. In a normal ovulatory cycle, secretion of glycodelin usually begins on the 5^{th} d after ovulation (70–72). Because of its inhibitory activity on sperm-egg binding, glycodelin-A may contribute to the contraceptive activity of the uterus during the latter half of the secretory phase (150, 151). This activity probably depends on the unique oligosaccharides present in glycodelin-A but absent from seminal plasma-derived glycodelin-S that has no contraceptive activity (44). It is possible that the absence of glycodelin-A is required for the fertile window to be open to allow the sperm to maintain their fertilizing capacity on their way to encounter the egg in the fallopian tube (150, 151).

C. Fertility and infertility

The initial studies describing cyclical changes of glycodelin in endometrial tissue raised legitimate optimism for the development of a biochemical test for endometrial function (29, 30, 35, 54, 56, 70, 112, 146).

1. Endometrium biopsy specimens. In a precisely timed study of women suffering from unexplained infertility, the glycodelin-positive areas were examined by immunohistochemical staining and semiquantitated image analysis in endometrial biopsies taken at LH +4, +7, +10 and +13 d (152). Normal and retarded endometrium were identified in 16 and 8 women, respectively. Although both groups exhibited a significant increase of the area of glycodelin staining from d LH +4 to LH +13, two-way ANOVA showed that endometrial glycodelin was significantly lower at LH +10 and LH +13 in the retarded-endometrium group. In this particular study, serum glycodelin level was also significantly lower in the women with retarded endometrial development at LH +13, and the same women had a lower concentration of cumulative saliva progesterone from LH +3 to LH +5. It is believed that subnormal uterine glycodelin expression may adversely affect uterine receptivity during implantation and early placentation (152). However, most studies show a poor correlation between serum and endometrial glycodelin levels. Furthermore, there is irregular variation of glycodelin in endometrium from different parts of the uterus (153), limiting the diagnostic value of glycodelin measurement in endometrial tissue.

2. Uterine flushings. The measurement of glycodelin in uterine luminal fluid reflects secretion into a compartment directly in contact with the endometrium, sperm, and embryos (143). The relationship between glycodelin concentration in uterine flushing and endometrial morphology in the midluteal phase has been assessed in a prospective study involving precise timing of all samples by the LH surge and histological dating and morphometric criteria of endometrial development (80). The glycodelin levels were consistently below the sensitivity of the assay when histological dating was before d LH +5, or when the glandular lumen occupied less than 20% of the biopsy. These results are compatible with the studies showing that glycodelin appears in endometrial glands when histological maturation corresponds to LH +6 (71). Whereas the serum glycodelin concentrations are not related to histological dating or morphometric analyses, or to normal or retarded endometrial development, the detectable concentrations of glycodelin in uterine flushings are significantly correlated with normal histological dating (80). Significantly lower glycodelin levels in uterine flushings have been observed on d LH +10 and LH +12 in women with unexplained infertility compared with fertile women, whereas no difference is found in the flushing samples taken on d LH +7, or in the serum samples taken on d LH +7, LH +10, and LH +12. It is concluded that, compared with fertile women, those with unexplained infertility have reduced glycodelin concentrations in uterine flushing but not in peripheral plasma and, hence, for the assessment of endometrial function, glycodelin measurement in uterine flushings may be more valuable than measurement in plasma. An additional factor in favor of local measurements is that tissues other than the endometrium may also contribute to the circulating glycodelin compartment (154).

3. Circulating levels and effects of treatment

a. Polycystic ovaries. Women with polycystic ovary syndrome often present with hyperinsulinemia and anovulatory cycles associated with infertility and early pregnancy loss. A

single-blind placebo-controlled study has shown that insulin reduction with metformin increases luteal phase serum glycodelin concentration in women with the polycystic ovary syndrome (155). At the same time, insulin-regulated IGF-binding protein-1 (IGFBP-1) concentration increases in serum. Both these proteins are produced in ovarian granulosa cells and endometrium. In endometrium, glycodelin is produced in the epithelial compartment, whereas IGFBP-1 is synthesized in the stroma. There is evidence that, in the presence of basement membrane extract, stromal factors regulate epithelial differentiation and induce glycodelin secretion in the normal endometrium (116). As there is no evidence of direct effects of insulin on glycodelin secretion, the increased glycodelin concentration in serum during metformin treatment is probably a consequence of improved endocrine milieu for the establishment and maintenance of pregnancy. Given the importance of stromal factors in controlling epithelial differentiation (116), it would be of interest to learn whether IGFBP-1 is one of the stromal factors involved in the regulation of glycodelin secretion in endometrial epithelial cells.

b. In vitro fertilization (IVF). The first report on serum glycodelin levels in pregnancies after IVF and embryo transfer [IVF-ET (33)] showed a steep rise in the levels after implantation. During clomiphene and human menopausal gonadotropin (hMG) stimulation for IVF, the glycodelin levels decline first, reaching nadir at the time of oocyte retrieval, and then they rise (112). Serum glycodelin levels have been measured in serum samples taken at 2- to 3-d intervals throughout the implantation window in women treated by IVF. No difference was found in the glycodelin levels before implantation between fertile and infertile treatment cycles, whereas the levels markedly rose once pregnancy had become established by the hMG test (156). This suggests that the rise in serum glycodelin level does not predict implantation but rather represents an endometrial response to it.

There also are other studies on the glycodelin concentration with respect to endometrial maturation and receptivity in IVF. Late-luteal-phase serum glycodelin levels were compared in conceivers and nonconceivers, and higher levels were found in the conceivers (157). However, the results were inconclusive about the greater likelihood of continuing pregnancy with higher luteal glycodelin levels. Correlation between serum glycodelin levels and endometrial biopsies has been analyzed in a study to investigate whether subnormal glycodelin levels could be improved by the same therapies that are used to correct endometrial defects. Again, no significant correlations were found (99). In a group of infertile women who had luteal phase defects but in whom follicular maturation was deemed normal, the serum glycodelin levels were compared with endometrial histology and conceptions (158). From these studies, it is concluded that serum glycodelin measurements do not accurately reflect endometrial differentiation and should not replace histological evaluation (159, 160).

Serum glycodelin levels have been measured during the implantation period in successful and unsuccessful cycles, and the effects of two treatment regimens, clomiphene citrate, and pituitary down-regulation and subsequent administration of gonadotropins compared (161). All the patients who underwent superovulation therapy exhibited an endometrial response as indicated by a rise in serum levels of glycodelin. The rise was the same regardless of either implantation or the method of ovulation induction used. These results suggest that the measurement of serum glycodelin does not reflect the local changes occurring at the implantation site, but rather reflects the overall endometrial output of glycodelin and, thus, is unlikely to provide a clinical tool for early detection of pregnancy or successful implantation.

Differences in serum glycodelin concentration have been found during the first trimester depending on the method of ovarian stimulation used (162). Three groups of pregnant women were investigated: 1) natural conception; 2) pituitary desensitization with buserelin and ovarian stimulation with human menopausal gonadotropin followed by IVF-ET; and 3) ovarian stimulation with clomiphene citrate and hMG, followed by IVF-ET. A 7- to 8-fold increase in serum glycodelin levels was observed in normal pregnancies between wk 4 and 10. This increase was earlier and less marked in group 1 and absent in group 3. These results suggest that endometrial function is altered in pregnancies achieved after ovarian stimulation.

In conclusion, the results on the significance of glycodelin as a predictor of success in IVF practice are heterogeneous and even contradictory. It needs to be re-emphasized in this context that the circulating glycodelin levels do not necessarily reflect the local situation at the implantation site because there is variation of glycodelin expression in different parts of the uterus (153, 163). Even more importantly, poor embryo quality is the most important confounding factor in clinical studies to determine the significance of glycodelin in endometrial receptivity, because 60–90% of embryos are aneuploid and this increases with age (164). Perimenopausal women receiving donor oocytes from younger women do not have reduced implantation rates, and such recipients who had no own ovarian function and received estrogen/progesterone treatment have shown rapid histological advancement of endometrial glandular elements as well as progressive $\alpha v\beta 3$ integrin and glycodelin expression (165). There is still a place for evaluation of glycodelin in properly controlled patient populations and for employing the elective single embryo transfers that have recently shown remarkable success (166).

D. Seminal plasma glycodelin and fertilization in vitro

The original observation that glycodelin inhibits sperm-egg binding made no biological sense, because seminal plasma was known to contain a high concentration of glycodelin. However, detailed characterization of glycodelin from male and female reproductive systems clarified this finding. The glycosylation differences appeared to be related to biological activity so that only the female-type glycodelin-A interfered with the gamete interaction (44). The possibility that seminal plasma from infertile men could also contain glycodelin with A-type glycosylation had to be excluded as a possible cause of male infertility (167). Interestingly, the total glycodelin concentration in seminal plasma was found to be significantly elevated in the quartile of men

with the lowest IVF rate (167). However, logistic regression analysis showed that the seminal plasma glycodelin concentration does not significantly contribute to the information obtained by a simple measurement of the sperm concentration alone. Specific lectin immunoassays have been developed (43) to determine whether increased glycodelin-A type glycosylation in seminal plasma might be associated with the failure to fertilize. No such relationship has been found (167). It is concluded that whereas glycodelin-S appears in seminal plasma at high concentrations, the significance of this molecule for fertilization remains to be investigated in comparative studies involving binding kinetics on spermatozoa of both uterine glycodelin-A and seminal plasma glycodelin-S.

E. Contraception

The sperm-egg binding inhibitory activity of glycodelin-A and its absence from the uterus during the fertile window has made glycodelin an interesting model for contraceptive research. One of the questions is whether any of the currently used contraceptives can induce "inappropriate" glycodelin secretion in endometrium during the normally glycodelin-negative, fertile midcycle.

1. Oral contraceptives. The changes in serum levels and immunohistochemical staining of glycodelin in endometrial tissue have been investigated in women taking various combined oral contraceptives (163). Although, in the normal menstrual cycle, there is a greater than 3-fold increase between low basal serum levels at midcycle and those in late luteal phase, the concentration in the pill users remained at the low basal level throughout the cycle, regardless of progestogen in the formulation. This has been noted in serial and individual samples from both monophasic and triphasic oral contraceptive preparations. However, immunohistochemical staining has shown evidence of induction of glycodelin production in endometrium. Again, serum levels of glycodelin obviously do not reflect local endometrial production of the protein.

2. Intrauterine contraception. The use of a levonorgestrel-releasing intrauterine device (IUD) is consistently accompanied by "inappropriate" expression of glycodelin-A in endometrium between d 7 and 16 of the menstrual cycle (168). Also, women using the copper-releasing IUD occasionally express "inappropriate" glycodelin at this phase of the cycle, but this is less frequent than in women with a hormone-releasing IUD. In women with a levonorgestrel IUD, *in situ* hybridization localized glycodelin mRNA in glands of midcycle endometrium, confirming the cellular site of synthesis. Thus, it is possible that the presence of glycodelin in uterine glands (and, consequently, in uterine fluid) of hormone-releasing IUD wearers may contribute to the contraceptive activity of that IUD.

3. Subdermal contraceptive implants. The contraceptive activity of subdermal levonorgestrel implants is believed to be based on interference with sperm transport, ovum maturation, ovulation, endometrial development, and expression and secretion of proteins and peptides (169–171). Endometrial glycodelin expression has been studied in 108 women wearing subdermal levonorgestrel implants (172). Overall, 80% of the specimens stained positive for glycodelin. Interestingly, 79% of the specimens with proliferative endometrial morphology were glycodelin positive, and 71% of the endometria that had inactive/weakly proliferative morphology also stained glycodelin positive. All endometrial specimens with menstrual or regenerating histological patterns contained glycodelin. Nineteen of the tissue specimens were taken during the midcycle, during which glycodelin is not normally expressed; of these, 89% stained positive for glycodelin, demonstrating that subdermal levonorgestrel implants can induce "inappropriate" glycodelin expression. In view of the inhibitory activity of glycodelin-A on sperm-egg binding, the induction of endometrial glycodelin by subdermal levonorgestrel-releasing implants may contribute to the contraceptive effect.

4. Emergency hormonal contraception. Emergency hormonal contraception has become increasingly available. There are three hormonal approaches: the first is the so-called Yuzpe regimen, which employs high doses of estrogen and progestogen; the second is the use of levonorgestrel only; and the third involves the administration of antiprogesterone, mifepristone. The mechanism by which the contraceptive effect is achieved has remained elusive, because there is no method to identify fertilization before implantation. Therefore, knowledge on the effects of treatment on markers of uterine receptivity should be of great interest.

The results of two reports (173, 174) on the effects of the Yuzpe regimen on the endometrium appear conflicting. Both studies involved two subsequent cycles of healthy ovulatory women. Results of the first (control) and the second (hormone intake) cycle were compared with each other. In the first study (173), high-dose ethinyl estradiol and norgestrel treatment given on luteal d 9 effectively suppressed glycodelin concentration in serum and uterine lavage specimens taken on luteal d 11. The second study (174) also included a control cycle and, in the second cycle, each participant took 100 μg ethinyl estradiol and 1 mg levonorgestrel on the day of the urinary LH surge and repeated the dose 12 h later. In both cycles, endometrial biopsy and vaginal sonogram were performed 8–10 d after the urinary LH surge. No significant difference was found between untreated and treated cycles in most measures, including endometrial histology, endometrial expression of β3 integrin subunit, leukemia inhibitory factor, glycodelin, or PRs, as assessed by immunohistochemical techniques. However, statistically significant changes were observed in treated cycles *vs.* controls with regard to some of the markers: a reduction in endometrial MUC-1 mucin expression, an increase in endometrial ERs, lower luteal-phase serum estrogen level, reduced endometrial thickness, and greater proportion of glandular supranuclear vacuoles (174). The discordant results in the two studies are likely due to the remarkable differences in timing between steroid administration and taking of the samples. In the first study, the interval between steroids and the samples was only 2 d, and the steroids were given when glycodelin secretion had already begun. In the latter study, each participant took the steroids on the day of the urinary LH surge, *i.e.*, when the fertile window was open, with no glycodelin

secretion, and the samples were taken 8–10 d later. Thus, whereas the first study showed immediate suppression of ongoing normal glycodelin secretion, the second study found no suppression of glycodelin secretion under the circumstances that mimic the need to take emergency contraception after unprotected sex during the fertile window. Nevertheless, both studies showed that the massive steroid doses used in the Yuzpe protocol are not without endometrial effects, whereas it remains unclear which, if any, of these changes has any relationship with the contraceptive action of the Yuzpe regimen.

F. Pregnancy and pregnancy disorders

1. Normal pregnancy. The first quantitative values of glycodelin in pregnancy serum demonstrated that, in single samples from 201 women with uncomplicated pregnancy, there was a marked increase in concentrations of both glycodelin and hCG during the first 8–9 wk gestation (30). The high levels of both proteins were maintained until 14–15 wk, after which the concentrations declined progressively and remained at low detectable levels until term gestation. This pioneering study shed optimism for a new blood test of endometrial responsiveness to products from the corpus luteum in infertility and abnormal pregnancy.

A study of 340 pregnant women expanded these observations (33). Glycodelin levels were found to be highest in maternal serum during 9–10 wk gestation, decreasing thereafter (Fig. 3). In amniotic fluid, the levels were considerably higher than in maternal serum, peaking at 16 wk (33). Importantly, whereas glycodelin and hCG show a similar pattern of circulating levels, they have a striking difference in their compartmental distribution. Thus, glycodelin is synthesized in maternal decidua and appears in high concentrations in amniotic fluid but in low concentrations in maternal serum and urine, whereas trophoblastic hCG is mainly secreted into maternal blood and urine, not to amniotic fluid. Perhaps this is the reason why glycodelin purified from the placenta (4) contains traces of hCG (55). This compartmental difference in glycodelin and hCG concentrations has been exploited for the purification of hCG-free glycodelin from midtrimester amniotic fluid (55, 175, 176).

An interesting set of observations on the regulation of glycodelin secretion during pregnancy resulted from the observation that the circulating glycodelin level is very low, if detectable at all, in a woman with Turner's syndrome, pregnant after donor embryo transfer (104). This occurred despite steroid replacement with transdermal estradiol and vaginal-micronized progesterone and normal maternal blood levels of the steroid hormones necessary for maintenance of early gestation. The observation suggested that factors other than decidual products and steroid hormones, possibly those originating from the maternal ovary, are involved in the production of glycodelin. It also demonstrated that very low serum glycodelin levels are not incompatible with a successful pregnancy. These observations were extended (105) to include pregnancies after gonadotropin down-regulation (177). However, the latter report on subnormal glycodelin levels in pregnancies after pituitary down-regulation for assisted reproduction has not been reproduced in other studies (178). Data on the glycodelin concentrations in endometrial tissue and amniotic fluid of such pregnancies should be more meaningful, but these issues remain to be addressed.

2. Pregnancy termination and disorders

a. Pregnancy termination. When termination of first trimester pregnancies is carried out with mifepristone and prostaglandin, the circulating progesterone level starts falling within 4 h after administration of mifepristone, whereas the glycodelin levels remain unaltered until 2 d, then increase (95). This has been proposed to indicate that decidual secretion of glycodelin might be independent of progesterone. However, the above example does not provide sufficient evidence for the independence. Resorption into maternal serum of uterine contents may explain such a phenomenon, similar to what occurs in Rh-immunization and has been reported for α-fetoprotein after pregnancy termination. Therefore, these results cannot be taken as evidence that ovarian and placental progesterone secretion are not specifically counteracted by mifepristone. It is noteworthy in this context, however, that mifepristone can directly increase glycodelin gene transcription *in vitro* (66). This observation needs to be confirmed.

b. Spontaneous miscarriage. Late-luteal-phase serum glycodelin levels have been compared amongst unsuccessful and successful pregnancies. No differences in midluteal progesterone and late-luteal-phase glycodelin levels have been found between the two groups (179). During pregnancy, the glycodelin serum concentration rises after implantation, but the rise does not differ according to whether pregnancy progresses normally or subsequently miscarries (161).

c. Recurrent miscarriage. A proportion of women who recurrently miscarry have an associated endometrial defect (180). Approximately one third of women with recurrent miscarriage exhibit elevated CD56+ NK cells at the implantation site (181), and the number of NK cells has been reported to be increased in peripheral blood of women with a history of recurrent miscarriage. Significantly increased NK (CD56+, CD56+/CD16+) and B cells (CD19+) have also been demonstrated (182). In view of these studies and the documented immunosuppressive properties of glycodelin (64, 119, 125, 127), a high local concentration of glycodelin suggests a role for this immunoregulatory protein at the fetomaternal interface (133).

Serum glycodelin levels have been studied during a normal preconception cycle in 50 women with a history of recurrent miscarriage, defined as at least three consecutive miscarriages (183). The blood specimens were taken on d 6–9, 12–15, and 19–23 of the cycle, and 2 d before the expected onset of the next period. Compared with the controls without a history of abortion, the glycodelin levels in the samples taken during luteal phase from habitual aborters were found to be subnormal, with no correlation to the observed luteal-phase defects determined by endometrial biopsies taken on day LH +10–12. However, not all studies support this finding (184).

Studies have also addressed the glycodelin levels in uter-

ine flushings in prepregnancy cycles of women with recurrent miscarriage. Endometrial flushings done on d LH +10 or LH +12 indicate that the difference in concentrations of glycodelin in the flushings is more significant than those seen on d LH +7. Glycodelin concentrations are significantly lower in the flushings from the recurrent miscarriage patients than those from fertile controls on both d LH +10 and LH +12 (184), indicating that patients with recurrent miscarriage have defects in endometrial maturation more frequently than normal fertile women.

The glycodelin concentration in uterine flushings may also have bearing on the subsequent obstetric outcome. In the pooled data obtained on d LH +10 and LH +12, there were significantly lower glycodelin concentrations in the uterine flushings from the patients who subsequently miscarried compared with those who had a live birth (184). The immunosuppressive properties of glycodelin (64, 119, 120, 123, 125) would predict a role for glycodelin in controlling the maternal immune system during implantation and during pregnancy (64, 133). In view of this background, it is difficult to understand how the low glycodelin levels in serum and tissue would be associated with both improved IVF success (185, 186) and an increased rate of recurrent miscarriage (183). Because the time of the LH surge varies in clinical situations and there is discordance between the published results, serum glycodelin measurements in women with recurrent miscarriage are likely to be of little clinical value, whereas detection of a low glycodelin concentration in uterine flushings could be more informative.

d. Early-pregnancy bleeding, ectopic pregnancy. Serum glycodelin concentration has been used to evaluate whether decidual function is different in women with early-pregnancy bleeding compared with normal pregnant women (187). A reference range for serum glycodelin concentrations was established on the basis of single samples from 236 women with normal pregnancy in whom gestational age was ultrasonically confirmed. Serum glycodelin levels of 128 pregnant women with vaginal bleeding between 6 and 18 wk gestation, and with verified fetal heart activity by ultrasound examination, were not different from those at the corresponding week of normal pregnancy. However, women with vaginal bleeding and subnormal glycodelin levels appeared to have a 5-fold higher risk of preterm delivery compared with women with bleeding and a normal serum glycodelin level. Thus, a subnormal serum glycodelin concentration appears to be an unfavorable sign in pregnancy associated with uterine bleeding.

Decidualized endometrium and fallopian tubal mucosa produce glycodelin (37, 82). In ectopic pregnancy, the endometrium is decidualized in response to pregnancy-related hormonal chances. The circulating glycodelin levels have been measured in 59 women with laparoscopically verified ectopic pregnancy and the levels have been compared with those in 98 women with uncomplicated pregnancy. Lower serum glycodelin levels were found in women with ectopic pregnancy. Interestingly, in ectopic pregnancy, a significant correlation was found between serum glycodelin and progesterone concentrations, whereas no such correlation is present in normal intrauterine pregnancies (188). Here, the relationship between glycodelin and progesterone may only be seen at lower progesterone concentrations. Other studies have confirmed the subnormal serum glycodelin levels in ectopic pregnancies after IVF, and it has been suggested that a combination of hCG and glycodelin analyses can distinguish between normal and abnormal implantation as early as 15 d after oocyte retrieval (189). Here, the levels of both hCG and glycodelin are subnormal in ectopic pregnancy, whereas in intrauterine pregnancies subsequently resulting in spontaneous abortion the glycodelin levels are similar to those in a normal pregnancy. Although these observations are of theoretical interest, they have little significance for the clinical diagnosis of ectopic pregnancy that can effectively be identified by ultrasound and hCG determination.

3. Late pregnancy disorders. In pregnancies with intrauterine growth retardation the serum glycodelin levels tend to be lower than in normal pregnancy, reaching significance at 36–38 wk gestation (31). No correlation has been found between glycodelin levels and the sex of the child, parity, maternal age, or birth weight (190). Interestingly, some women with preeclampsia show an increased glycodelin concentration during advancing pregnancy, compared with the normal pattern of decreasing values (31). In a study on gestational hypertension, the serum glycodelin levels were significantly higher than in normal pregnancy, being above the normal median in 80% of cases and above the normal 90th percentile in 38% of cases (191), but again, not all studies have confirmed this (192).

G. Endometriosis

As in eutopic endometrium, glycodelin expression can be cyclical in endometriosis (46, 87, 193). During the menstrual cycle, the temporal appearance of glycodelin in endometriosis correlates with its appearance in eutopic endometrium (87), suggesting that endometriosis tissue can respond to the same hormonal stimuli as the eutopic endometrium. However, in eutopic endometrium, immunohistochemical staining patterns of glycodelin exhibit better concordance with the plasma levels than in endometriotic lesions. Glycodelin expression in endometriosis lesions is often discordant with that of synchronously sampled eutopic endometrium in that the lesions can be glycodelin positive also during the proliferative phase and midcycle, whereas in proliferative eutopic endometrium, glycodelin is consistently undetectable (36, 71). Therefore, it is not surprising that, in women with advanced endometriosis, the serum glycodelin concentration also is elevated during midcycle (194), a time when the circulating levels are normally undetectable. These results agree with the findings that endometriosis lesions may have different endocrine dependency due to their low concentration of steroid receptors (195) or altered expression of receptor isoforms (196), resulting in a relative resistance to progestogens. Whether the elevated glycodelin levels during midcycle have bearing on endometriosis-related infertility remains to be investigated.

In many cases of endometriosis, the cellular localization of glycodelin is comparable to that in eutopic endometrium: epithelial cells express the protein, whereas stromal cells are

negative. In endometriosis, positive immunostaining is restricted to apical secretory granules of the epithelioid cells. There also are lesions in which some glandular cells contain glycodelin but other glands do not. The positive staining is observed more frequently (60%) in superficial lesions with *in situ* secretory differentiation compared with deep lesions, of which only 36% contained detectable glycodelin (193). Moreover, atrophic implants rarely express glycodelin. These findings are in agreement with the suggestion that superficial endometrial lesions come first and are more consistent biochemically with the eutopic endometrium. Perhaps another reason for the more frequent expression of glycodelin in superficial lesions is their closer contact with peritoneal fluid that contains cyclically higher progesterone concentrations than serum does (197).

Like the progesterone concentration (197), the glycodelin concentration in peritoneal fluid is at least 10-fold higher than the corresponding serum concentration, and the peritoneal fluid glycodelin concentrations are highest in the late luteal phase. Superficial endometriosis appears to secrete glycodelin mainly toward the peritoneal fluid, whereas endometriomas and deeply infiltrating endometriosis lesions secrete it mainly into blood (198). In endometriosis, the peritoneal fluid glycodelin concentration has been found to correlate with the pelvic area of endometriosis lesions, whereas the serum glycodelin concentration correlates with the presence and the volume of endometriomas and deeply infiltrating endometriosis lesions (198). In patients with more advanced stages of disease, the elevated serum glycodelin levels at midcycle decline after surgery or medical treatment (194). Interestingly, the concentrations also decrease after GnRH agonist therapy, oral contraceptives, danazol and medroxyprogesterone acetate, and they are lower after GnRH agonist than after danazol (194, 198, 199). In conclusion, most studies show that serum glycodelin level is elevated in patients with advanced endometriosis, particularly those with deeply infiltrating lesions, and the level declines after treatment. Assessment of clinical utility of these observations would need to be focused on the midcycle, during which glycodelin secretion from eutopic endometrium is minuscule. So far, data on the sensitivity of glycodelin in the diagnosis and monitoring treatment of endometriosis are not available.

H. Postmenopausal hormone replacement treatment (HRT)

Endometrial and circulating glycodelin levels are very low in postmenopausal women. The first study reporting serum glycodelin levels in response to cyclical HRT showed that, in women with an intact uterus, serum glycodelin level does not increase during estrogen treatment alone. However, when estrogen is given in combination with progestogen after estrogen priming, the glycodelin level rises toward the end of the estrogen-progestogen phase. No similar increase is found in hysterectomized women (11), indicating that the endometrium is the major source of circulating glycodelin. There is considerable variation in the responsiveness of individual glycodelin values of different women, some of them exhibiting a 2.5-fold rise in response to cyclical HRT, whereas other women with an intact uterus show no rise at all (11). In estrogen-primed cycles, treatment with a conventional dose of levonorgestrel produces a greater rise than medroxyprogesterone acetate (MPA). However, there is overlap between the basal and the highest values in one third of the women with an intact uterus (11).

In a study of 12 months' continuous percutaneous estrogen monotherapy of postmenopausal women, compared with no treatment or placebo, serum glycodelin level remained undetectable (200). In contrast, continuous estrogen/progestogen (norethisterone acetate, NETA) therapy showed a small but significant increase in serum glycodelin concentration over the treatment period of 24 months. In 15 premenopausal and 30 postmenopausal women taking 3 different doses of 17β-estradiol cyclically combined with NETA, the highest levels were found on d 1 after the onset of menstrual bleeding and on d 7 during NETA administration (201). The authors could estimate the area under serum glycodelin curve from one or two blood samples taken at optimal times. They found that the higher the estradiol dose in relation to the fixed NETA dose, the higher the serum glycodelin response. It was concluded that serum glycodelin concentration might reflect the quantitative development of the endometrium in the secretory phase in both premenopausal and postmenopausal women. Sequentially combined estrogen/levonorgestrel HRT has been compared with continuously combined estradiol/cyproterone acetate HRT or placebo. All the women taking the continuous combination HRT or placebo had inactive or atrophic endometrium, and their serum glycodelin levels were low (202). Those women who took cyclical HRT showed cyclical elevation of serum glycodelin concentration that was significantly correlated with the levels of 17β-hydroxysteroid dehydrogenase and isocitrate hydrogenase. Moreover, serum glycodelin levels at 3 months and 2 yr of HRT were significantly correlated, reflecting long-term validity of the measurement. Also, the longitudinal effects of continuous combined estradiol plus cyproterone acetate HRT have been addressed (203). Serum glycodelin concentration increased after the first month of treatment at the occurrence of bleeding. Again, the glycodelin concentration was significantly correlated with the serum concentration of estradiol. However, a longer use of continuous combined HRT resulted in low glycodelin levels. Semiquantitative estimation of the glandular area in endometrial tissue expressing glycodelin during the late progestogenic phase was significantly higher compared with the early progestogenic phase (204).

Serum glycodelin concentrations have been measured in postmenopausal women receiving 1 yr of 100–200 μg of percutaneous estradiol for 28 d each month and 10 mg oral MPA given during the last 12 d of estrogen administration (205). A small but significant increase in glycodelin levels was found after estrogen plus MPA, but because of substantial overlap between basal and post-progestogen serum glycodelin concentrations, the authors concluded that the glycodelin levels are not useful for monitoring postmenopausal replacement therapy with percutaneous estrogen and oral MPA.

Four different HRT regimens have been used in 36 cycles in which serum glycodelin was measured on d 1, 15, 19, and 29. Among the 18 women with premature ovarian failure,

there were 6 associated with Turner's syndrome and 12 with idiopathic premature ovarian failure. In the cycles with standard HRT, the glycodelin levels were similar to those of the natural cycle, *i.e.*, there was cyclical elevation toward the end of the cycle when combined estrogen/progestogen were taken. Interestingly, the subjects with Turner's syndrome did not show elevated serum glycodelin levels in response to HRT, whereas those with idiopathic premature ovarian failure had elevated levels on d 29 of the cycle. The levels appeared to depend on the dosage, as they were lower when either the doses of estradiol valerate were reduced to one third, or the doses of progesterone were reduced to one fifth, of the standard HRT. In keeping with the results of a previous study (84), the authors conclude that the circulating glycodelin level is dependent not only on progesterone but also on adequate estrogen priming (90).

Glycodelin levels also have been measured in uterine flushings of 30 postmenopausal women randomized to receive either continuous combined estradiol valerate and norethisterone HRT or tibolone HRT daily (206). The glycodelin concentrations in uterine flushings were considerably increased after the administration of both types of HRT. The continuous combined estradiol valerate and norethisterone HRT was associated with a greater increase (150-fold) in the glycodelin concentration in the flushings compared with tibolone HRT (a 6-fold increase). This difference was related to secretory histology of the endometrium. Remarkably, women with a higher post-HRT uterine glycodelin concentration were more likely to have irregular bleeding. As postulated previously, these authors suggest that increased glycodelin in uterine flushings may suggest endometrial stimulation of some form and predict the predilection to irregular bleeding.

The effect of two different doses of progestogen on endometrial morphometric parameters and glycodelin secretion has been studied in postmenopausal women receiving estrogen for HRT (207). The starting levels of glycodelin were low. Although both 25 and 50 μg of progestogen in combination with estrogen induced a significant increase in glycodelin concentration in uterine flushing, no between-group difference in the rise was observed. The addition of cyclical progestogen to estrogen therapy has been found effective in reducing the risks of endometrial cancer. Besides withdrawal bleeding, the induction of differentiation likely confers a protective effect of progestogens against endometrial cancer, supported by the induction of glycodelin secretion.

The above clinicopathological studies shed light for understanding the glycodelin concentrations in long-term, combined estrogen-progestogen administration. Such treatments in postmenopausal women result in atrophic changes in endometrium with "glandular exhaustion" and decidualized stroma. Thus, the expected pattern of a glandular secretory protein would have been expected to be suppressed but, interestingly, this was not the case (Ref. 206 and Table 4).

I. Tumors

Immunohistochemical staining has been used to identify glycodelin in various tumors. This glycoprotein has been observed in benign and malignant tumors of the ovary, uterus breast and other tissues (21, 23, 24, 40, 47, 87).

1. Endometrial adenocarcinoma. Malignant endometrium does not appear to synthesize glycodelin, nor can its synthesis be induced by MPA (208). Interestingly, only occasionally during MPA treatment can this protein be found in regions of normal histology among specimens from endometrial cancers. The absence of glycodelin expression probably reflects failure of normal differentiation in the progestogen-resistant microenvironment that prevails in the cancerous endometrium of such patients. In keeping with the observations on endometrial adenocarcinoma tissue, no elevation in circulating glycodelin levels has been found in patients with endometrial cancer (209). Cell lines derived from human endometrial adenocarcinomas (*e.g.*, Ishikawa) do not express glycodelin under standard culture conditions (68, 210), and expression cannot be induced by incubation with ovarian steroids, relaxin, hCG, and a variety of cytokines (R. N. Taylor and J. L. Vigne, unpublished data). However, when glycodelin-negative Ishikawa cells are placed in coculture in contact with normal endometrial stromal cells and basement membrane extract, production of glycodelin is induced at the same time as the adenocarcinoma cells differentiate to more closely resemble normal endometrial epithelium (211). This indicates that glycodelin expression is associated with differentiation and growth inhibition in these adenocarcinoma cells.

An endometrial carcinoma cell line, MFE-280, has been reported to expresses glycodelin protein and the mRNA (212), and RL95–2 endometrial carcinoma cells react with a polyclonal antipeptide antibody [anti-glycodelin peptide (Gp) antibody] generated against synthetic Gp H2N-(Y)KKVLGEKTENPKKFK-COOH (213–215). Using this polyclonal antibody directed against the synthetic Gp sequence, measurement by an ELISA has revealed a significant

TABLE 4. Clinical perspectives in glycodelin research

Observation (Ref.)	Area of clinical relevance
Inhibition of sperm-egg binding (44, 128)	Fertilization, infertility, contraception
Absence/low expression in endometrium during midcycle (36, 56, 70, 71, 80, 150)	Fertilization, infertility
Inappropriate expression in endometrium during midcycle (168, 172)	Infertility, contraception
Immunosuppressive activity (119–123)	Implantation
Induction of expression in endometrial tissue and secretion into uterine fluid and serum reflect prior endometrial exposure to estrogen and progesterone/progestogens (11, 73, 80, 89, 90, 97, 201)	Implantation
Decreased immunostaining in histologically retarded endometrium (152, 180)	Implantation failure
Decreased concentration in uterine flushings (184)	Recurrent miscarriage

increase in plasma Gp levels in endometrial, ovarian, and cervical cancer patients compared with controls (214). Strong expression of glycodelin mRNA and Gp protein has been found in the endometrial and ovarian tumor tissues. Blood vessels of endometrial cancer tissues show strong immunostaining with the anti-Gp antibody, whereas immunoreactivity is very low in normal endometrial tissues (215). It has been speculated that, given glycodelin's immunosuppressive properties, an increased level of Gp may facilitate tumor growth in gynecological malignancies. These results are at striking variance with those obtained with anti-glycodelin antibodies generated against the whole glycodelin molecule.

2. Ovarian tumors. Early studies have shown that serous ovarian cyst fluids contain higher concentrations of glycodelin than mucinous cyst fluids. In these cases, benign or borderline tumors have higher concentrations than malignant tumors (40). Both glycodelin mRNA and protein have been found in benign, borderline, and malignant serous ovarian cystadenomas, whereas mucinous tumors are negative (21). In serous tumors, the expression pattern differs from that seen in the normal ovary. In tumors, expression of glycodelin mRNA and protein is strong in well-differentiated epithelial cells. The epithelium of ovarian serous tumors resembles that of the fallopian tube, and the epithelium of endometrioid ovarian tumors resembles eutopic endometrium in some respects (216, 217). Normal epithelium of all these tissues contains glycodelin protein and mRNA, lending biochemical support to their morphological similarity. Irrespective of the occasional detection of glycodelin in cancerous ovarian tissue, the circulating levels are not different from those in normal controls (208), suggesting that the measurement of circulating levels is not helpful for monitoring of treatment.

Ovarian cancers also show positive immunostaining with the anti-Gp antibody, but in different cell types (214). Immunoreactive Gp appears in blood vessels and is colocalized with immunostaining with anti-von Willebrand factor antibody, suggesting the presence of Gp in endothelial cells of blood vessels. Interestingly, conventional antiglycodelin antibodies have not previously been reported to react with endothelial cells.

3. Breast cancer. Immunohistochemistry, Northern blotting, and RT-PCR analyses have been used to study glycodelin expression in malignant breast tissue, and the results have been compared with the expression of ERs and PRs, p53 tumor suppressor protein, and the proliferation marker, Ki67. Glycodelin was found in 6 of 6 benign lactating adenomas, 21of 35 ductal carcinomas, 9 of 9 tubular carcinomas, 9 of 9 mucinous carcinomas, 3 of 3 mixed ductal/tubular carcinomas, and 7 of 11 lobular carcinomas (47). In lobular carcinomas, the appearance of glycodelin in paranucleolar vacuoles of the carcinoma cells is notable. Glycodelin immunostaining is not related to the presence or absence of ER, PR, or p53, as glycodelin was found in 69% ER/PR-negative tumors compared with 74% ER/PR-positive tumors, and in 75% of the p53-positive tumors compared with 68% of the p53-negative tumors. Northern blot analysis of fresh, frozen tissues revealed the normal full-length 0.9-kb mRNA of glycodelin in ductal breast carcinoma. Using RT-PCR analysis, glycodelin mRNA was found in 13 of 13 ductal and in 3 of 3 tubular tumor tissues. A splicing variant lacking exon 4 was also identified. Exon 4 includes the nucleotide sequence encoding the potential N-glycosylation site at Asn-85. The possible relationship between glycodelin and patient age (35–84 yr) was also analyzed, and no correlation was found (47). The presence of glycodelin in normal breast tissue and breast cancer shows that glycodelin cannot serve as a tumor marker. As transfection of glycodelin cDNA into a glycodelin-negative breast cancer cell line has resulted in differentiation and restricted proliferation (23), it may be anticipated that glycodelin-expressing breast cancers would be more differentiated and have better prognosis. So far no published report has addressed this question.

4. Synovial sarcomas. These are malignant mesenchymal tumors that are classified on histomorphological criteria as monophasic tumors consisting of undifferentiated spindle cells and biphasic tumors with areas of epithelial differentiation (218). The histogenesis of biphasic synovial sarcoma involves epithelial differentiation of mesenchymal cells (219). In 18 synovial sarcomas, of which 11 were classified as biphasic, immunoreactive glycodelin was found in all biphasic sarcomas. The glycodelin-expressing areas exhibited epithelial or glandular differentiation, whereas the sarcomatous spindle cells were glycodelin-negative (220). Glycodelin was also found in 1 of 7 histologically monophasic synovial sarcomas, where glycodelin was localized in some flattened spindle cells. The glycodelin expression was confirmed by the demonstration of mRNA by RT-PCR. These findings raise the possibility that activation of the glycodelin gene may be involved in mesenchyme-to-epithelium differentiation. It remains to be studied whether glycodelin expression is related to aggressiveness on these tumors.

5. Trophoblastic tumors. Serial determinations of glycodelin have been performed in 31 patients with a trophoblastic tumor (20 hydatidiform moles, 4 invasive moles, and 7 choriocarcinomas; Ref. 209). As expected for a pregnancy-related disorder with decidual reaction, the circulating levels of glycodelin were elevated, more so in hydatidiform mole than in choriocarcinoma, and the levels decreased in response to treatment. However, no data are available to demonstrate that glycodelin level would give any additional information to hCG in the monitoring of trophoblastic disease. To serve as an effective tumor marker, a protein needs to be produced by the tumor cells, and this evidence is not available for trophoblastic tumors.

6. Other tumors. In 21 women with carcinoma of the cervix and 2 women with carcinoma of the vulva, the circulating levels of glycodelin were similar to those in normal controls (209). Patients with various tumors including cancers of the cervix, endometrium, ovarian granulosa cell tumor, and dysgerminoma had low serum glycodelin levels (221).

7. Collective conclusions for glycodelin in cancer. The results obtained with purified glycodelin and its conformational antibodies suggest that glycodelin cannot be used as a tumor marker. This is because glycodelin is rarely expressed in carcinoma tissue and, when present, it is associated with

differentiated areas of tumor tissue rather than in poorly differentiated carcinoma cells. The same is seen in synovial sarcomas in which biphasic tumors with epithelial differentiation express glycodelin. A notable example is induced epithelial differentiation and decreased proliferation after transfection of glycodelin cDNA into glycodelin-negative MCF-7 breast cancer cells (23).

Given the striking differences in the results obtained with antiglycodelin and anti-GP antibodies, comparative studies are needed to clarify the differences and to resolve their significance in tumor cells, normal cells, blood vessels, and angiogenesis. Angiogenesis plays an important role in neovascularization of tumors, regulated by vascular endothelial factor (VEGF). Increased migration and tube formation of human umbilical cord vein endothelial cells (HUVECs) have been found in the presence of the synthetic Gp, and this increase is blocked by the anti-Gp antibody and by an anti-VEGF antibody, suggesting that angiogenic effects of Gp are mediated by VEGF (215). In support of this, Gp significantly increases the release of VEGF protein and mRNA expression in HUVECs, RL-95 (human endometrial carcinoma cells), OVCAR-3 (human ovarian carcinoma cells), EM42 (human endometrial epithelial cells), and MCF-7 and MDA-MB-231 (human breast adenocarcinoma) cell lines. In experiments employing anti-Gp antibodies for immunodetection, an endogenous growth factor that stimulates phospholipase D activity and cell proliferation (lysophosphatidic acid, LPA) induces glycodelin gene expression in MDA-MB-231 breast adenocarcinoma cells, HeLa cervical carcinoma cells, RL-95 endometrial carcinoma cells, OVCAR-3 ovarian carcinoma cells, and K562 erythroleukemia cells (222). As plasma LPA concentration is frequently elevated in patients with gynecological cancer, LPA may be related to endogenous stimulation of Gp expression in gynecological tumors (222).

Contrary to the results obtained with anti-Gp antibodies, no immunostaining was found in HUVEC, OVCAR-3, and MDA-MB-231 cells with the use of polyclonal and monoclonal antibodies against native glycodelin, not even after stimulating the cells with LPA or phorbol 12-myristate 13-acetate. Moreover, no stimulation of the normally occurring VEGF expression was found in the presence of glycodelin (R. Koistinen, unpublished observation). The conflicting results between Gp and glycodelin clearly show that anti-Gp antibodies (213) are different from antiglycodelin antibodies (43, 47, 165, 172, 176, 193, 211). This is not surprising in view that linear and conformational epitopes are known to differ, as antibody's recognition of the antigen is finely specific and is affected by antigenic variation (223, 224). The Gp sequence corresponds to amino acids 69–83 of the glycodelin sequence (18, 213), and it is located between the first two of the three cysteines of glycodelin. Unlike in glycodelin, Gp has no cystein residues for S-S bridging (213), and it is known that a mere loop formation of oxidized cysteines can alter peptide immunogenicity (225). In some reports (215), the N-terminal amino acid of the 15-amino acid Gp sequence contains an additional tyrosine. This is not present in glycodelin, and the reasons for its inclusion are not clear, unless added for radiolabeling with iodine. If used for immunization, it is not clear whether and how this difference affects structural mimicry by the Gp peptide with the conformational epitope in glycodelin. Although information from epitope mapping is not available for glycodelin, its contacting amino acid residues are likely to form also discontinuous epitopes. Finally, carbohydrates in glycodelin may also have an impact on immunogenicity, as deglycosylation is known to cause conformational changes in glycoproteins and result in enhanced immunoreactivity (226).

Given the above considerations, it is possible that the immunodominant epitopes of Gp are not exposed in the conformational epitopes of glycodelin, leading to different antibody specificities. The results obtained with the use of Gp and anti-Gp antibodies are therefore restricted to Gp and are not applicable to glycodelin in every respect. Nevertheless, the results on Gp are of interest because they show that Gp-immunoreactive molecules are found *in vivo*, notably in cancer (214, 215), and the linear Gp has VEGF-stimulating activity (215). Future studies will be needed to clarify the relationship of endogenous Gp immunoreactivity with glycodelin—whether it is part of a larger molecule(s) generated by proteolysis from glycodelin, or from other proteins with structural mimicry of the Gp epitopes. Obviously, research employing antibodies against linear Gp and conformational glycodelin will be crucial in uncovering the structure/function relationships that may exist in different parts of the glycodelin molecule.

J. Immunosuppression and prevention of HIV transmission

Sexual activity is a frequent route of adult HIV transmission, the target cells being T lymphocytes, monocytes/macrophages, and dendritic cells (227). Aspects of reproductive-tract biology and retroviral diseases both involve immunosuppression. In the reproductive system, this takes place in a spatially and temporally regulated fashion, whereas in HIV the loss of immune response is disseminated and unregulated. The observed immunosuppressive activity of glycodelin is of interest in the light of its highly unusual N-linked oligosaccharides that manifest immunosuppressive activities. It is noteworthy that some of the unusual sequences associated with glycodelin also are expressed on HIV-infected T lymphocytes. For instance, Lewis^y active biantennary glycans are present in 53% of all glycodelin-S monomers (44), and the percentage of CD8+ lymphocytes expressing the Le^y epitope increases from 3–5% in uninfected lymphocytes to 20–25% in patients with AIDS (228). CD4+ cells also undergo similar changes in the Le^y expression after HIV infection. The increase in the percentage of Le^y-positive cells is directly correlated with the severity of clinical immunosuppression in HIV-infected patients, and the percentage of human H9 lymphoblastoid cells reactive with anti-Le^y-specific antibody increases from 12% under normal circumstances to 97% after exposure to HIV (229).

Schistosomiasis is a parasitic disease that, in advanced stages, can immunocompromise the host. The specific defects are blunting of type 1 T-helper cell responses and enhancement of type 2 T-helper cell responses (230, 231). A similar shift in immune response has been observed in HIV infection (232) and pregnancy. Schistosomes and glycodelin-A share the same unusual surface expression of terminal-fucosylated lacdiNAc sequences (20, 133, 233), and it has been postulated

that the expression of such glycans may confer a protective effect on intravascular parasites, enabling them to circumvent potential immune responses by the host (133, 234).

The amino acid similarity between β-lactoglobulins and glycodelin has led to an interesting reason to modify glycodelin. The concept was sparked from the studies demonstrating that nanomolar concentrations of bovine β-lactoglobulin chemically modified with 3-hydroxyphthalic anhydride blocked the binding site on CD4 for the HIV surface glycoprotein gp120 (235). Glycodelin is homologous with bovine β-lactoglobulin (9, 18, 38), so it was plausible to study whether the antifertility effects of glycodelin could be combined with these antiviral effects. To prevent sexual transmission of HIV, an ideal supplement to local barrier contraception would be topical application of a nonirritating substance with both contraceptive and antiviral effects. Material for purification of bovine β-lactoglobulin is abundantly available; however, its potential sensitizing effects in long-term intravaginal use are not known. It is not known whether bovine β-lactoglobulin has any contraceptive effect in terms of inhibition of sperm-egg binding. Therefore, glycodelin was modified with 3-hydroxyphthalic anhydride to find out whether it too would have antiviral properties. Although native glycodelin-A and glycodelin-S were inactive against HIV transmission, the chemically modified 3-hydroxyphthalic anhydrides of glycodelin-A and glycodelin-S dose-dependently inhibited gp120-CD4 binding at nanomolar concentrations (Fig. 5). They also inhibited HIV nucleocapsid p24 production and the cytopathic effects of HIV-1IIIB (236). Finally, chemically modified glycodelin-A and glycodelin-S potently inhibited infection of peripheral blood mononuclear cells by the primary HIV isolate THA/93/051 belonging to the subtype E. This subtype grows more efficiently in the Langerhans' cells, suggesting that it might be preferentially spread by sexual transmission (237).

VIII. Concluding Remarks and Future Directions

Glycodelin synthesis is stimulated by progesterone. Endometrial glands contain both type A (PRA) and type B (PRB) PR isoforms. Classically, it has been assumed that epithelial PRs are down-regulated by progesterone (238), whereas stromal PRs are not down-regulated by progesterone and play an important role in mediating the effects of progesterone from stroma to epithelium. A recent study (91) indicates that PRB levels are maintained in secretory endometrial epithelium, where glycodelin is synthesized. It is possible that other factors, such as relaxin, also promote the steep rise that takes place in serum glycodelin level during the first 10 wk of pregnancy. Contribution to the circulating glycodelin pool by other glycodelin-synthesizing tissues is possible, whereas the magnitude by which nonuterine tissues contribute to glycodelin serum levels is likely to be small, as shown in hysterectomized women (11).

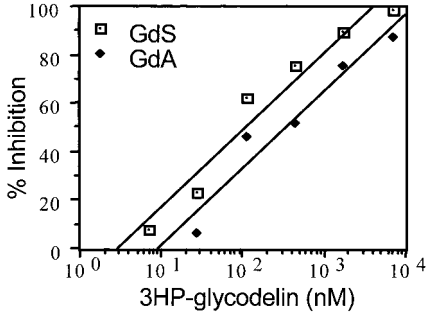

FIG. 5. Inhibition of gp120-CD4+ binding by 3-hydroxyphthalic anhydride-modified glycodelins. GdA, Glycodelin-A; GdS, glycodelin-S. [Reproduced with permission from M. Seppälä *et al.: Lab Invest* 77: 127–130, 1997 (236).]

Glycodelin-A potentially inhibits fertilization by interfering with sperm-egg binding, and it may also modulate the process of implantation via its immunosuppressive activity. Because of its cyclical secretion, absence of glycodelin during the fertile periovulatory phase makes glycodelin an interesting lead molecule for research in infertility and contraception. Here, any drug or substance that induces midcycle glycodelin secretion in the uterus would potentially decrease fertility. Studies addressing "inappropriate" secretion of glycodelin during otherwise fertile midcycle are only beginning to emerge, and systematic studies on women with unexplained infertility featuring midcycle expression of glycodelin are still scarce. Moreover, the contraceptive effects, if any, of a sustained elevation of glycodelin level in serum or endometrium, such as occurs during sustained administration of relaxin (101) or progestogens (172), remain to be studied.

There is a demand for contraceptive methods that cannot be classified as abortifacients. Glycodelin research has given an interesting model to study the mechanisms by which some of the currently used methods of contraception work. Based on current evidence, the IUDs exert contraceptive action by interfering with sperm transport, ovum development, fertilization, and implantation. By consensus definition, a woman becomes pregnant when the embryo attaches in her body (239). Consequently, after IVF-ET, a woman is not pregnant before the embryo has implanted. The same applies to natural fertilizations. The best evidence for implantation is detection of hCG in serum or urine. Among IUD-wearing women, hCG or other trophoblast products are rarely detected (240), showing that the major mechanism by which the IUDs provide contraception is not via interruption of early pregnancy. The levonorgestrel-releasing intrauterine system induces glycodelin secretion from uterine glands over the fertile midcycle, potentially contributing to a contraceptive microenvironment within the uterus before fertilization, whereas the frequency with which a copper-releasing IUD does the same has not been determined.

The anti-HIV activity of chemically modified glycodelin should be of interest in view of the development of antiviral contraceptive strategies. The contraceptive effect of chemically modified antiviral glycodelin is yet to be tested. If no contraceptive activity is found in antiviral glycodelin, then another possibility is to mix it with contraceptive glycodelin for local use. Recombinant glycodelin produced in CHO cells does not express the glycodelin-A-type glycosylation (69), whereas recombinant glycodelin produced in HEK293 human embryonic kidney cells contains the same glycosylation pattern and, hence, is expected to be suitable for further contraceptive research.

Glycodelin has also elucidated the actions of immuno-

regulatory proteins. Being abundant at the fetomaternal interface, its NK cell immunomodulatory functions are likely to play a role in fetomaternal defense mechanisms. Glycodelin is one of those immunoregulatory proteins that directly inhibits T cells on an early phase of activation (125), and glycodelin also induces T cell apoptosis (127). Glycodelin increases the T cell activation threshold, *i.e.*, it renders T cells less sensitive to stimulation (241). Recently, glycodelin has been localized to the contact sites between antigen-presenting cells and the T cell receptor, where glycodelin negatively regulates T cell activation by diminishing T cell responses in the contact site at the time of T cell receptor triggering. This indicates that glycodelin may interfere with the organization of the immune synapse (242).

Glycodelin research has elucidated the biology of epithelial differentiation. The glandular association of native glycodelin indicates a fundamental role in glandular morphogenesis (23), and induced glycodelin expression in glycodelin-negative endometrial adenocarcinoma cells is associated with differentiation toward normal endometrial epithelium and restricted cell growth (211). Obviously, the growth-restrictive association of glycodelin requires further investigation because of the discrepant findings with anti-Gp antibodies and Gp that show few functional similarities to glycodelin.

Finally, the importance of differential glycosylation on biological activity is illustrated by glycodelin isoforms. This too has broad implications for cell biology. The glycobiological aspect is likely to become increasingly important in the understanding of how posttranslational events affect glycoprotein bioactivity in health and disease (243). Many unexplored areas still remain in glycodelin research, such as its roles in tumors, fetal development, and prepubertal age, and the biological role of glycodelin expressed outside the reproductive system. Further investigation into this exciting and multifunctional glycoprotein is bound to yield new insights into human reproductive biology.

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