American Journal of Pathology, Vol. 146, No. 1, January 1995

A Mucin-Like Glycoprotein Identified by MAG (Mouse Ascites Golgi) Antibodies

Menstrual Cycle-Dependent Localization in Human Endometrium

Harvey J. Kliman,*† Ronald F. Feinberg,†‡ Lisa B. Schwartz,† Michael A. Feinman,§ Ehud Lavi,*‡ and Erika L. Meaddough*

From the Departments of Pathology and Obstetrics and Gynecology,† Yale University School of Medicine, New Haven, Connecticut; the University of Pennsylvania Medical Center,‡ Philadelphia, Pennsylvania; and the Pacific Fertility Center,§ Westlake Village, California*

Human endometrial glands synthesize and secrete a high molecular weight mucin-like glycoprotein in a menstrual cycle-dependent fashion. A novel moiety within this Golgi-associated glycoprotein is strongly reactive with IgG antibodies in numerous murine ascites, and has been termed MAG (mouse ascites Golgi). Immunohistochemical staining of 201 endometrial biopsies revealed the following patterns: MAG first appeared in the Golgi on cycle day 5, peaked on day 15, was present on the surface of the luminal epithelium between days 17 and 19, and was no longer detectable after day 19. MAG was also present in cervical, prostate, seminal vesicle, and lacrimal glands, pancreatic acinar cells, gall bladder and bile duct epithelium, and certain cells of the salivary and sweat glands. Interestingly, only tissues from blood group A individuals exhibited this staining. As a common link among all these cell types is the expression of mucins, we speculated that the MAG epitope could be a mucin-associated blood group A-related epitope. This hypothesis was tested by absorption experiments with a variety of glycoconjugates and erythrocytes and by immunoblots of MAG-rich material. The absorption studies demonstrated that only type III porcine mucin (<1% sialic acid) and blood type A or AB erythrocytes were able to absorb the anti-MAG antibody. Inasmuch as N-acetyl-galactosamine alone, the terminal blood group A carbohydrate, did not block MAG antibody binding, the MAG epitope appears to involve N-acetyl-galactosamine plus other determinants. Immunoblots of endometrial extracts and saliva from blood type A individuals revealed MAG-reactive material with a molecular weight >200 kd under reducing conditions. Because the MAG epitope appears on the endometrial surface during the purported implantation window, we speculate that mucin-like epitopes could play a role in the earliest apposition phases of conceptus-endometrial interaction. (Am J Pathol 1995, 146:166–181)

The molecular basis for the apposition and adhesion phases of implantation is not precisely understood but may involve cell-cell recognition moieties.[1] Synchronization between embryonic and uterine development leading to the receptive phase, or "implantation window," appears to be necessary for successful implantation in the rodent[2] and human.[3–5] In preparation for the implantation process, the uterine epithelium produces both secreted and membrane-bound glycoproteins,[6] some of which are hormonally regulated.[7–9] Biochemical changes, such as a decrease in the thickness and charge of the uterine epithelial glycocalyx, have been shown to precede implantation.[10–12] The carbohydrate complexes of the luminal endometrial surfaces (glycocalyx) have also been shown to change during the adhesive

Accepted for publication September 6, 1994.

Current address of Dr. Schwartz: Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, New York University Medical Center, New York, NY.

Address reprint requests to Dr. Harvey J. Kliman, Developmental and Perinatal Pathology Unit, Departments of Pathology and Obstetrics and Gynecology, Yale University School of Medicine, 310 Cedar Street, New Haven, CT 06520-8023.

phase,[13,8] as demonstrated by changes in lectin binding. Lindenberg and colleagues,[14,15] have demonstrated the presence of a specific oligosaccharide determinant, lacto-*N*-fucopentaose I, on the mouse endometrium that appears at the time of implantation and specifically binds to a lectin receptor on the trophoblast surface of the blastocyst. These workers showed that the addition of lacto-*N*-fucopentaose I inhibited blastocyst attachment to endometrial monolayers.[16] In addition to binding to specific receptor molecules, endometrial cell surface glycoproteins may act as substrates for cell surface glycosyltransferases. Galactosyl transferase has been identified on the surface of early mouse preimplantation embryos.[17] As demonstrated in sperm-egg binding,[18] glycosyltransferases present at the embryonic-endometrial interface could facilitate the complex heterotypic cell-cell interactions required for implantation.

While characterizing the immunolocalization pattern of oncofetal fibronectin in human implantation sites,[19] we observed highly specific Golgi staining in certain endometrial biopsies. Although initially attributing this Golgi staining to the anti-oncofetal fibronectin antibody, we later identified IgG antibodies in other non-fibronectin-related murine ascites that exhibited the same specific Golgi-associated staining. This fortuitous finding led us to further characterize the mouse ascites Golgi moiety, termed MAG. In this study, detailed analysis of multiple endometrial samples revealed that MAG has a unique pattern of expression in the normal hormonally responsive endometrium. Furthermore, MAG is present in various secretory epithelia and appears to be part of a large mucin-like glycoprotein. The specific temporal regulation and secretion of MAG by normal cycling endometrial glands suggests an important role for mucin-like glycoconjugates in early embryo-endometrial interactions.

Materials and Methods

Specimens

Endometrial tissue was examined from endometrial biopsies, endometrial curettings, and hysterectomies performed at the Hospital of the University of Pennsylvania, Yale-New Haven Hospital, and the Pacific Fertility Medical Center. A total of 201 specimens were examined, including specimens throughout the entire menstrual cycle. Endometrial specimens with stromal/glandular dyssynchrony, hyperplasia, atypia, or carcinoma were not included. Occasionally, patients were biopsied in sequential cycles, and one patient was biopsied five times during the follicular phase of one cycle. Tissues from other organs were examined from the files of both the Hospital of the University of Pennsylvania and the Yale-New Haven Hospital. Brain tissue was examined from resections performed at the Hospital of the University of Pennsylvania. The specimens were fixed in either Bouin's solution or 10% neutral buffered formalin overnight at room temperature and either embedded immediately or stored in 70% ethanol until embedding (usually within a few days of tissue collection). All human material was collected with the approval of the Institutional Review Boards at each institution.

Endometrial Dating

Standard hematoxylin and eosin stained sections were examined for dating. The endometrial samples were dated according to the general principles of Noyes et al,[20] as more fully detailed in Hendrickson and Kempson's[21] Decision Tree for Endometrial Dating. As described by these authors, only the portions of each biopsy from the functionalis layer were used for dating, and the most advanced area was used to assign the final date.

Ascites, Antibodies, and Lectins

Mouse ascites were generated by first priming male and female retired breeders with the mineral oil pristane (Sigma Chemical Co., St. Louis, MO), followed by intraperitoneal inoculation with 1×10^6 hybridoma cells (see Table 1) 10 to 14 days after the pristane injection. Mice were observed daily for ascites development. When sufficient fluid had accumulated in the peritoneal cavity (5 ml or more) of each

Table 1. *Frequency of MAG-Positive Ascites in Balb/c Mice**

Mouse	Sex	Hybridoma	MAG Reactivity
A	male	9018	–
B			–
C			–
D1	female	X18A4	+
D2			±
D3			–
E1	female	P20	–
E2			+
E3			+
E4			–
F1	male	9018	–
F2			–

*Ascites were generated in mice as described in Materials and Methods. The resultant ascites were tested by immunohistochemistry against known MAG-positive endometrial biopsies. –, no MAG staining; ±, weakly MAG-positive; +, strongly MAG-positive. Only ascites that were MAG-positive for endometrium were able to stain other MAG-containing tissues (*see Table 3*).

mouse, it was tapped by inserting an 18-gauge 1-inch needle intraperitoneally. Mice were repeatedly tapped in this way as long as the ascitic fluid reaccumulated and the animal remained healthy. A variety of ascites from both private and commercial sources was also tested (Table 2). Mouse ascites were used at 1:1000 dilutions. Anti-A, -B, and -O (H) monoclonal antibodies from Dako Corp. (Carpinteria, CA) were used, the former at a dilution of 1:1000, the latter two at 1:250. Biotinylated anti-mouse α, γ, and μ specific secondary antibodies from Vector Laboratories (Burlingame, CA) were used at a final concentration of 2.25 μg/ml, as instructed by the manufacturer. Biotinylated lectins from Vector were used at the following dilutions: *Ulex europaeus* I, 1:250; *Dolichos biflorus*, 1:500; *Bandeiraea simplicifolia*, 1:200; and *Vicia villosa*, 1:200.

Immuno- and Lectin Histochemistry

Sections (5 μ) from paraffin-embedded tissue were placed on glass slides previously coated with a film of 1% poly-d-lysine, 30,000 to 70,000 molecular weight (Sigma), dried for 30 minutes at temperatures no greater than 60 C, and stored at room temperature until used. Immunoperoxidase staining was performed by the avidin-biotin detection method with kits from Vector with diaminobenzidine (Sigma) as the chromogen. The temperature at which the immunoperoxidase procedure was performed proved to be critical. If the slides were incubated for as little as 10 minutes at 37 C in the blocking, primary, or secondary antibody steps, as much as 80% of the staining intensity was lost. The best protocol was achieved when blocking, secondary, and avidin-biotin complex steps were performed at 22 to 24 C for 45 to 60 minutes and the primary antibody step at 4 C overnight. Biotinylated lectins were incubated for either 1 hour at room temperature (20 to 22 C) or 4 C overnight, followed directly by avidin-biotin complex and then diaminobenzidine. Slides were counterstained with hematoxylin. Commercially available ascites, ascites acquired as gifts, and ascites generated by pristane priming followed by intraperitoneal hybridoma inoculation were tested for MAG activity (Tables 1 and 2). Those ascites that intensely stained select human endometrial gland Golgi were used to test the remaining specimens. Control slides were incubated with either undiluted P3X63Ag8 mouse myeloma cell line supernatant (American Type Culture Collection, Bethesda, MD) or control ascites that were unreactive to known positive endometrial samples. All immunohistochemical studies included known positive control slides.

Absorption Studies

For IgG absorption, 10 μl of MAG-positive and -negative ascites, diluted 1:10 in phosphate-buffered saline-0.1% bovine serum albumin, were added to 10 μl of protein G Sepharose beads (Pharmacia, Piscataway, NJ), incubated overnight at 4 C with gentle agitation, centrifuged, and used for immunohistochemistry. For glycoconjugate absorption, MAG-positive ascites was diluted to an IgG concentration of approximately 5 μg/ml. Between 2 and 4 μl of glycoconjugate were added to 100 μl of antibody solution to make a 1:1 molar ratio of IgG to glycoconjugate (Sigma), incubated overnight at 4 C, and centrifuged in a microcentrifuge at maximal speed for 5 minutes, and the resultant supernatant was used to perform MAG immunohistochemistry. Absorption studies with red blood cells RBCs were performed in a similar manner. Whole blood from types O, A, B, and AB individuals were washed two times with cold phosphate-buffered saline (150 mmol/L NaCl and 10 mmol/L phosphate buffer, pH 7.4) by centrifugation and pelleted, and the RBCs were diluted 1:1 with cold phosphate-buffered saline. A total of 50 μl of this 50% suspension was added to 100 μl of diluted MAG antibody solution, incubated overnight at 4 C, and centrifuged, and the supernatant was used to perform MAG immunohistochemistry.

Table 2. *MAG Reactivity of Ascites From Various Sources**

Designation	Strain:Sex	Source	MAG Reactivity
H4C4	$CB6F_1/J \times$ C57Black/BALB/c:unknown	J. Thomas August, Johns Hopkins University	+
H4.8	Balb/c:female	Stephen Warren, Yale University	–
A137	Not known	Adeza Biomedical Corporation, Sunnyvale, CA	+
Pooled Control	Balb/c:female	Zymed, San Francisco, CA	–
Pooled Control	Balb/c:female	Immunovision, Springdale, AR	+
Pooled Control	Not known	International Bio Sphere, Springdale, AR	+
NS-1, pooled	Balb/c:female	Biomakor, Rehovot, Israel	+
SP2/0	Balb/c:female	Harlan Bioproducts for Science, Indianapolis, IN	+
NMA	Balb/c:male and female	Sigma Chemical Coop St. Louis, MO	+

*Ascites acquired as gifts and commercial sources were tested for MAG reactivity by immunohistochemistry against known MAG-positive endometrial biopsies. –, no MAG staining; +, strongly MAG-positive.

Sialidase (from *Clostridium perfringens*) and α-*N*-acetylgalactosaminidase (from chicken liver; Oxford Glycosystems, Rosedale, NY)-treated ovine submaxillary mucin (OSM; a generous gift from V. P. Bhavanandan, Hershey Medical Center, Hershey, PA) was prepared according to the manufacturer's instructions. This treatment sequentially converted the OSM carbohydrate side chains from sialyl-(2′6)-*N*-acetyl-galactosamine (GalNAc)[22] to GalNAc to core protein free of carbohydrates.[23] GalNAc (Sigma) was used at a concentration of 200 mmol/L.

Immunoblotting

Immediately after hysterectomy, a uterus from a premenopausal 33-year-old gravida 1, para 1 woman (day 17 by dates) with a history of dysmenorrhea and menorrhagia was opened sterilely, and endometrial samples were removed and fixed for later histological dating. The remaining endometrial tissue was curetted and placed in 10 ml of Dulbecco's modified Eagles' medium, containing 25 mmol/L glucose and 25 mmol/L HEPES supplemented with gentamicin (50 μg/ml), glutamine (4 mmol/L), and 20% (v/v) heat-inactivated fetal calf serum, and transported to the laboratory. Pathological examination of the uterus revealed two small leiomyomas and day-15 endometrium by histological criteria. The freshly removed endometrium, 0.9 grams, was homogenized with a model PT 15/35 Brinkman homogenizer (Brinkman Instruments, Westbury, NY) for 3 minutes at a setting of 3.5 in 2 volumes of room temperature 4X solubilizing buffer (250 mmol/L Tris, 280 mmol/L sodium dodecyl sulfate, 160 mmol/L dithiothreitol, 40% glycerol (v/v), and 0.02% bromphenol blue, pH 6.8) and stored at 4 C until used. Saliva from healthy donors was collected into sterile 50-ml centrifuge tubes, immediately solubilized, 1:1 (v/v), with 2X solubilizing buffer with dithiothreitol and used immediately or stored at −80 C until used. From 25 to 100 μl of the solubilized endometrial tissue or saliva was electrophoresed on 6% sodium dodecyl sulfate polyacrylamide gel electrophoresis gels under reducing conditions. Rainbow protein molecular weight markers (Amersham, Arlington Heights, IL) were run on each gel. The gels were electrotransferred to nitrocellulose (Schleicher and Schuell, Keene, NH) overnight at 0.5 volts at 4 C, incubated with MAG-positive ascites as primary antibody (1:1000 dilution), undiluted P3X63Ag8 mouse myeloma cell line supernatant, or MAG-negative ascites as the negative controls, with immunodetection using a biotinylated anti-mouse IgG or γ-specific secondary antibody (ABC Vectastain, Vector Laboratories) and developed with the substrate diaminobenzidine.

Results

Identification and Characterization of Murine Ascites Containing Anti-MAG Antibodies

Our initial identification of MAG staining in normal cycling human endometrium followed a detailed immunohistochemical analysis with A-137,[19] an ascites generated with the anti-oncofetal fibronectin hybridoma FDC-6 (see Table 2). Initially, the highly specific staining pattern described below led us to conclude that oncofetal fibronectin was synthesized and secreted by the endometrium and that this adhesive protein was present on the endometrial surface at the initiation of implantation. However, studies comparing A-137 ascites with pure FDC-6 antibody obtained from hybridoma supernatant demonstrated that MAG immunostaining was not due to the FDC-6 antibody *per se* (Figure 1) but due to some other component in the ascites. We have also confirmed that the pure FDC-6 specifically identifies trophouteronectin, an implantation site oncofetal fibronectin isoform derived from trophoblasts.[24] In addition, the pure FDC-6 does not react with endometrium (Figure 1B). These results

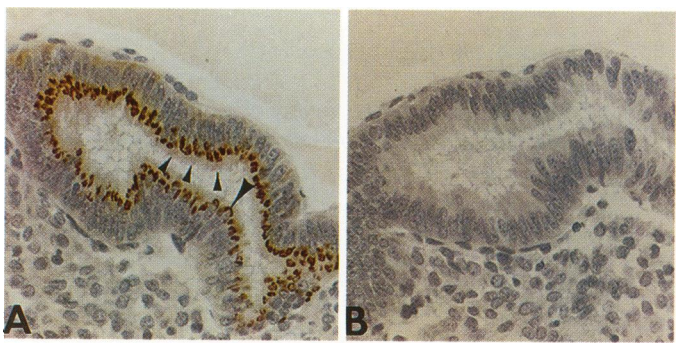

Figure 1. *MAG immunohistochemistry with ascites* versus *supernatant forms of FDC-6. Serial sections of formalin-fixed, paraffin-embedded normal human endometrium was stained with FDC-6 monoclonal antibody made as an ascites* (**A**) *or as a supernatant* (**B**). *Only the ascites form of the antibody* (**A**) *resulted in supranuclear immunoreactivity* (*MAG reactivity*), *suggesting that the anti-oncofetal fibronectin antibodies were not responsible for the MAG staining. Note the supranuclear, cap shape of the MAG-reactive material* (*large arrowhead*). *Also note the distance between the MAG-reactive material and the apical portion of the endometrial gland cells* (*small arrowheads*). *Magnification,* ×*350.*

suggest that the anti-oncofetal fibronectin antibodies were not responsible for the MAG staining.

When γ-, μ-, and α-chain-specific secondary anti-mouse antibodies were used in the immunohistochemical staining procedure against known MAG-positive tissue specimens, only γ-chain-specific secondary antibodies yielded Golgi staining with the MAG-positive ascites. This result suggested that the MAG staining component in the ascites was one or more IgG antibodies generated in the mice before or during the ascites manufacture. We confirmed that the MAG-reactive material is an IgG by demonstrating that protein G sepharose beads were able to absorb out the MAG-reactive material from known positive ascites (data not shown).

To evaluate whether the generation of the MAG-active IgG was specific to the sex or strain of mice, or possibly related to the hybridoma used to generate the ascites, we injected male and female Balb/c mice with a variety of hybridomas and tested the resultant ascites for MAG activity (Table 1). MAG-positive ascites was generated in 50% of the female mice but in none of the male mice tested. MAG antibody production appeared to be independent of the hybridoma injected to promote the ascites. In addition to our own ascites, we tested ascites from a variety of sources (Table 2). Analogous to our result with A-137, we identified a number of ascites with MAG immunoactivity, especially pooled ascites in which both MAG-reactive and nonreactive ascites were likely to have been blended together. This survey of MAG immunoreactivity demonstrated that MAG antibodies are not a universal phenomenon but are produced only by certain mice.

MAG Expression in the Normal Human Menstrual Cycle

Examination of 201 endometrial biopsies revealed that MAG expression was linked to the specific cycle phase of each sample. Of the 201 biopsies examined, MAG staining was demonstrated in 81 (40.3%). The earliest expression of MAG was on day 5 of the menstrual cycle (Figures 2 and 3). The staining first appeared in the endometrial glands in an apical perinuclear location—consistent with a Golgi staining pattern (Figure 2B, C). Biopsies on day 6 began to show similar perinuclear staining in the luminal surface glands. Between days 6 and 15, the Golgi staining became more diffuse throughout the specimens examined, and more intense in each individual gland. Maximal MAG staining for both endometrial glands and surface epithelium was on day 15 of an idealized 28-day cycle (Figure 2C). Beginning on day 16, when subnuclear vacuoles could be identified in greater than 50% of the glandular epithelial cells,[21] the MAG staining began to spread throughout the apical portion of the glandular cells, suggesting movement of the MAG epitope from the Golgi to secretory vesicles (Figure 2D). At this time, the MAG staining in the surface epithelium still remained within the Golgi. Beginning on day 17, MAG staining could be identified within the gland lumens (Figure 2E) and associated with scattered patches of apical surfaces on the luminal epithelial cells—possibly deposited there in part by secretion of the MAG-positive material out of the glands onto the luminal surface of the endometrium (Figure 2F). On days 18 and 19, almost no MAG activity remained in the Golgi of the gland cells but could be identified more frequently in the Golgi of the cells of the luminal epithelium. MAG staining could still be identified on luminal apical surfaces on day 19. After day 19, no MAG staining could be identified in the cells of the functionalis or surface epithelium, presumably because it had all been secreted from the epithelial cells. Occasionally, deeper basalis glands, which appeared morphologically to be either inactive or proliferative, could be MAG-positive during the later part of the menstrual cycle. Menstrual endometrium, although negative for MAG in the functionalis layer, also occasionally contained MAG-positive basalis glands.

Occasionally an endometrial biopsy showed variability of morphological date from one part of the biopsy to another. Interestingly, the MAG staining reflected the date of each individual gland, not an average of the entire biopsy. For example, in biopsy specimens that contained glands that spanned several days by histological dating, MAG staining also spanned these dates (Figure 4). These results suggest that MAG expression is related to the specific state of biochemical differentiation of each individual cell and not to the ambient conditions of the endometrium as a whole.

MAG Tissue Survey

We next examined a variety of other tissues to determine whether the MAG epitope was specific to the endometrium or was more generally distributed throughout the body (Table 3). MAG staining was found in a wide variety of tissues. It was mostly present in epithelial cells—especially secretory epithelium—and was not detectable in mesenchymal

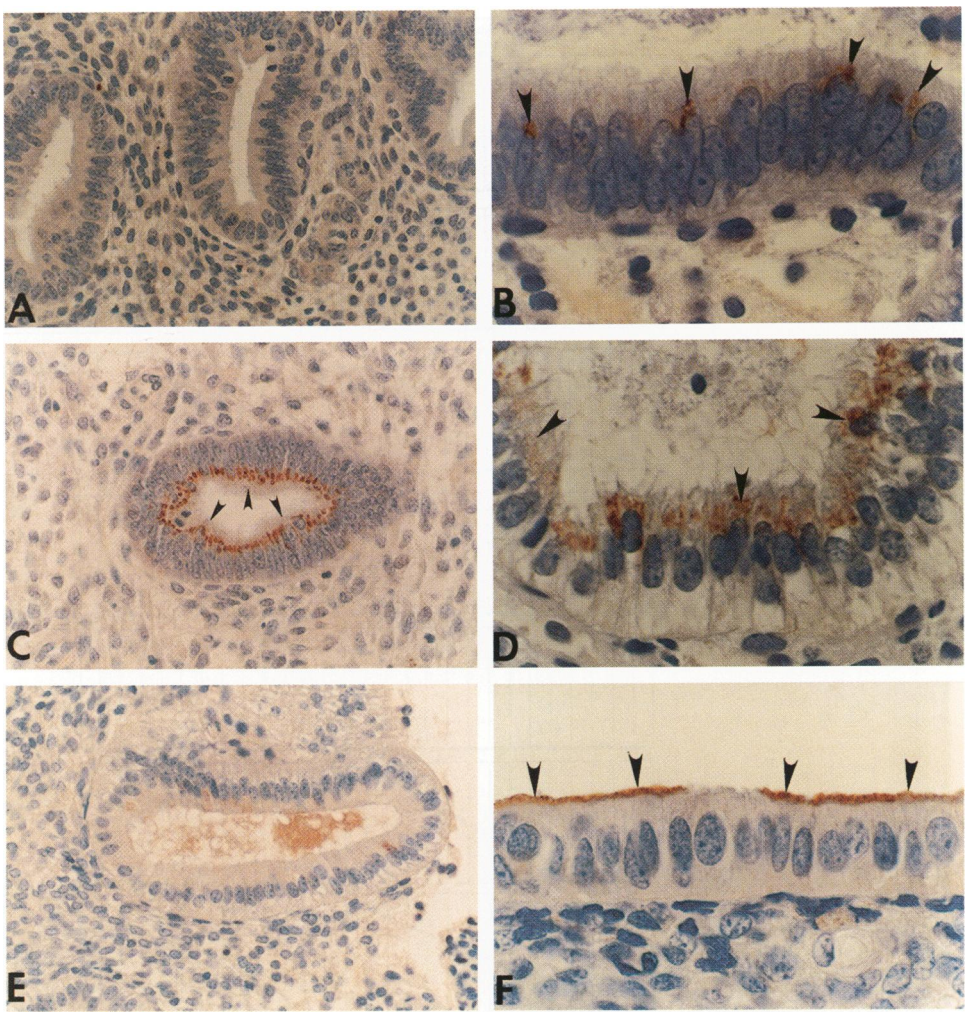

Figure 2. *MAG expression throughout the menstrual cycle. Endometrial biopsies were fixed and processed for MAG immunohistochemistry as described in Materials and Methods.* **A:** *No staining was noted in menstrual to day-4 endometria.* **B:** *Patchy, light Golgi staining (arrowheads) was first identified in glands on day 5.* **C:** *Golgi staining progressively increased in intensity during the proliferative phase to day 15 and could be identified in the Golgi of both endometrial glands and the surface epithelial cells. The staining was seen only close to the nuclei in a supranuclear distribution, leaving the apical cytoplasm free of staining (arrowheads).* **D:** *Day-16 and -17 endometria exhibited a loss of tight supranuclear glandular Golgi staining with an increase in diffuse apical staining (arrowheads), suggesting transport of MAG to apical secretory vesicles.* **E:** *Gland lumens on days 17 and 18 exhibited positively staining material, suggesting secretion of the MAG epitope.* **F:** *Day-19 endometria exhibited almost no glandular Golgi staining, had gland lumen secretion positivity, retained surface epithelium Golgi staining, and also exhibited occasional strong staining of the apical surface of luminal epithelial cells (arrowheads). Beyond day 19, no staining could be identified in either the glands or surface epithelial cells. Magnifications, ×180 (A, C, and E) and ×450 (B, D, and F).*

cells. One exception to this pattern was the staining of the perivascular astrocytes of the central nervous system. One of the common links between all of the sites that were MAG-positive was the ability of these cells to produce and secrete mucins. Mucins are high molecular weight glycoproteins containing peptide cores surrounded by numerous oligosaccharide chains.[25] The protein cores (ranging in molecular weight between 0.3×10^6 to 0.5×10^6 kd) are connected to each other via disulfide bonds, resulting in complex supramolecular structures that range in molecular weight between 10^6 and $>45 \times 10^6$ kd.[26] To examine whether the MAG antibody was recognizing a mucin-like glycoprotein, we next performed immunoblotting of MAG-containing extracts.

MAG Immunoblotting

When the endometrium from the day-15 hysterectomy specimen was electrophoresed under reducing conditions and immunostained with either control antibody or MAG-positive ascites, a high molecular weight smear (>200 kd) was seen only in the MAG-incubated blot (Figure 5). This smear is typical of highly glycosylated proteins and suggests a marked

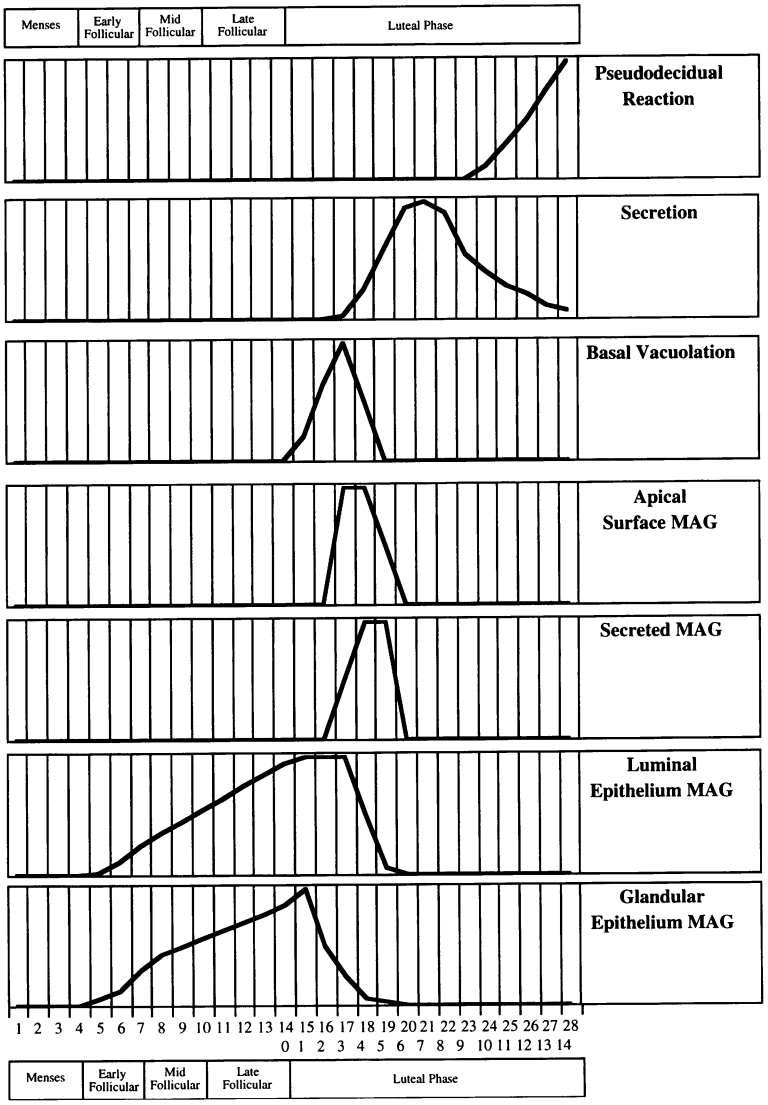

Figure 3. *MAG variation throughout the normal menstrual cycle. A total of 201 endometrial biopsies were immunohistochemically stained for MAG. Relative expression (from no staining to maximal staining) of MAG in glandular cells, surface epithelium, and lumens and on the apical surface of the luminal epithelium in the 81 biopsies that contained any MAG staining was plotted throughout an idealized 28-day ovarian cycle. Data for the top three graphs (basal vacuolation, secretion, and pseudodecidual reaction) were adapted from Noyes et al*[17] *and matched to the histological dating seen in the analyzed biopsy specimens.*

heterogeneity of glycosylations on each polypeptide core. Although many nonspecific lower molecular weight bands were noted in both the control and MAG-positive ascites blots, an additional band at approximately 35 kd was also seen in the MAG-treated membrane. This band may either represent a proteolytic fragment of the higher molecular weight band or a distinct MAG-reactive protein.

Because we had shown by immunohistochemistry that salivary gland Golgi stained with the MAG antibodies (Table 3), we postulated that saliva might contain MAG-reactive material. Saliva was collected from seven healthy adult male and female donors, immunoblotted, and stained with MAG-positive and MAG-negative ascites (Figure 6). All three male donors expressed MAG-positive saliva, whereas one out of the four females had MAG-positive saliva. The MAG-reactive bands appeared as smears from a molecular weight of >200 kd to approximately 70 kd. Why didn't all of the samples show MAG reactivity? When we obtained the blood types of the donors, we were surprised to find that only samples from blood type A individuals, male and female, contained any MAG-reactive material. At first we considered that this would limit the usefulness of the antibody, but further analysis proved that this result was actually an opportunity to elucidate the nature of the MAG epitope. This blood type association suggested that the MAG epitope may be related to the blood group A oligosaccharide. We therefore next set out to determine the oligosaccharide specificity of the MAG-positive ascites.

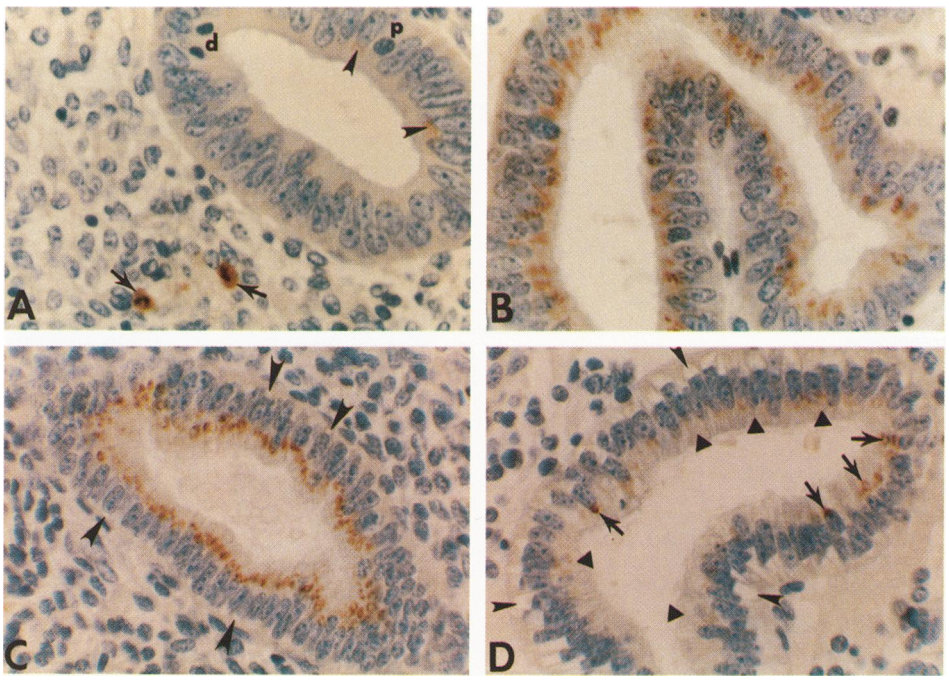

Figure 4. *MAG expression in endometrial glands is dependent on the maturity of each individual gland. Early proliferative* (A *and* B) *and early secretory* (C *and* D) *endometrial biopsies were fixed and stained for MAG as described in Materials and Methods. Every endometrial biopsy shows a small degree of variability throughout the specimen. When MAG expression was analyzed and compared with the specific histological pattern of each gland we found that MAG expression varied in relation to the histological date of each portion of the biopsy.* A: *Simple, early proliferative gland with surrounding stroma containing neutrophils (arrows), consistent with day 6. Only two glandular epithelial cells show any supranuclear MAG reactivity (arrowheads). Note daughter cells (d) and prophase nucleus (p), consistent with proliferation.* B: *Folded, early proliferative gland consistent with day 8 from same biopsy as shown in* A. *Virtually all of the glandular epithelial cells now show supranuclear MAG reactivity.* C: *Interval gland with a few, minute subnuclear vacuoles (arrowheads), consistent with a day-15 biopsy. Note uniform, strong supranuclear MAG staining throughout the entire gland.* D: *Early secretory gland showing almost uniform subnuclear vacuoles from a region near the gland shown in* C. *The fact that the nuclei are not lined up, but greater than 50% of the cells are vacuolated (eg, arrowheads) makes this gland consistent with day 16 (possibly 16.5). Unlike the day-15 gland in this same biopsy* (C), *this gland shows only a few cells with residual, tightly supranuclear MAG reactivity (arrows). Some cells show light, diffuse MAG reactivity (triangles; see also Figure 2D), suggestive of movement of the epitope into apical secretory vesicles. Magnification, ×350.*

Absorption Studies: Glycoproteins, RBCs, and GalNAc

Blood group-related oligosaccharides can be found on erythrocytes and glycoproteins, especially the mucin glycoproteins. Mucins are glycosylated through *O*-linked carbohydrates attached to either a serine or threonine in the protein core.[27] These oligosaccharides are similar to the carbohydrates seen on erythrocytes and terminate with several sugars, including galactose, GalNAc, fucose, and sialic acid. To determine whether a carbohydrate structure is a component of the MAG epitope, we next performed a series of absorption experiments with a variety of natural and enzymatically produced glycoconjugates as well as erythrocytes of the major blood types.

We examined the ability of *N*-linked, *O*-linked, and erythrocyte glycoconjugates, as well as the monosaccharide GalNAc, to neutralize MAG antibody from known positive ascites (Table 4), by immunohistochemical analysis of known MAG-positive endometrial biopsies. None of the *N*-linked glycoproteins absorbed out the MAG activity. Heparin and chondroitin sulfate, two members of the proteoglycan family, also had no effect. Types I and I-S bovine submaxillary mucins (which contain 12 and 5% terminal sialic acid, respectively) did not absorb any MAG activity. In contrast, type III porcine stomach mucin, which contains less than 1% sialic acid, was able to absorb out all of the MAG activity. Because this mucin contains oligosaccharides that resemble human blood group oligosaccharides,[28] we next examined the ability of erythrocytes (RBCs) from A, B, O, and AB donors to absorb out the MAG antibody activity. O erythrocytes contain the H oligosaccharide and terminate with a β-linked galactose. B erythrocytes express the H oligosaccharide with an additional terminal α-linked galactose. A erythrocytes differ from B cells by terminating with an α-linked GalNAc in place of galactose. AB cells contain a roughly equal mixture of A and B oligosaccharides. O and B RBCs had no effect on the anti-MAG activity, whereas A RBCs absorbed out all

Table 3. *Tissue Survey of MAG Reactivity**

Tissue	MAG Staining
Endometrial glands (menstrual cycle dependent)	+
Cervical glands	+
Fallopian tube epithelium	+
Ovary	–
Prostate glands	+
Seminal vesicle glands	+
Lacrimal glands	+
Pancreatic acinar cells	+
Pancreatic islets	–
Liver (parenchyma)	–
Intrahepatic bile ducts	+
Gall bladder epithelium	+
Common bile duct epithelium	+
Stomach mucosa	–
Duodenal mucosa	–
Duodenal submucosal glands	+
Small intestine mucosa	–
Large intestine mucosa	–
Kidney (proximal collecting ducts only)	+
Salivary glands (exocrine cells)	+
Breast (lobules, lactating, or nonlactating)	+
Breast (ducts)	–
Sweat glands (eccrine only)	+
Nasal mucosa glands	+
Bronchial glands	+
Smooth muscle	–
Skeletal muscle	–
Endothelial cells	–
Adipose tissue	–
Lymph node	–
Spleen	–
Squamous epithelium and mucosa (skin, oral, and vaginal)	–†
Brain (only perivascular astrocytes	+

*Tissues only from blood group A patients were processed and stained for MAG as described in Materials and Methods.

†For an unknown reason, occasional squamous epithelium specimens showed MAG reactivity in between the keratinocytes.

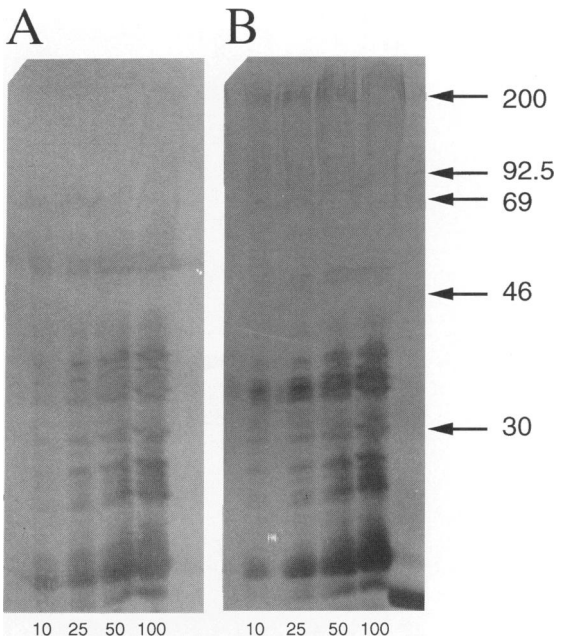

Figure 5. *Immunoblot showing MAG expression by day-15 human endometrium. Endometrial tissue (0.9 g) removed from a hysterectomy specimen was solubilized with sodium dodecyl sulfate and reducing agents. Amounts of 10, 25, 50, and 100 µl were loaded per lane in two sets of lanes. After electrophoresis, the proteins were electrotransferred from the gel to nitrocellulose and immunochemically stained with either* (A) *control mouse myeloma supernatant or* (B) *with an ascites with anti-MAG activity. Notice the diffuse band in gel* B *corresponding to the high molecular weight (>200 kd) MAG mucin-like glycoprotein. An approximately 35 kd band can also be seen in gel* B, *possibly representing a proteolytic fragment of the mucin-like glycoprotein or a distinct MAG-reactive protein. Molecular weight markers are in kd.*

of the MAG activity. AB RBCs absorbed out most, but not all, of the MAG activity.

These results suggested that GalNAc must be at least part of the MAG epitope. We therefore next investigated whether GalNAc alone would be sufficient to compete with the MAG activity. When GalNAc was added to the primary antibody at a concentration of 200 mmol/L, there was no effect, suggesting that, although GalNAc is necessary for MAG binding, it is not sufficient. Because the first carbohydrate in each *O*-linked glycoprotein is GalNAc linked to either a serine or threonine residue, we next evaluated whether a GalNAc linked to the protein core was sufficient to absorb out MAG or whether the full oligosaccharide with GalNAc as the terminal sugar was necessary for MAG absorption. To test these two possibilities, we used ovine submandibular mucin (OSM). OSM has the particular characteristic that it only contains the disaccharide sialyl-(2′6)-GalNAc linked to either a serine or threonine. By treating OSM with either sialidase alone or sialidase with α-*N*-acetylgalactosaminidase, one can produce OSM with only GalNAc attached or with no sugars attached. All forms of OSM, including native and enzymatically treated forms, were unable to absorb out the MAG activity (Table 4), suggesting that a single GalNAc attached to a mucin protein core is not the MAG epitope. It appears, therefore, that the MAG epitope is closely related to the blood group A oligosaccharide that is found on type A RBCs and material containing mucin oligosaccharides that terminate with a GalNAc.

MAG Staining and the Blood Type of the Patient

We speculated that the apparent A oligosaccharide specificity of MAG might explain why we could identify MAG staining in only 40.3% of the endometrial biopsies examined (see above). When we sorted the immunohistochemical results according to blood type, the correlation became clearer (Table 5). We

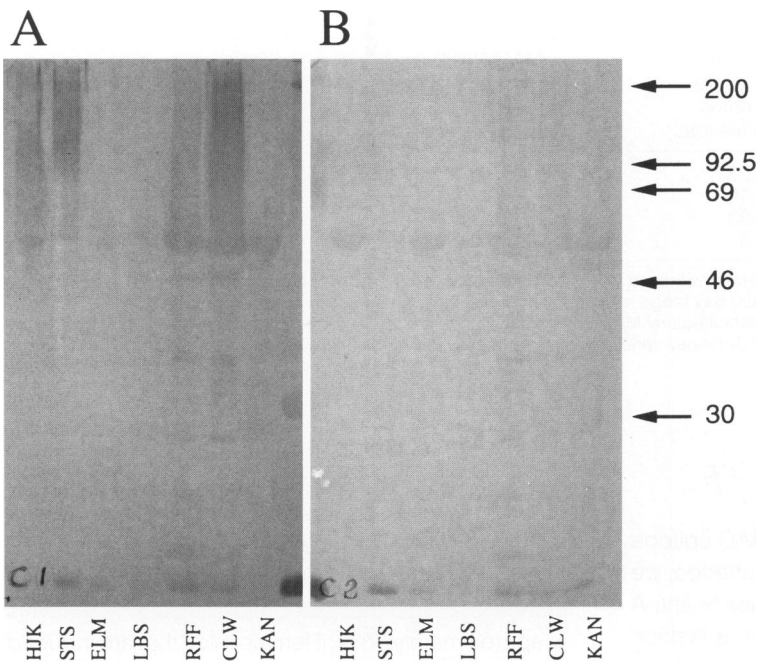

Figure 6. *Immunoblot showing MAG expression in saliva. Saliva from seven normal adults, three male (HJK, RFF, and CLW) and four female (STS, ELM, LBS, and KAN), was immunoblotted in two parallel sets for MAG expression as described in Materials and Methods. The sets were separated and reacted with either MAG-positive ascites* (**A**) *or MAG-negative ascites* (**B**). **A** *Several of the samples revealed a MAG-reactive smear between >200 kd to approximately 70 kd. Only blood type A individuals (HJK, STS, RFF, and CLW) expressed the high molecular weight smear. Although weak in this particular blot, a distinct band was always seen with the RFF sample. ELM is blood type B, whereas LBS and KAN are blood type O. Notice that the approximately 35-kd band that was seen in the endometrial sample (Figure 5B) was not seen here.* **B:** *No high molecular weight smear was seen in the MAG-negative blot for any of the samples. Very light, identical nonspecific bands were noted in both MAG-positive and MAG-negative blots.*

Table 4. *Absorption Studies*

Glycoconjugate/Sugar	Linkage	Terminal carbohydrates†	Absorbs MAG?
Ovalbumin	GlcNAc-Asp	Gal, GlcNAc, Man	No
α1-Acid glycoprotein	GlcNAc-Asp	Gal	No
Bovine serum albumin	GlcNAc-Asp	Gal, GlcNAc, Man	No
Heparin	Xylose-Ser	$GlcNSO_4$, GlcUA, GlcNAc	No
Chondroitin sulfate	Xylose-Ser	GlcUA	No
Type I* bovine submaxillary mucin	GalNAc-Ser(Thr)	Sialic Acid (12%), Gal, GlcNAc, GalNAc	No
Type I-S* bovine submaxillary mucin	GalNAc-Ser(Thr)	Sialic Acid (5%), Gal, GlcNAc, GalNAc	No
Type III* porcine stomach mucin	GalNAc-Ser(Thr)	Sialic Acid (1%), Gal, GlcNAc, GalNAc	>95%
Type O RBCs	GalNAc-Ser(Thr)	Galβ-GlcNAc	No
Type B RBCs	GalNAc-Ser(Thr)	Galα-Galβ-GlcNAc	No
Type A RBCs	GalNAc-Ser(Thr)	GalNAcα-Galβ-GlcNAc	>95%
Type AB RBCs	GalNAc-Ser(Thr)	Galα-Galβ-GlcNAc GalNAcα-Galβ-GlcNAc	~50%
GalNAc			No
OSM	GalNAc-Ser(Thr)	Sialyl-GalNAc-	No
OSM, Sialidase Rx'd	GalNAc-Ser(Thr)	GalNAc	No
OSM, Sialidase & GalNAcase Rx'd	Ser(Thr)	None	No

† Gal, galactose; GlcNAc, *N*-acetyl-glucosamine; Man, mannose; $GlcNSO_4$, glucosamine sulfate; GlcUA, glucuronic acid; Sialyl, *N*-acetyl neuraminic acid[29]
*Sigma designation

were able to positively determine the blood types of only 90 of the patients examined. Of these, 29 were A, 13 were B, 45 were O, and 3 were AB, in general agreement with the distribution of ABO blood types in the United States.[30] All of the biopsies from the blood group A patients between cycle days 5 and 19 were MAG (Golgi)-positive (23 of 23). In addition, 5 of the 10 blood type A and 2 of the 2 blood type AB biopsies between days 17 and 19 had apical surface MAG reactivity. In contrast, all the biopsies from patients with blood groups B and O (n = 58) were MAG (Golgi)-negative at all times of the cycle, as were all day-20 to -28 and menstrual biopsies from all blood types. Interestingly, 3 non-A biopsies from days 17 to 19, 1 from a blood group B patient and 2 from blood group O patients, did show MAG reactivity but only in the region of the apical surface of the luminal epithelium. These results suggest that, although blood group B and O patients are not capable of generating the MAG epitope in their Golgi, they may be capable of generating the epitope on their surface epithelium between days 17 and 19 of the menstrual cycle.

Table 5. *MAG Staining and Patient Blood Type**

Patient blood type	Number (%) of patients (total = 90)	MAG-Positive day-5 to -19 endometria (No. positive/total)	MAG-Positive menstrual and day -20 to -28 endometria (No. positive/total)	Day-17 to -19 endometria with MAG-positive surface (No. positive/total)
A	29 (32%)	23/23	0/6	5/10
B	13 (14%)	0/10	0/3	1/2
O	45 (50%)	0/30	0/15	2/6
AB	3 (3%)	3/3	0/0	2/2

*Patient blood types of 90 of the total of 201 biopsies examined were able to be obtained from Blood Bank records. The number of MAG-positive biopsies out of the number of biopsies in each day range is expressed for days 5 to 19 and menstrual and days 20 to 28. A separate listing of the biopsies between days 17 and 19 that exhibited any MAG epitope staining of the apical surface luminal epithelium is given in the last column. The amount of surface epithelium in each biopsy varied greatly and may partly explain why not all of these day-17 to -19 biopsies expressed luminal apical MAG.

Anti-Blood Group and Lectin Immunohistochemistry

Because we now had evidence that the MAG epitope was similar to the blood group A oligosaccharide, we investigated whether other anti-A antibodies or anti-A lectins might stain human endometrium in a fashion similar to the MAG antibody. Anti-A monoclonal antibody stained tissue from blood group A patients but not from B or O patients (data not shown). When these endometrial biopsies were examined, staining could be identified in the endothelial cells, RBCs, lumens of the vessels, surfaces of the glands, surface epithelium, and occasionally the Golgi of the glandular epithelial cells. Antibodies to blood group B and O antigens stained the endothelial cells and RBCs of the corresponding blood type patients, stained the surfaces and occasionally the lumens of the glands, but did not stain the Golgi of any of the endometrial biopsies examined. Anti-A lectins (*D. biflorus*, *V. villosa*, and *B. simplicifolia*) produced similar results with endothelial cells, vessel lumens, and cell surfaces of the endometrial glandular epithelium. Only *V. villosa* stained any of the Golgi in the endometrial epithelial cells. Although GalNAc had no inhibitory effect when added to the MAG-positive ascites at 200 mmol/L (Table 4), GalNAc at this same concentration completely abolished the lectin staining of all the tissue samples examined. These results suggest that MAG staining is a specific subset of the staining seen with anti-A antibody and *V. villosa* and that neither anti-A antibodies nor lectins can selectively react with the MAG epitope.

Discussion

Examination of an unexpected Golgi-reactive immunoglobulin in mouse ascites has led us to characterize a menstrual cycle-dependent mucin-like glycoprotein in human endometrium. MAG first appears in endometrial gland Golgi as early as day 5, is secreted on days 16 to 18, and becomes apparent on the apical aspect of the surface epithelium only on days 17 to 19. The antibody that recognizes the MAG substance is an IgG that can be identified in high levels in approximately 50% of female mice that are induced to form pristane-hybridoma ascites. None of the five male mice we used to make ascites formed MAG antibodies. The menstrual cycle distribution of MAG appeared similar when different active ascites were used to perform the immunohistochemical studies. Our data do not exclude the possibility that different ascites react with slightly different epitopes, or even different proteins, albeit with the same endometrial staining pattern.

Our results suggest that MAG antibodies bind to a mucin-associated moiety, on the basis of the following observations: 1), the tissue distribution survey demonstrated MAG in many cells and tissues of endodermal origin that are known to secrete mucins[31]; 2), the absorption results that showed that only material that contained mucins with blood group A oligosaccharides were effective in absorbing out the MAG activity, specifically, an extract of porcine gastric mucins and A and AB erythrocytes (which contain the transmembrane mucin glycophorin); and 3), the immunoblots that exhibited high molecular weight smears, a pattern typical of highly glycosylated glycoproteins such as mucins. An interesting exception to the endodermal association of MAG-reactive tissues was the finding of MAG reactivity in the perivascular astrocytes of the brain. Although not normally thought of as a site of mucin production, a high molecular weight mucin related to the Tamm-Horsfall protein has been identified at this site[32] where it may function in the blood-brain barrier.

Although we have demonstrated the critical nature of a terminal GalNAc residue in the MAG epitope, it

appears that the MAG antibody is identifying more than just GalNAc inasmuch as a single GalNAc residue attached to a mucin peptide backbone or GalNAc alone is not sufficient to absorb or compete with the MAG activity. In addition, anti-A antibody and GalNAc-reactive lectins do not stain in a MAG-like pattern. It appears, therefore, that MAG reactivity is a subset of more general GalNAc probes. The MAG epitope may include more proximal carbohydrates and possibly a portion of the mucin polypeptide backbone.

Previous Descriptions of MAG-Like Activity and Blood Group A-Like Antigens

MAG-like antibodies appear to have been noted previously, sometimes without inferences being drawn by the investigators. The first example of an endogenous antibody in mouse ascites with glycoprotein reactivity was described by Gooi and Feizi.[33] These workers demonstrated that many samples of ascites contained natural antibodies that reacted with fetal glycoproteins (meconium) and other blood group substances. The first definitive characterization of an endogenous mouse ascites Golgi-reactive antibody was made by Smith et al,[34] who showed that ascites and serum from multiple strains of mice contained endogenous IgG antibodies to Golgi-specific epitopes. They proved by monensin treatment and electron microscopy that these antibodies reacted specifically with the Golgi of pancreatic acinar cells and with many other exocrine tissues in the rat. Interestingly, they observed that the titer of these anti-Golgi antibodies increased with the length of time the mice were kept in captivity, suggesting to them that the antigenic stimulation came from some environmental exposure. It is also of interest to note that mucins are particularly immunogenic in mice.[27]

The next demonstration of MAG-like activity came in a series of papers from several laboratories that were working on P-glycoprotein,[35–39] an integral membrane pump that mediates multidrug resistance in tumor cells. The common finding in this work was that ascites antibodies from two different commercial sources, purportedly specific for P-glycoprotein, stained the Golgi of endometrial glands. Although P-glycoprotein was known to be a surface glycoprotein, these workers speculated that the endometrium had a specialized Golgi form of the protein. As this work developed, the staining seemed to be related to the blood group of the patient from whom the tissue was derived.[35,36] Interestingly, Axiotis et al[37] demonstrated that their P-glycoprotein staining was menstrual cycle dependent in the endometrium and that staining peaked on days 15 and 16 of the cycle. The meaning of this work became clear from the elegant studies of Finstad et al[40] who demonstrated that several of the particular lots of ascites antibodies used by these P-glycoprotein workers contained high titers of an endogenous IgG anti-A blood group-specific antibody that was responsible for the Golgi staining pattern described previously. The MAG antibody we describe here appears to be similar, if not identical to, that described by Finstad et al.[40] In spite of the caution implied by that work, papers continue to be published showing Golgi staining for P-glycoprotein in endometrial gland Golgi with the same source of antibodies.[41]

Does Endometrial MAG Production Correlate with Other Endometrial Mucin Epitopes?

Other mucin glycoprotein epitopes have been identified in the human endometrium.[42] TAG-72 (tumor-associated glycoprotein) is a mucin glycoprotein found in malignant and normal human tissues. In the human endometrium it is not detectable by immunohistochemistry during the proliferative phase, first appears around or shortly after the time of ovulation, then peaks during the late luteal phase.[43,44] The cellular distribution of TAG-72 appeared to be apical and cytoplasmic. CA-125, an epitope on a mucin glycoprotein found in many epithelial tissues of Müllerian origin,[45] has been shown to have maximal expression during the early proliferative and mid-secretory phase, times when MAG levels are low, and lowest during the early secretory phase, a time at which MAG expression is high.[46,47] In addition to the temporal differences between CA-125 and MAG, CA-125, unlike MAG's supranuclear distribution, is present in the infranuclear region of the epithelial cells during the proliferative phase. A mucin sialoglycoprotein identified by monoclonal antibody D9B1 has also been identified in human endometrium.[48] The D9B1 epitope first appears on approximately day 15 in a basal distribution, peaks on day 20, and then is seen in the gland lumens on days 21 and 22, a pattern different from that of MAG (Figure 2). In addition to these specific mucin glycoproteins, Ravn et al[49] have shown that human endometrium expresses a mucin type-3 chain A antigen in a menstrual cycle-dependent fashion. Using the HH5 antibody, these workers demonstrated that this A antigen was first expressed in mid-proliferative endometrium, peaked

during late proliferative and interval phases, but reappeared during the mid-secretory phase, making it similar but not identical to the MAG epitope. All of these epitopes, although apparently part of high molecular weight glycoproteins, do not appear to parallel exactly the expression of MAG in the human endometrium.

In contrast, Rye et al,[50] using two different monoclonal antibodies, have recently described the immunohistochemical expression of polymorphic epithelial mucin in the human endometrium. One antibody (NCRC 11), which reacts with a human breast cancer-associated antigen,[51] showed staining only in the luteal phase, much like TAG-72 and D9B1. The other antibody (HMFG 1), which reacts with the milk fat globule membrane antigen,[52] stained endometrial glands between days 5 and 18, as does MAG. The similarity in menstrual cycle expression between these two epitopes suggests that MAG may be related or identical to what has now come to be known as the MUC-1 family of mucin glycoproteins.[27]

The MUC-1 mucins are a family of highly glycosylated, high molecular weight (>200 kd) glycoproteins present on the surfaces of many epithelial cells.[27,28] The basic MUC-1 structure consists of a straight protein backbone that contains many highly glycosylated regions. Carbohydrates are attached to the mucin protein backbone via an α1,3 linkage between *N*-acetyl galactosamine and the oxygen atom of serine or threonine. Many of the carbohydrates found on epithelial mucin glycoproteins are similar to the blood group oligosaccharides. MUC-1 synthesis has been shown to be hormonally regulated in mammary epithelial cells.[53] More recently, additional types of MUC-1 mucin molecules have been described, the membrane-associated mucins.[54] These include such molecules as leukosalin (CD43), glycophorin, ascites sialoglycoprotein-1 (ASGP-1), epiglycanin, and episialin. Episialin, like some of the mucins described above, is encoded by MUC-1 and has been defined by a variety of names, including polymorphic epithelial mucin (PEM) and epithelial membrane antigen (EMA). Unlike regular mucins, which are secreted by a variety of cells and combine to form large polymeric gels in the extracellular space, membrane-associated mucins remain attached to the surfaces of cells where they may act as ligands for a variety of receptor molecules.[55] For example, a membrane-associated mucin (GlyCAM-1) found on the apical surface of endothelial cells of high endothelial venules in lymph nodes is the ligand for lymphocyte L-selectin,[56] whereas leukosalin, a neutrophil membrane-associated mucin, interacts with the endothelial lectin domain-containing protein CD62.[55,57] Once these mucin-lectin interactions are initiated, tighter, integrin-mediated interactions are established.[58]

A Role in Implantation?

Could similar mucin-lectin interactions take place between the conceptus and the endometrium during the earliest phase of implantation? The work of Lindenberg suggests this possibility.[1,10–12] Like the lacto-*N*-fucopentaose I epitope found in the mouse endometrium, one function for mucin glycoproteins such as MAG may be to promote embryo-endometrial interaction. This hypothesis proposes that lectin-like proteins exist on the human preimplantation embryo, as has been shown for the macrophage asialoglycoprotein-binding protein,[59] and that perhaps this lectin specifically reacts with the MAG epitope. If this is so, how do type O and B women acquire the terminal *N*-acetyl galactosamine necessary to make up this epitope? Our finding of the MAG epitope on the apical surface of the luminal epithelium in some type O and B patients suggests the possibility of an enzyme or enzymes that are capable of modifying or adding to pre-existing oligosaccharides on the cell surface. Glycotransferases, usually thought of as Golgi-localized oligosaccharide synthesizers, are also found on cell surfaces and the extracellular space.[60–62] Therefore, it is possible that a cell surface glycotransferase in a non-A endometrium may be able to convert a cell surface mucin oligosaccharide to a MAG mucin-containing oligosaccharide. Alternatively, the presence of apical surface epithelial staining in type O and B patients may reflect the presence of additional IgGs in the ascites that are specific for these two blood types. Additional work will be necessary to assess these possibilities.

The MAG glycoprotein, observed at first as an artifact, appears to be a hormonally regulated mucin-like glycoprotein expressed on the endometrial surface during the purported implantation window. Like the lacto-*N*-fucopentaose I epitope described in the mouse,[1] the essential GalNAc epitope recognized by the MAG antibody may be important for the initial interaction between the human conceptus and the endometrium. Interestingly, endometrial integrins $\beta 3$[63] and $\alpha 4$[64] are also expressed during the implantation window. Thus, as has been demonstrated for neutrophil-endothelial interactions,[55,57,58] sequential interactions between endometrial mucins and trophoblast lectins followed by integrin-mediated adhesion could contribute to a fertile, receptive endometrium. Prospective studies correlating MAG

expression with ovulation induction and assisted reproduction technology protocols should help us understand the relationship between the MAG epitope and successful implantation in humans.

Acknowledgments

We thank Drs. Frederick Naftolin, Jon S. Morrow, David L. Olive, Alan S. Penzias, David Keefe, and Eugene Davidson for helpful comments on this work; Minxia Liu, Edward Buchanan, and Cai-Liang Wang for their excellent technical assistance; the histotechnologists in the Departments of Pathology at the Hospital of the University of Pennsylvania and the Yale-New Haven Hospital for their continuous efforts in preparing unstained slides for this work; Drs. J. Thomas August, Stephen Warren, and the many companies for supplying ascites samples; Dr. V. P. Bhavanandan for supplying the OSM; Dr. Bernice W. Kliman for proofreading the manuscript; and especially Sandra T. Stein for her unceasing willingness to supply repeated control endometrial samples for these studies.

References

1. Lindenberg S: Ultrastructure in human implantation: transmission and scanning electron microscopy. Bailliere's Clin Obstet Gynaecol 1991, 5:1–14
2. Vanderhyden BC, Armstrong DT: Decreased embryonic survival of in-vitro fertilized oocytes in rats is due to retardation of preimplantation development. J Reprod Fertil 1988, 83:851
3. Navot D, Scott RT, Droesch K, Veeck LL, Liu HC, Rosenwaks Z: The window of embryo transfer and the efficiency of human conception *in vitro*. Fertil Steril 1991, 55:114–118
4. Navot D, Bergh PA, Williams M, Garrisi GJ, Guzman I, Sandler B, Fox J, Schreiner EP, Hofmann GE, Grunfeld L: An insight into early reproductive processes through the *in vivo* model of ovum donation. J Clin Endocrinol Metab 1991, 72:408–414
5. Harper MJ: The implantation window. Baillieres Clin Obstet Gynaecol 1992, 6:351–371
6. Aplin JD: Glycans as biochemical markers of human endometrial secretory differentiation. J Reprod Fertil 1991, 92:525–541
7. Carson D, Farrar JD, Laidlaw J, Wright DA: Selective activation of the *N*-glycosylation apparatus *in uteri* by estrogen. J Biol Chem 1990, 265:2947–2955
8. Munakata H, Isemura M, Yosizawa Z: Enzymatic sulfation of exogenous high molecular weight glycopeptides by microsomal fraction of the rabbit uterine endometrium. J Biol Chem 1985, 260:6851–6856
9. Mani SK, Carson DD, Glasser SR: Steroid hormones differentially modulate glycoconjugate synthesis and vectoral secretion by polarized uterine epithelial cells *in vitro*. Endocrinology 1992, 130:240–248
10. Enders AC, Schlafke S: Surface coat of the mouse blastocyst and the uterus during the preimplantation period. Anat Rec 1974, 181:31–46
11. Hewitt K, Beer AE, Grinnell F: Disappearance of anionic sites from the surface of the rat endometrial epithelium at the time of blastocyst implantation. Biol Reprod 1979, 21:691–707
12. Anderson TL, Olsen GE, Hoffman LH: Stage specific alterations in the apical membrane glycoproteins on endometrial epithelial cells related to implantation in rabbits. Biol Reprod 1986, 34:701–720
13. Chavez DJ, Anderson TL: The glycocalyx of mouse uterine luminal epithelium during estrus, the periimplantation period, and delayed implantation. I. Acquisition of RCA-1 binding sites during pregnancy. Biol Reprod 1985, 32:1135–1142
14. Lindenberg S: Experimental studies on the initial trophoblast endometrial interaction. Dan Med Bull 1991, 38:371–380
15. Kimber SJ, Lindenberg S: Hormonal control of a carbohydrate epitope involved in implantation in mice. J Reprod Fertil 1990, 89:13–21
16. Lindenberg S, Sundberg K, Kimber SJ, Lundblad A: The mil oligosaccharide, lacto-*N*-fucopentaose I, inhibits attachment of mouse blastocysts on endometrial monolayers. J Reprod Fertil 1988, 83:149–158
17. Sato M, Muramatsu T, Berger EG: Immunological detection of cell surface galactosyltransferase in preimplantation mouse embryos. Dev Biol 1984, 102:514–518
18. Miller DJ, Macek MB, Shur BD: Complementarity between sperm surface β-1,4-galactosyltransferase and egg-coat ZP3 mediates sperm-egg binding. Nature 1992, 357:589–593
19. Feinberg RF, Kliman HJ, Lockwood CL: Is oncofetal fibronectin a trophoblast glue for human implantation? Am J Pathol 1991, 138:537–543
20. Noyes RW, Hertig AT, Rock J: Dating the endometrium. Fertil Steril 1950, 1:3–25
21. Bennington JL: Surgical pathology of the uterine corpus. Major Prob Pathol 12:80–85
22. Graham ERB, Gottschalk A: Studies on mucoproteins. I. The structure of the prosthetic group of ovine submaxillary gland mucoprotein. Biochim Biophys Acta 1960, 38:513–524
23. Gottschalk A, Schauer H, Uhlenbruck G: Immunological properties of ovine submaxillary glycoprotein. Hoppe-Seyler's Z Physiol Chem 1971, 352:117–124
24. Feinberg RF, Kliman HJ: Tropho-uteronectin (TUN): a unique oncofetal fibronectin deposited in the extracellular matrix of the tropho-uterine junction and regulated *in vitro* by cultured human trophoblasts. Trophoblast Res 1993, 7:167–179
25. Silbergerg A, Meyer FA: Structure and function of mucus. Mucus in Health and Disease II. Edited by EN

Chantler, JB Elder, and M. Elstein. New York, Plenum Press, 1982, p 53–74
26. Carlstedt I, Sheehan JK: Macromolecular properties and polymeric structure of mucus glycoproteins. Ciba Found Symp 1984, 109:157–172
27. Devine PL, McKenzie FC: Mucins: structure, function, and associations with malignancy. BioEssays 1992, 14:619–625
28. Rose MC: Mucins: structure, function, and role in pulmonary diseases. Am J Physiol 1992, 263:L413–L429
29. Rodén L: Structure and metabolism of connective tissue proteoglycans. The Biochemistry of Glycoproteins and Proteoglycans. Edited by WJ Lennarz. New York, Plenum Press, 1980, pp 267–371
30. Walker, RH (Ed): ABO, H and P Blood Groups and Structurally Related Antigens: Technical Manual, ed 11. Bethesda, MD, American Association of Blood Banks, 1993, p 204
31. Strous GJ, Dekker J: Mucin-type glycoproteins. Crit Rev Biochem Mol Biol 1992, 27:57–92
32. Zalc B, Collet A, Monge M, Ollier HMP, Jacque C, Hartmann L, Baumann NA: Tamm-Horsfall protein, a kidney marker is expressed on brain sulfogalactosylceramide-positive astroglial structures. Brain Res 1984, 291:182–187
33. Gooi HC, Feizi T: Natural antibodies as contaminants of hybridoma products. Biochem Biophys Res Commun 1982, 106:539–545
34. Smith ZD, D'Eugenio-Gumkowski F, Yanagisawa K, Jamieson JD: Endogenous and monoclonal antibodies to the rat pancreatic acinar cell Golgi complex. J Cell Biol 1984, 98:2035–2046
35. Weinstein RS, Coon JS, Dominquez JM, Jakate SM, Lebovitz MD, Chang MA, Kluskens LF: Correlation between ABO blood type and Golgi P-glycoprotein expression in epithelia (letter). Lancet 1990, 336:54–55
36. Weinstein RS, Kuszak JR, Jakate SM, Lebovitz MD, Kluskens LF, Coon JS: ABO blood type predicts the cytolocalization of anti-P-glycoprotein monoclonal antibody reactivity in human colon and ureter. Hum Pathol 1990, 21:949–958
37. Axiotis CA, Guarch R, Merino MJ, Laporte N, Neumann RD: P-glycoprotein expression is increased in human secretory and gestational endometrium. Lab Invest 1991, 65:577–581
38. Axiotis CA, Monteagudo C, Merino MJ, LaPorte N, Neumann RD: Immunohistochemical detection of P-glycoprotein in endometrial adenocarcinoma. Am J Pathol 1991, 138:799–806
39. Finstad CL, Saigo PE, Rubin SC, Federici MG, Provencher DM, Hoskins WJ, Lewis JLJ, Lloyd KO: Immunohistochemical localization of P-glycoprotein in adult human ovary and female genital tract of patients with benign gynecological conditions. J Histochem Cytochem 1990, 38:1677–1681
40. Finstad CL, Yin BW, Gordon CM, Federici MG, Welt S, Lloyd KO: Some monoclonal antibody reagents (C219 and JSB-1) to P-glycoprotein contain antibodies to blood group A carbohydrate determinants: a problem of quality control for immunohistochemical analysis. J Histochem Cytochem 1991, 39:1603–1610
41. Schneider J, Efferth T, Centeno MM, Mattern J, Rodriguez-Escudero FJ, Volm M: High rate of expression of multidrug resistance-associated P-glycoprotein in human endometrial carcinoma and normal endometrial tissue. Eur J Cancer 1993, 29A:554–8
42. Fay TN, Grudzinskas JG: Human endometrial peptides: a review of their potential role in implantation and placentation. Hum Reprod 1991, 6:1311–1326
43. Thor A, Viglione MJ, Muraro R, Ohuchi N, Schlom J, Gorstein F: Monoclonal antibody B72.3 reactivity with human endometrium: a study of normal and malignant tissues. Int J Gynecol Pathol 1987, 6:235–247
44. Lessey BA, Pidzola JA: Tumor-associated glycoprotein (TAG-72) in endometriotic implants. J Clin Endocrinol Metab 1993, 1075–1079
45. Kabawat SE, Bast RC, Welch WR, Knapp RC, Colvin RB: Immunopathologic characterization of a monoclonal antibody that recognizes common surface antigens of human ovarian tumors of serous, endometrioid, and clear cell types. Am J Clin Pathol 1983, 79: 98–104
46. Weintraub J, Bischof P, Tseng L, Redard M, Vassilakos P: CA 125 is an excretory product of human endometrial glands. Biol Reprod 1990, 42:721–726
47. Zeimet AG, Müller-Holzner E, Marth C, Daxenbichler G, Dapunt O: Tumor marker CA-125 in tissues of the female reproductive tract and in serum during the normal menstrual cycle. Fertil Steril 1993, 59:1028–1035
48. Aplin JD: Glycans as biochemical markers of human endometrial secretory differentiation. J Reprod Fertil 1991, 92:525–541
49. Ravn V, Teglbjaerg CS, Visfeldt J, Bock JE, Sorensen H, Dabelsteen E: Mucin-type carbohydrates (type 3 chain antigens) in normal cycling human endometrium. Int J Gynecol Pathol 1992, 11:38–46
50. Rye PD, Bell SC, Walker RA: Immunohistochemical expression of tumour-associated glycoprotein and polymorphic epithelial mucin in the human endometrium during the menstrual cycle. J Reprod Fertil 1993, 97: 551–556
51. Ellis IO, Robins RA, Elston CW, Blamey RW, Ferry B, Baldwin RW: A monoclonal antibody, NCRC 11, raised to human breast carcinoma. I. Production and immunohistological characterization. Histopathology 1984, 8:501–506
52. Taylor-Papadimitriou J, Peterson J, Arklie J, Burchell J, Ceriani RL, Bodmer WF: Monoclonal antibodies to epithelium-specific components of the human milk fat globule membrane: production and reaction with cells in culture. Int J Cancer 1981, 28:17–21
53. Parry G, Li J, Stubbs J, Bissell MJ, Schmidhauser C, Spicer AP, Gendler SJ: Studies of Muc-1 mucin expression and polarity in the mouse mammary gland demonstrate developmental regulation of Muc-1 glycosylation and establish the hormonal basis for mRNA

expression. J Cell Sci 1992, 101:191–199
54. Hilkens J, Ligtenberg MJL, Vos HL, Litvinov SV: Cell membrane-associated mucins and their adhesion-modulating property. Trends Biochem Sci 1992, 17: 359–363
55. Shimizu Y, Shaw S: Mucins in the mainstream. Nature 1993, 366:630–631
56. Lasky LA, Singer MS, Dowbenko D, Imai Y, Henzel WJ, Grimley C, Fennie C, Gillett N, Watson SR, Rosen SD: An endothelial ligand for L-selectin is a novel mucin-like molecule. Cell 1992, 69:927–938
57. Williams AF: Out of equilibrium. Nature 1991, 352: 473–474
58. Butcher EC: Leukocyte-endothelial cell recognition: three (or more) steps to specificity and diversity. Cell 1991, 67:1033–1036
59. Ii M, Kurata H, Itoh N, Yamashina I, Kawasaki T: Molecular cloning and sequence analysis of cDNA encoding the macrophage lectin specific for galactose and *N*-acetylgalactosamine. J Biol Chem 1990, 265: 11295–11298
60. Patt LM, Grimes WJ: Cell surface glycolipid and glycoprotein glycosyltransferases of normal and transformed cells. J Biol Chem 1974, 249:4157–4165
61. Roth J, Greenwell P, Watkins WM: Immunolocalization of blood group A gene specified α1,3*N*-acetylgalactosaminyltransferase and blood group A substance in the trans-tubular network of the Golgi apparatus and mucus of intestinal goblet cells. Eur J Cell Biol 1988, 46:105–112
62. Lopez LC, Youaki A, Evans SC, Shur BD: Evidence for a molecular distinction between Golgi and cell surface forms of β1,4-galactosyltransferase. J Biol Chem 1991, 266:15984–15991
63. Lessey BA, Damjanovich L, Coutifaris C, Castelbaum A, Albelda SM: Integrin adhesion molecules in the human endometrium: correlation with the normal and abnormal menstrual cycle. J Clin Invest 1992, 90:188–195
64. Lessey BA, Castelbaum AJ, Buck CA, Lei Y, Yowell CW, Sun J: Further characterization of endometrial integrins during the menstrual cycle and in pregnancy. Fertil Steril 1994, 62:497–506