Characterization of Placentation-specific Binucleate Cell Glycoproteins Possessing a Novel Carbohydrate

EVIDENCE FOR A NEW FAMILY OF PREGNANCY-ASSOCIATED MOLECULES*

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Yvelle H. Atkinson, Kathryn J. Gogolin-Ewens, Elizabeth F. Hounsell, Michael J. Davies, Mal R. Brandon, and Robert F. Seamark

From the Department of Obstetrics and Gynaecology, University of Adelaide, Adelaide 5001, South Australia, Australia, the Centre for Animal Biotechnology, School of Veterinary Science, The University of Melbourne, Parkville 3052, Victoria, Australia, and the Clinical Research Centre, Glycoconjugates Section, Harrow, Middlesex HA1 3UJ, United Kingdom

The ovine binucleate cell-specific glycoproteins recognized by the monoclonal antibody SBU-3 first appear at the initiation of placentation, and their expression continues throughout gestation. These placenta-specific proteins have not been detected in any other adult or fetal sheep tissues and are specific to the materno-fetal interface. The SBU-3 monoclonal antibody recognizes the carbohydrate epitope common to a group of proteins ranging in molecular mass from 30 to 200 kDa whose function during pregnancy remains undefined. The biochemical properties of these uniquely expressed glycoproteins were investigated by analyzing both the carbohydrate and protein portion of the molecules. Analysis of phytohemagglutinin and concanavalin A binding to electrophoretically separated SBU-3 proteins revealed that the major proteins between 40 and 70 kDa bind phytohemagglutinin. In contrast, concanavalin A bound only to minor proteins in the SBU-3 glycoprotein preparation. Analysis of the carbohydrate conjugated to the SBU-3 glycoproteins revealed that the major chains are siaIylated O-linked and complex partially siaIylated multiple antennary N-linked chains. The presence of N-glycolylneuraminic acid in an N-linked structure indicates the unique nature of this carbohydrate epitope. The differential binding to phytohemagglutinin and concanavalin A provides a method for further purification and characterization of the major protein components with monoclonal antibody immunoaffinity-purified SBU-3 proteins being further separated by concanavalin A-Sepharose chromatography. Microsequence analysis of the major non-concanavalin A-binding proteins (69, 62, and 57 kDa) revealed partial homology to ovine and bovine pregnancy-associated glycoprotein and rabbit pepsinogen F. Immunoblot analysis of the SBU-3 proteins showed cross-reactivity with polyclonal antisera directed against ovine placental-associated glycoprotein and pregnancy-specific glycoprotein B. These results suggest that together these glycoproteins represent members of a binucleate cell-derived family of pregnancy-associated molecules in the ruminant placenta.

Fetal binucleate cells are unique to the ruminant placenta (Wooding, 1992). In the sheep, they differentiate from the mononucleate cells of the trophoderm as it establishes close contact with the uterine epithelium on days 14-15 of gestation (Wooding, 1992). At least two subpopulations of binucleate cells are present in the ovine placenta, as defined by the monoclonal antibody (mAb)1 SBU-3 which recognizes a proportion of binucleate cells present in the areas where the placenta develops (placentomes), and not those present in the interplacental regions (Lee et al., 1986). This mAb, which was raised against trophoblast microvilli isolated from the fetal portion of midgestation ovine placentomes, has been shown to recognize a carbohydrate moiety present on a group of proteins with molecular weights ranging between 30,000 and 200,000 (Gogolin-Ewens et al., 1985). Extensive studies using this antibody failed to detect the presence of the SBU-3 carbohydrate antigen in a wide range of ovine tissues other than in restricted regions of the placenta.

Cell surface carbohydrates attached to glycoproteins have been implicated in a wide range of biological processes. The concept of tissue-specific recognition of carbohydrates was first introduced with the discovery of the asialoglycoprotein receptor on hepatocytes (Kawasaki and Ashwell, 1976). With tissue-specific carbohydrate recognition came the concept of tissue-specific carbohydrate expression. The involvement of carbohydrates in cell-cell interaction provides an excellent illustration of this phenomenon. For example, it was observed that the SSEA-1 antigen expressed on the preimplantation mouse embryo is actually a Galβ1-4(Fucα1-3)GlcNAcβ1- structure (Gooi et al., 1981). Exogenous addition of oligosaccharides inhibited morulae compaction and decompacted morulae (Fenderson et al., 1984). Another reproductive process in which carbohydrates are involved includes the adhesion of sperm to eggs during fertilization (Wassarman, 1987).

Carbohydrate modification that is site-specific has been described for other placental glycoproteins. Examples include human chorionic gonadotropin (Endo et al., 1979), human placental alkaline phosphatase (Endo et al., 1988), and human placental fibronectin (Takamoto et al., 1989a) which contain only one type of sialyl linkage, whereas their serum glycoprotein counterparts express alternative sialyl linkages. The ex

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1 The abbreviations used are: mAb, monoclonal antibody; bPAG, bovine pregnancy-associated glycoprotein; CAPS, 3-(cyclohexyIamino)-1-propanesulfonic acid; CHAPS, 3-(3-cholamidopropyl)dimethylammonio-1-propanesulfonate; ConA, concanavalin A; HPAlE-PAD, high performance anion exchange chromatography with pulsed amperometric detection; IL-2, interleukin 2; NeAc, N-acetylneuraminic acid; NeD, N-glycolylneuraminic acid; oPAG, ovine pregnancy-associated glycoprotein; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; PBS/T, phosphate-buffered saline + 0.05% Tween 20; PGC-HPLC, porous graphic carbon high performance liquid chromatography; FHA, phytohaemagglutinin; PSC, pregnancy-specific β glycoprotein; PSpB, pregnancy-specific glycoprotein B.
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amination of the glycans of fibronectin purified from human plasma (Takasaki et al., 1980), amniotic fluid (Takamoto et al., 1989b), and placenta (Takamoto et al., 1989a) revealed differences in sialyl linkages, the ratio of bi-, tri-, and tetraantennae, and fucosylation.

The unique and pregnancy-specific expression of the SBU-3 carbohydrate epitope which is common to a group of placental glycoproteins, raises the possibility of placental specific glycosylation. We sought to gain an insight into this question by biochemically characterizing some of these glycoproteins. In addition, the expression of a placenta-specific carbohydrate epitope warrants further investigation with a view to defining the molecular mediators of placation.

**EXPERIMENTAL PROCEDURES**

**Monoclonal Antibody Immunoaffinity Purification of SBU-3 Conjugated Proteins**—The SBU-3 proteins were mAb immunoaffinity-purified as previously described (Gogolin-Ewens et al., 1986). Briefly, SBU-3 mAb was purified from ascites fluid and coupled to Affi-Gel 10 (Bio-Rad) at 10 mg of protein per mg of beads. Preparation of total tissue microsomal microvilli isolated from midgestation ovine placentomes were centrifuged to yield a supernatant and a membrane fraction. The membrane pellet was diluted and applied to the affinity column. Specifically bound proteins were eluted with 4 mM sodium thiocyanate (Ajax Chemicals, Australia), and column fractions were immediately desalted into distilled water on a PD-10 column (Pharmacia, Sweden). Column fractions containing SBU-3 protein were identified on Western blots stained with anti-SBU-3 mAb (Gogolin-Ewens et al., 1986), pooled, and concentrated.

**Lectin and Immunoblotting of SBU-3 Proteins**—SBU-3 proteins were separated by SDS-PAGE on 10% gels (8 x 7 x 1.5 cm) according to the method of Laemmli (1970) and transferred onto 0.2-μm or 0.45-μm nitrocellulose membranes (Schleicher & Schuell) in electrophoretic transfer buffer containing 25 mM Tris base (Sigma), 192 mM glycine (Sigma), 0.01% v/v SDS (Sigma), and 10% (v/v) methanol (Towbin et al., 1979). Proteins were transferred at 70 V for 2 hr. For lectin and immunoblotting, unbound membrane slices were blocked with phosphate-buffered saline (PBS) containing 0.2% bovine serum albumin (BSA) or 3% (w/v) skim milk. Following incubation with anti-SBU-3 mAb (Gogolin-Ewens et al., 1986), pooled, and concentrated, the membranes were washed three times in PBS + 0.05% Tween 20 (PBS/T) and incubated for 1 hr in SBU-3 hybridomas supernatant fluid, anti-ovine placental-associated glycoprotein (oPAG) polyclonal rabbit sera (a generous gift from Prof. J. F. Beckers, Département d’Endocrinologie et de Reproduction Animales, Université de l’Etat à Liege), or anti-pregnancy-specific glycoprotein B (PSPB) polyclonal rabbit sera (a generous gift from Dr. R. Garth Sasser, Dept. of Animal and Veterinary Science, University of Idaho). Following three washes in PBS/T, the membrane was incubated in horseradish peroxidase-α-linked rabbit anti-mouse immunoglobulin (Dako, Glostrup, Denmark), or horseradish peroxidase-conjugated goat anti-rabbit (1:500 in PBS/T, Dako), or horseradish peroxidase-conjugated goat anti-rabbit (1:500 in PBS/T, Sigma) for 1 hr at room temperature. Following two washes in PBS/T and two washes in PBS, the blot was developed with the chromogen 3,3'-diaminobenzidine (Dako, 0.6 mg/ml + 0.0075% H2O2) at room temperature. For lectin blots, a similar procedure was used, except the first incubation was with 6.25 μg/ml biotinylated ConA (Sigma) or biotinylated PHA (Sigma) in PBS/T. The second incubation was with horseradish peroxidase-streptavidin conjugate (Amersham), diluted 1:1000 with PBS/T. Lectin blots were developed as described above for immunoblots. For carbohydrate profil...
were probed with either anti-SBU-3 antibody or biotin-labeled PHA or ConA. The reaction was detected by horseradish peroxidase-conjugated second antibody and developed with diaminobenzidine.

Blots separated by 10% SDS-PAGE and transferred to nitrocellulose. Blots were probed with either anti-SBU-3 antibody or stained with biotin-labeled PHA or ConA which stain with SBU-3 antibody also bind PHA. In contrast, ConA bound only to minor proteins in the SBU-3 preparation. Notably, ConA did not bind to the major SBU-3-reactive proteins localized between 40 and 70 kDa. These binding patterns are consistent with the differential intensities of binding seen in the dot blots. Lower molecular mass proteins (<40 kDa) were lost during this transfer due to the use of 0.45-pm nitrocellulose and a longer transfer time which was employed to enable us to focus on the higher molecular mass proteins.

**Table I**

| Carbohydrate | Nanomol/300 µg | Molar ratio | Micrograms |
|--------------|----------------|-------------|------------|
| Fucose       | 6.2            | 0.6         | 0.34       |
| Mannose      | 30.1           | 2.0         | 1.81       |
| Galactose    | 32.4           | 3.2         | 1.94       |
| Glucose      | 10.1           | 0.3         | 0.61       |
| N-Acetylgalactosamine & N-Acetylglucosamine | 49.1 | 4.9 | 3.62 |
| Sialic acid  | 41.3           | 4.1         | 4.25       |

Analysis does not distinguish N-deacylated hexosamines from their N-acetylated counterparts or differentiate types of sialic acid from each other.

The structure has been proposed based on the molar ratios of monosaccharides, lectin binding, and elution time on HPAE and PGC chromatography.

Fig. 2. The generalized structure of N-linked oligosaccharides present on SBU-3 glycoproteins. The structure has been proposed based on the molar ratios of monosaccharides, lectin binding, and elution time on HPAE and PGC chromatography.

nositained with SBU-3 mAb or stained with biotin-labeled PHA or ConA (Fig. 1). The major proteins between 40 and 70 kDa which stain with SBU-3 antibody also bind PHA. In contrast, ConA bound only to minor proteins in the SBU-3 preparation. Notably, ConA did not bind to the major SBU-3-reactive proteins localized between 40 and 70 kDa. These binding patterns are consistent with the differential intensities of binding seen in the dot blots. Lower molecular mass proteins (<40 kDa) were lost during this transfer due to the use of 0.45-μm nitrocellulose and a longer transfer time which was employed to enable us to focus on the higher molecular mass proteins.

**Carbohydrate Analysis of the SBU-3 Proteins**—Gas chromatographic (GC) analysis of the immunoaffinity-purified SBU-3 proteins revealed that they contained 18% carbohydrate with the molar ratios shown in Table I. The large amount of N-acetylgalactosamine suggests that O-linked chains are present, having the core linkage GalNAc1-Ser/Thr. The ratio of the remaining monosaccharides with respect to mannose at 3.0 suggests a generalized N-linked chain having three to four GalNAc antennae, with additional GlcNAc (bisection), terminal sialylation of branches, and the possibility of a core or terminal fucose (Fig. 2). Binding of the SBU-3 glycoproteins to the lectin PHA suggests that not all N-linked antennae are sialylated, but that some of the sialic acid is on O-linked GalNAc. Analysis of the sialic acid component of the carbohydrate indicated that this residue is N-glycoly neuraminic acid (NeuGc; Fig. 3). These results are consistent with the oligosaccharide profiles obtained by both HPAE and PGC chromatography. In the former technique, N-glycoly neuraminic acid elute later than their N-acetylated counterparts. Comparison of the HPAE elution profile of the oligosaccharides released from SBU-3 (Fig. 4A) and a standard glycoprotein, fetuin (Fig. 4B), shows that except for a peak at 26 min in the region of disialylated oligosaccharides containing NeuAc (25–30 min), the SBU-3 oligosaccharides elute later than trisialylated oligosaccharides containing NeuAc (36–40 min). The peaks for SBU-3 at around 46, 51, and 56 min (Fig. 4A) are therefore consistent with multiple sialylated complex oligosaccharides containing NeuGc. The various peaks represent different numbers of sialic acid residues, different linkage isomers, and a different number of antennae as shown for peaks around 29, 38, and 50 min in the standard (Fig. 4B) for oligosaccharides having N-acetylmuramic acids. On PGC chromatography, sialylated oligosaccharides differing only in their content of NeuAc or NeuGc co-elute: monosialylated oligosaccharides chromatograph from 22–25 min, disialylated from 29 to 32 min, and trisialylated from 30 min.2 The oligosaccharide profile of SBU-3 (Fig. 5) is therefore consistent with the presence of mainly mono- or disialylated oligosaccharides with either NeuAc or NeuGc.

**Enrichment and Microsequence Analysis of the Major SBU-3**

2 M. J. Davies, K. D. Smith, R. A. Carruthers, W. Chai, A. M. Lawson, and E. F. Hounsell, manuscript in preparation.
Proteins—The differential binding of SBU-3 glycoproteins to PHA and ConA provided a method for further purification of the major protein components. The SBU-3 proteins were applied to a ConA-Sepharose column, and the flow-through was collected, desalted, and analyzed by SDS-PAGE. A proportion of the SBU-3 glycoproteins, ranging in molecular mass from 97 kDa to 37 kDa, did not bind to the ConA-Sepharose column (Fig. 6). Major bands of 69, 62, and 57 kDa were easily detected, whereas the 97-kDa (not seen in this photograph), 84-kDa, 64-kDa, 51-kDa, and 37-kDa bands represented minor components of the non-ConA-binding SBU-3 proteins. The major 69-kDa, 62-kDa, and 57-kDa proteins were separated further on SDS-PAGE for subsequent amino-terminal microsequence analysis.

Microsequence analyses of the 69-kDa, 62-kDa, and 57-kDa non-ConA-binding SBU-3 proteins determined 27, 25, and 23 amino acids, respectively (Fig. 7). These sequences are homologous to each other to varying degrees. SBU-3\(^{69}\) is 84% homologous (identical and conserved amino acids) to SBU-3\(^{62}\) and 57% homologous to SBU-3\(^{57}\). SBU-3\(^{62}\) and SBU-3\(^{57}\) are less homologous to each other, showing only 48% identity. Residues 4 and

FIG. 4. HPAE-PAD analysis of the PNGase F digest of the carbohydrate conjugated to the SBU-3 glycoproteins. The ethanol precipitate of the PNGase F digest of the SBU-3 glycoproteins was taken up in 0.1 M NaOH and injected onto a CarboPac PA1 column. Oligosaccharides were eluted at 1 ml/min in a gradient of 0.1 M NaOH and 0.1 M NaOH, 1 M sodium acetate. A represents the elution profile of oligosaccharides from the SBU-3 carbohydrate, and B represents the standard N-linked chain mixture from fetuin, showing elution of di- (26–29 min), tri- (38–40 min), and tetra- (49–52 min) sialylated (NeuAc) oligosaccharides.

FIG. 5. Porous graphitic carbon HPLC analysis of the PNGase F digest of the carbohydrate conjugated to the SBU-3 glycoproteins. The SBU-3 oligosaccharides were injected onto a Hypercarb S column, eluted in a gradient of 0.05% aqueous trifluoroacetic acid and acetonitrile, 0.05% trifluoroacetic acid, and detected at 206 nm. The elution profile revealed the presence of mainly mono- or disialylated oligosaccharides with either NeuAc or NeuGc.

FIG. 6. SDS-PAGE analysis of non-ConA-binding immunoaffinity-purified SBU-3 proteins. One hundred µg of SBU-3 mAb immunoaffinity-purified glycoprotein was applied to ConA-Sepharose under gravity flow and washed with 20 mM Tris-HCl, 0.5 M NaCl, pH 7.4. Fractions containing SBU-3 proteins that did not bind to ConA were pooled and desalted, then resolved on a 12% polyacrylamide gel, and visualized following silver staining.
Biochemical Characterization of the SBU-3 Proteins

| 69 Kd | 62 Kd | 57 Kd |
|-------|-------|-------|
| Gln-Gly-Ser-Val-Thr-Ile-Leu-Pro-Leu-Arg-Aan-Met-Lys | Asp-Ile-Phe-Tyr-Val-Gly-Asp-Leu-Thr-Arg-Gly-Val-XxX-Ile-Arg-Thr-Thr-Pro | Gln-Gly-Ser-Val-Thr-Ile-Leu-Pro-Leu-Arg-Aan-Met-Lys | Asp-Ile-Phe-Tyr-Val-Gly-Asp-Leu-Thr-Ile-Arg-Thr-Thr-Pro |
| Ser-Leu-Thr-Ile-Leu-Pro-Leu-Arg-Aan-Met-XxX | Asp-Ile-Phe-Tyr-Arg-Gly-Asp-Leu-Thr-Ile-Thr-Arg-Gly-Val-Gly-Pro |
| Pro-Leu-Aan-Aan-Met-XxX | Pro-Leu-Aan-Aan-Thr-Lys-Gly-Leu-Thr-Thr-Met-XxX-Ile-Arg-Thr-Thr-Pro |
| Thr-Lys-Gly-Leu-Thr-Thr-Met-XxX-Ile-Arg-Thr-Thr-Pro |

**FIG. 7.** Microsequence analyses of the major PHA-binding SBU-3 glycoproteins. Amino-terminal analyses were performed by sequential Edman degradation on SBU-3 proteins immobilized on PVDF membrane, following electrophoretic separation on a 12% polyacrylamide gel. The residues *underlined* in the SBU-3 sequence represent a possible N-myristoylation site. The *borders* outline areas of sequence that are identical or where conserved substitutions occur.

| 69 Kd | 62 Kd | 57 Kd |
|-------|-------|-------|
| Gln-Gly-Ser-Val-Thr-Ile-Leu-Pro-Leu-Arg-Aan-Met-Lys | Asp-Ile-Phe-Tyr-Val-Gly-Asp-Leu-Thr-Arg-Gly-Val-XxX-Ile-Arg-Thr-Thr-Pro | Gln-Gly-Ser-Val-Thr-Ile-Leu-Pro-Leu-Arg-Aan-Met-Lys | Asp-Ile-Phe-Tyr-Val-Gly-Asp-Leu-Thr-Ile-Arg-Thr-Thr-Pro |
| Ser-Leu-Thr-Ile-Leu-Pro-Leu-Arg-Aan-Met-XxX | Asp-Ile-Phe-Tyr-Arg-Gly-Val-XxX-Ile-Arg-Thr-Thr-Pro |
| Pro-Leu-Aan-Aan-Met-XxX | Pro-Leu-Aan-Aan-Thr-Lys-Gly-Leu-Thr-Thr-Met-XxX-Ile-Arg-Thr-Thr-Pro |
| Thr-Lys-Gly-Leu-Thr-Thr-Met-XxX-Ile-Arg-Thr-Thr-Pro |

**FIG. 8.** Sequence homology between the SBU-3 proteins and placental associated glycoprotein (PAG) and pepsinogen F. Peptide and DNA sequence databases were searched, and homologies computed at the NCBI using the BLAST network service (Altschul et al., 1990). The *borders* outline areas of sequence that are identical or where conserved substitutions occur.
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Fig. 9. Antibodies recognizing oPAG and PSPB cross-react with the immunoaffinity-purified SBU-3 proteins. Proteins were separated by 10% SDS-PAGE and then transferred to nitrocellulose. After incubation with either anti-SBU-3, anti-oPAG, or anti-PSPB, binding was detected with a horseradish peroxidase-conjugated second antibody and developed using diaminobenzidine.

21 in the 69-kDa protein and 2 and 19 in the 62-kDa protein yielded no identifiable amino acid derivative, but are followed by the consensus sequence for an N-glycosylation site (N-X-[ST]-X, X cannot be a P). The SBU-3 proteins contain a consensus sequence for an N-myristoylation site (GSXVTI), and all three protein sequences contain the consensus sequence for sulfation of carbohydrate (PLR).

A search of the protein and DNA sequence databases reveals a 61–78% identity between the three SBU-3 proteins and the binucleate cell-specific protein, ovine (and bovine) pregnancy-associated glycoprotein (oPAG, Fig. 8). In addition, the SBU-3, SBU-3, and SBU-3 sequences are 66–68% homologous to rabbit pepsinogen F. The amino-terminal sequence of SBU-3 starts at Arg-39 in the oPAG sequence, which is the proposed cleavage site for the aspartic protease.

To determine the extent of homology between the SBU-3 proteins, oPAG, and PSPB, immunoblotting of the SBU-3 proteins was performed. Immunoaffinity-purified SBU-3 proteins were separated by SDS-PAGE and transferred onto 0.2-μm nitrocellulose. The blots were then probed with SBU-3 mAb or polyclonal antisera directed against oPAG or PSPB as shown in Fig. 9. Both the oPAG and the PSPB antibodies recognized the three major SBU-3 proteins characterized in this paper, indicating that a high degree of homology exists between these proteins. The oPAG antibody also recognizes all of the minor SBU-3 proteins (both ConA-binding and non-ConA-binding), whereas the PSPB antibody recognizes only five such bands.

DISCUSSION

The SBU-3 glycoproteins represent a group of at least 10 proteins linked to a fatally derived pregnancy-specific carbohydrate antigen recognized by the SBU-3 mAb (Gogolin-Ewens et al., 1986). These glycoproteins are initially detectable in the binucleate cells of the fetal trophoblast at the time of placentome formation, and their expression continues throughout gestation (Gogolin-Ewens et al., 1986; Morgan et al., 1987). It remains to be established whether their expression profile varies as placentome progresses. In this paper, we show that within this glycoprotein family, the major PHA-binding proteins are homologous to oPAG and rabbit pepsinogen F. The SBU-3 carbohydrate antigen appears to be a complex tri- or tetra-antennary chain containing N-glycolyneuraminic acid. These results provide a clue as to the identity of the SBU-3 glycoproteins. The unique structure of the SBU-3 carbohydrate antigen, combined with a restricted distribution in large quantities at the feto-maternal interface, suggests that modification of the glycoprotein may play an important role in the establishment and/or maintenance of pregnancy.

Our preliminary study of the immunosuppressive properties of the SBU-3 glycoproteins revealed an ability to suppress PHA-induced mitogenesis without affecting basal proliferation. However, these proteins had no effect in a mixed lymphocyte reaction, which represents an IL-2-driven proliferative response, or on IL-2-dependent lymphoblast proliferation, suggesting that the SBU-3 proteins do not influence IL-2-dependent lymphocyte proliferation. Dot-blot analyses revealed that the SBU-3 proteins bind to PHA at concentrations which are inhibitory in the mitogenesis assay. These findings together indicate that the SBU-3 proteins may inhibit PHA-induced mitogenesis by binding and sequestering the PHA rather than by directly influencing lymphocyte function. However, these results do not preclude the possibility that the SBU-3 glycoproteins play an immunosuppressive role during pregnancy.

Analysis of the carbohydrate moiety conjugated to the SBU-3 proteins was performed in order to define the carbohydrate content and structure. The results were consistent with the major chains being sialylated, O-linked, and complex partially sialylated, multiple antennary N-linked chains. The sialic acid assay indicated that this is all present as N-glycolyneuraminic acid. NeuGc is reported to be completely absent from human serum and from serum glycoprotein, such as α1-acid glycoprotein (Corfield and Schauer, 1982). Human erythrocytes contain only NeuAc linked to glycoproteins (Corfield and Schauer, 1982). In contrast, high proportions of NeuGc are found in mice, rat, cat, pig, sheep, cow, horse, mule, and donkey erythrocytes (Corfield and Schauer, 1982). NeuGc on horse erythrocytes is the structure causing serum sickness in humans (Higashi et al., 1977), suggesting the absence of this moiety from all human tissues. The majority of NeuGc-containing molecules of non-human origin have been characterized as O-linked glycoprotein chains (Chai et al., 1992) and gangliosides. Few oligosaccharides of N-linked type containing NeuGc have been isolated and fully characterized, and no systematic studies have been carried out to look at their tissue distribution.

Analysis of the SBU-3 proteins revealed heterogeneity in their lectin binding properties. The major proteins between 40 and 70 kDa bind PHA, but not ConA, whereas minor proteins ranging in molecular mass up to 200 kDa bind only to ConA. These lectin-binding characteristics provide further information about the possible structures of the carbohydrate attached to the SBU-3 proteins. The biotinylated PHA preparation used in this study contains both PHA-L and PHA-E. PHA-L recognizes tri- and tetra-antennary N-linked chains with outer Gal and Kornfeld, 1982). PHA-E recognizes bi- or triantennary oligosaccharides containing a bisecting GlcNAc and a Galβ1–4GlcNAc sequence linked β-2 to Man1–6 (Yamashita et al., 1988). ConA does not bind to the major SBU-3 proteins indicating that they are not high mannosyl, hybrid, or biantennary oligosaccharides with 2-O-unsubstituted trimannosyl core residues. These lectin binding data are consistent with the carbohydrate structure causing serum sickness in humans (Higashi et al., 1977), suggesting the absence of this moiety from all human tissues. The majority of NeuGc-containing molecules of non-human origin have been characterized as O-linked glycoprotein chains (Chai et al., 1992) and gangliosides. Few oligosaccharides of N-linked type containing NeuGc have been isolated and fully characterized, and no systematic studies have been carried out to look at their tissue distribution.

Microsequence analyses of the 69-kDa, 62-kDa, and 57-kDa SBU-3 proteins demonstrated that they show partial identity with one another (Fig. 7). The SBU-3 and SBU-3 exhibit the highest degree of homology, followed by the SBU-3 and SBU-3. The alignment of these sequences with each other suggests that they may be a related family of pregnancy-specific glycoproteins.

Immunohistological analysis of ovine placentomes demonstrated that SBU-3 glycoproteins are expressed initially at the time of placentome formation, and for the duration of gestation (Morgan et al., 1987). However, it remains to be established whether some or all of the proteins recognized by the SBU-3 antibody are expressed simultaneously and/or in the same lo-
cation. For example, one form of SBU-3 may be expressed in migrating binucleate cells, another in fusing binucleate cells, and still another only in syncytiotrophoblast (Home et al., 1983; Bohn, 1971). Although the function of the PSGs is unknown, carcinoembryonic antigen has recently been shown to function as a Ca²⁺-independent homophilic cell adhesion molecule in vitro (Benchimol et al., 1989).

We have shown in the present study that the SBU-3 proteins expressed exclusively at the fetomaternal interface during placentation have sequences that are homologous to oPAG and contain a unique carbohydrate structure. Experiments are in progress to determine whether the SBU-3 epitope is cleaved or modified from oPAG or PSPB. If so, these results may provide an important insight into placenta-specific glycosylation patterns and their role in glycoprotein trafficking.

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