American Journal of Pathology, Vol. 160, No. 5, May 2002

Human Endomucin

Distribution Pattern, Expression on High Endothelial Venules, and Decoration with the MECA-79 Epitope

Ulrike Samulowitz,* Annegret Kuhn,* Gertrud Brachtendorf,* Roman Nawroth,* Attila Braun,† Agnes Bankfalvi,‡ Werner Böcker,‡ and Dietmar Vestweber*§

From the Institute of Cell Biology, ZMBE, University of Münster, Germany; the Max-Planck-Institute of Biochemistry,† München, Germany; the Gerhard-Domagk-Institute of Pathology,‡ University of Münster, Germany; and the Max-Planck-Institute of Vascular Biology,§ Münster, Germany*

Endomucin is a typical sialomucin that we recently identified on the surface of mouse endothelial cells and on putative hematopoetic clusters of the dorsal aorta in the embryo. We have generated a panel of monoclonal antibodies (mAbs) against the extracellular part of human endomucin and polyclonal antibodies against the cytoplasmic part. Using immunohistochemistry endomucin was specifically detected on endothelial cells of blood and lymphatic vessels of all analyzed human tissues. In addition, the polyclonal antibodies stained the epithelium of the epidermis as well as epithelial and myoepithelial cells of the eccrine and apocrine glands in the skin. This nonendothelial staining could only be seen with a subset of mAbs if the staining procedure was amplified. Although high endothelial venules (HEVs) were not significantly stained with mAbs against endomucin, the polyclonal antibodies clearly detected endomucin on HEVs in lymphatic organs of the mouse and human, suggesting HEV-specific glycosylation affecting recognition by the mAbs. Indeed, endomucin isolated from human and mouse lymphoid organs carried the MECA-79 epitope that defines a set of L-selectin ligands on HEVs called peripheral node addressins. We conclude that human and mouse endomucin are endothelial sialomucins with the potential to function as L-selectin ligands. ***(Am J Pathol 2002, 160:1669–1681)***

Sialomucins form a heterogeneous class of highly O-glycosylated sialic acid-rich glycoproteins that adopt an extended rod-like structure because of their extensive O-glycosylation. This feature enables them to be highly accessible on the cell surface and, therefore, allows some of them either to support or to prevent cell adhesion. Several examples for each of these two functions are known and some sialomucins have been found to support both functions depending on the way they are glycosylated.

One of the first sialomucins that was demonstrated to have anti-adhesive activity was the primarily epithelial mucin MUC-1/episialin that can inhibit the cell adhesive activity of integrins[1] and of E-cadherin.[2] Analyzing gene ablated mice allowed to show that T cells devoid of CD43 entered secondary lymphoid organs more readily than T cells with CD43, indirectly suggesting a function for this sialomucin in cell repulsion.[3] Most striking is the anti-adhesive function of the highly charged sialoprotein podocalyxin that was originally identified on the foot processes of podocytes in kidney glomeruli.[4] At these sites, podocalyxin is involved in maintaining the spacing of these subcellular structures, most likely by charge repulsion, and deficiency of the corresponding gene leads to defects in the filtration activity of glomeruli.[5]

Besides anti-adhesive activities, some sialomucins clearly serve proadhesive functions. Most proadhesive sialomucins, although not all, serve as ligands for the selectins, cell adhesion molecules that recognize carbohydrate structures and function as initiators of cell contacts between leukocytes and endothelial cells.[6,7] The best functionally characterized selectin ligand is the sialomucin P-selectin glycoprotein ligand-1 that is essential for leukocyte extravasation under most inflammatory conditions and is able to bind to the two endothelial selectins, E- and P-selectin, as well as to L-selectin on leukocytes.[8] L-selectin mediates the entry of leukocytes into inflamed tissue as well as the homing of lymphocytes into lymphatic tissue, a process that occurs in the specialized blood vessels known as high endothelial venules (HEVs).[9,10] L-selectin initiates the contact between lymphocytes and high endothelial cells by binding to certain carbohydrate-presenting glycoproteins. Searching for

Supported by grant C1 from the Interdisziplinäres Zentrum für Klinische Forschung, University of Münster (to D. V.) and by a Lise-Meitner-Scholarship (to A. K.).

Accepted for publication January 31, 2002.

Address reprint requests to D. Vestweber, Institute of Cell Biology, ZMBE, University of Münster, Von-Esmarch-Str. 56, 48149 Muenster, Germany. E-mail: vestweb@uni-muenster.de.

HEV-specific antigens that would support L-selectin-mediated binding of lymphocytes to HEVs, the monoclonal antibody (mAb) MECA-79 was found that blocked this interaction and the homing of lymphocytes into lymph nodes.[11] This antibody binds exclusively to endothelial cells of HEVs as well as to activated endothelium in chronically inflamed sites[12,13] and in rejected human heart transplants.[14] MECA-79 defines a carbohydrate epitope[15–17] that is specifically found on a small number of glycoproteins expressed on the surface of endothelial cells in HEVs. Because of the blocking function of MECA-79, these antigens were defined as peripheral node addressins (PNAds).

Independent of this approach, several glycoprotein ligands of L-selectin were identified by using L-selectin as an affinity probe. First, four major protein bands were affinity-isolated from detergent extracts of [^{35}S]-sulfate-labeled mouse lymph nodes.[18] All four of these bands reacted with MECA-79 and two of them were later identified as GlyCAM-1[19] and CD34.[20] In human tonsils, MECA-79 also recognizes four major bands of 60, 105, 165, and 205 kd with almost similar electrophoretic mobility as those isolated from mouse lymph nodes[21–23] and two of them were identified as CD34 (105 kd)[22] and podocalyxin (160 kd),[23] respectively. Interestingly, another protein band migrating close to CD34 became detectable on complete depletion of CD34 from a purified PNAd fraction.[22] The identity of this protein is unknown.

Mouse endomucin was originally identified by expression cloning with the help of three mAbs that had been raised against mouse endothelial cells.[24] Analyzing the tissue distribution of this sialomucin in the adult mouse, we detected the antigen exclusively on endothelial cells of any tissue or organ that was tested.[24] Tissues from various stages of mouse embryo development revealed early expression of endomucin on endothelia at E8.0 as well as on clustered putative hematopoetic cells associated with the luminal surface of the endothelium of the dorsal aorta.[25] In addition, endomucin is clearly expressed on endothelium of the aorta at stage E11.5, but only weakly detectable as patchy, focal staining on aortic endothelium at stage E15.5 and on the adult aorta.[25] In combination with anti-adhesive effects of endomucin ectopically expressed in transfected human embryonal kidney cells[26] it has been speculated that endomucin may play a role in the detachment of hematopoetic cells from endothelium during early hematopoiesis.[27]

The tissue distribution of human endomucin protein was unknown and no antibodies against this antigen were available. To identify and analyze human endomucin, we cloned the full-length cDNA and generated an endomucin-IgG fusion protein that we used to raise a panel of mAbs. Each of these antibodies recognized the extracellular domain of native endomucin on human umbilical vein endothelial cells (HUVECs) and most bound also to the unglycosylated, *in vitro* translated antigen. In addition, we raised polyclonal antibodies against the cytoplasmic tail of the protein that react with both the murine and the human antigen.

Similar to the tissue distribution of the mouse orthologue, the mAbs against human endomucin stained specifically and selectively endothelial cells in any human tissue that was analyzed. However, using the polyclonal antibodies, we found two important differences. First, these antibodies additionally detected the antigen on epithelium of the skin epidermis as well as in eccrine and apocrine glands. Only a subset of mAbs could verify this staining, and only if amplifying detection systems were used, suggesting that the epitopes were masked on most endomucin moieties on these epithelial cells. Second, similar to the distribution pattern for the mouse orthologue, human endomucin was not readily detectable on HEVs of lymphatic tissue with any of the mAbs. However, the polyclonal antibodies clearly detected endomucin on HEVs in human tonsils as well as in mouse lymph nodes. This demonstrates that endomucin is not absent from HEVs and suggests that endomucin on HEVs is differently modified than on other endothelial cells. Indeed, we found that endomucin isolated from lymphatic tissue carries the PNAd-specific carbohydrate epitope MECA-79, demonstrating that endomucin belongs to the group of glycoproteins defined as PNAds.

Materials and Methods

Cloning of Human Endomucin cDNA

Searching the human Expressed Sequence Tag (EST) database with BLAST at National Center for Biotechnology Information we found several clones with homology to the transmembrane and cytoplasmic region of mouse endomucin. AA426230, AA426155, and AA464807 matched between bp 572 to 945 of mouse endomucin (AF060883) corresponding to the 19 membrane proximal extracellular amino acids, the transmembrane and the cytoplasmic region, as well as part of the 3′UTR. AF464807 and AF121324 further extended the 3′UTR but with lower homology. Based on these EST sequences, we created the oligonucleotide HEM777–750 GCCTAGGTGTGGAGAGAATTCCTCAAGC as 3′ oligo for a 5′-rapid amplification of cDNA ends (5′RACE) on an adaptor-ligated human heart cDNA library (Marathon-Ready cDNA; Clontech, Palo Alto, CA), which was performed according to the manufacturer's instructions. The resulting fragment of ~900 bp was cloned and sequenced and the open reading frame of 783 bp was verified by comparison with an reverse transcriptase-polymerase chain reaction (PCR) product obtained with total RNA from HUVECs using the oligonucleotides HEM-5-15 GCACCATGGAACTGCTTCAA and HEM777-758 GCCTAGGTGTGGAGAGAATT. The 5′-untranslated region was cloned with the 5′RACE System for Rapid Amplification of cDNA Ends, Version 2.0 (Gibco Life Technologies, Karlsruhe, Germany) according to the manufacturer's instructions. Briefly, total RNA from HUVECs was reverse-transcribed with the oligonucleotide HEM457-439 TACCAGTTTTAGAAGGT, TdT-tailed and the first PCR was performed with the oligonucleotides HEM449-425 TTAGAAGGTGATGCATCTGGTTGTA and AAP from the kit. As the second nested PCR yielded no fragment, the previous PCR fragment was cloned and sequenced and predicted a 5′-untranslated region of 108 bp.

The 3′-untranslated region was cloned by reverse transcription of HUVEC total RNA with the oligonucleotide 3′RACE-Adapter primer (Gibco Life Technologies) followed by a PCR with the oligonucleotides HEM 641-660 GAATGTCTGGAAGGCAGAT and the Abridged Universal Amplification Primer (Gibco Life Technologies).

Generation of Human Endomucin IgG

A DNA fragment corresponding to nucleotides 553 to 1803 of GenBank entry J00228 spanning the genomic sequence of the hinge and the constant region 2 and 3 of the heavy chain of human immunoglobulin γ was amplified from hIgG:pCMV5[28] and cloned into pcDNA3 (Invitrogen, Gronigen, The Netherlands) resulting in the vector hIgG:pcDNA3. A cDNA fragment coding for the complete extracellular domain of human endomucin with the triplet for the membrane proximal serine residue AGT replaced by the serine-coding triplet TCA creating a splice consensus site was amplified with the oligonucleotides HEM5-IgG CGGGATCCATGGAACTGCTTCAAGTG and HEM3-IgG GGAATTCACTTACCTGAGGAATAAGACCGGCTGGTTG and cloned into hIgG:pcDNA3 in analogy to Hahne and colleagues.[28] The resulting expression vector was stably transfected into CHO-Pro^{-5} cells and the recombinant fusion protein was isolated as described.[28]

Generation of Antibodies

To generate mAbs, rats were immunized seven times within a time period of 36 days subcutaneously with 30 μg of purified human endomucin-IgG fusion protein and Freund's adjuvant per injection. Hybridoma fusions were done as described[29] using the mouse myeloma SP2/0. Supernatants were screened for antibody binding to immobilized endomucin IgG in enzyme-linked immunosorbent assays.[30] Binding to an immobilized selectin IgG[28] was used as a negative control. Positive clones were tested in a second screen for binding to HUVECs using an established cell surface enzyme-linked immunosorbent assay.[28]

To generate anti-endomucin antibodies against glycosylation-independent epitopes, a polyclonal rabbit antiserum was raised against a peptide covering the 15 C-terminal amino acids and containing an additional N-terminal cysteine residue for coupling with the bifunctional crosslinker 3-maleimidobenzoic acid N-hydroxysuccinimide ester (MBS) (Sigma, Munich, Germany). Immunization was performed with bovine serum albumin-conjugated peptide and affinity purification of the antibodies with the ovalbumin-conjugated peptide as described.[31] For specificity controls of the immunohistochemistry signals obtained with these antibodies, we depleted specific antibodies from the batches of affinity-isolated antibodies by reincubating them with the endomucin peptide column. Mock depletion was done by a similar incubation with an analogous MBS-ovalbumin peptide column carrying a peptide covering the last 15 C-terminal amino acids of mouse N-cadherin.

The mAb MECA-79[11] against a carbohydrate epitope on PNAds was purified from the corresponding hybridoma obtained from the American Type Culture Collection (Rockville, MD).

Immunohistochemistry

For immunoperoxidase staining, 2-μm paraffin-embedded sections of various 4% paraformaldehyde-fixed human tissues were cut on a microtome. After mounting the slides on poly-L-lysine-coated slides (Menzel-Gläser, Nuβloch, Germany), dewaxed specimens were immersed in 10 mmol/L of sodium citrate buffer, pH 6.0, in plastic Coplin jars and microwave-treated twice for 8 minutes. After cooling to room temperature, endogenous peroxidase was blocked by incubation with 0.1% H_2O_2/0.02 mol/L NaN_3 in phosphate-buffered saline (PBS) for 30 minutes at room temperature. Nonspecific binding was blocked by incubation with 2% bovine serum albumin (BSA), fraction V (Sigma) in PBS, pH 7.4. Tissue sections were incubated with primary antibodies, either as tissue culture supernatant or diluted in 1% BSA in PBS, pH 7.4, for 1 hour followed by incubation with affinity-purified peroxidase-conjugated donkey anti-rat IgG (H+L), goat anti-rat IgG+IgM, or goat anti-rabbit IgG (Dianova, Hamburg, Germany) diluted 1:1000 in 1% BSA in PBS, pH 7.4. After the reaction was visualized with 3-amino-9-ethylcarbazole, tissue sections were counterstained with Mayer's hematoxylin and mounted. For control purposes, the first antibodies were either omitted or replaced by an irrelevant isotype-matched reagent. Anti-human CD34, clone QBEnd10 (DAKO, Hamburg, Germany), was used for positive controls, and V.5C7 or V.7C7 against mouse endomucin,[24] which do not cross-react with the human antigen, were used as negative controls on human tissues. Tissues were derived from the archive of the Gerhard Domagk Institute for Pathology.

For staining of frozen tissues, 7-μm cryostat sections were cut and mounted on poly-L-lysine-coated slides and fixed in acetone for 10 minutes at 4°C, followed by blocking of endogenous peroxidase activity for 30 minutes at room temperature. Nonspecific binding was blocked with 2% BSA in PBS, pH 7.4, for 30 minutes. Tissue sections were incubated for 1 hour with hybridoma supernatant followed by incubation with affinity-purified peroxidase-labeled donkey anti-rat IgG+IgM (dilution 1:1000, Dianova). After visualization with 3-amino-9-ethylcarbazole, tissue sections were counterstained with Mayer's hematoxylin and mounted.

Alternatively, staining of serial sections of lymph nodes with primary antibodies was detected with tetramethylrhodamine B isothiocyanate-labeled goat anti-rat IgG or dichlortriazinyl amino fluorescein (DTAF)-labeled goat anti-rat IgG+IgM (dilution 1:100, Dianova) and visualized in a Zeiss-Axioscope fluorescence microscope. For double staining of mouse lymph node tissue sections were first incubated with mAb MECA-79 followed by incubation with a DTAF-labeled goat anti-rat IgM (μ chain, dilution 1:100; Dianova). Subsequently, the same sections were incubated with the polyclonal rabbit antiserum against

endomucin followed by incubation with a Cy3-labeled donkey anti-rabbit IgG (dilution 1:500, Dianova). For amplifying the peroxidase-staining procedure, the avidin-biotin-based Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA), was used. Briefly, paraffin sections were fixed and pretreated as described above. The sections were then incubated with appropriate primary antibodies diluted in PBS containing 1% BSA, followed by biotinylated secondary antibody. The sections were then washed with PBS and incubated with an avidin-biotin-peroxidase complex. The reactions were visualized and counterstained as described above.

Flow Cytometry

Fresh human peripheral blood samples were fractionated on Ficoll-Histopaque (1.077 g/ml and 1.119 g/ml; Sigma Chemical Co., St. Louis, MO) gradients according to standard procedures. Mononuclear cells and granulocytes were collected and washed twice before staining. Before staining, Fc-receptors of the cells were blocked with mAb 3G8 (mouse anti-human FcγRIII) and IV.3 (anti-human FcγRII) (MEDAREX Inc., Annandale, NJ) at doses of 1 μg per 10^6 cells for 10 to 15 minutes at 4°C. For two-color staining of the peripheral blood leukocytes the following mouse antibody conjugates, coupled with fluorescein isothiocyanate (FITC) or phycoerythrin were used: CD3 FITC (clone HIT3a, IgG_{2a}), CD14 FITC (clone MϕP9, IgG_{2b}), CD19 FITC (clone SJ25C1, IgG_1), CD56 FITC (clone NCAM16.1, IgG_{2b}), CD45 FITC or phycoerythrin (clone HI30, IgG_1), CD15 FITC (clone HI98, IgM). Directly conjugated antibodies were purchased from Becton Dickinson, San Jose, CA. All antibodies were used at 10 μg/ml per 10^7 cells/ml. For triple-stage staining, a biotinylated donkey anti-rat IgG, (Fab'_2) fragment (Dianova) was used at a dilution of 1:400. Streptavidin-phycoerythrin (Dianova) was used at a dilution of 1:100. Antibody incubations were done in fluorescence-activated cell sorting (FACS) buffer (PBS, pH 7.4, 0.5% dialyzed fetal calf serum, 0.02% NaN_3) for 20 to 30 minutes on ice. Cells were analyzed on a FACScalibur using Cellquest software (Becton Dickinson, Heidelberg, Germany).

Immunoprecipitations, Immunoblots, and in Vitro *Translation*

Cell surface biotinylation of HUVECs and immunoprecipitations and immunoblots were essentially done as described.[24,31] Human endomucin lacking posttranslational modifications was synthesized by a coupled *in vitro* transcription/translation reaction in a reticulocyte lysate using the TNT Quick Coupled Transcription/Translation System (Promega, Mannheim, Germany). Based on the manufacturer's instructions, each reaction containing 8 μl of TNT Quick Master Mix, 164 KBq of ^{35}S-methionine (Amersham, Freiburg, Germany), and 0.04 μg of vector DNA (either pcDNA3 containing full-length mouse endomucin cDNA or vector without insert) was filled up to a final volume of 10 μl with diethyl pyrocarbonate-H_2O and incubated for 90 minutes at 37°C. Subsequently reactions were subjected to immunoprecipitations as described above. As negative or positive standards, either 10 μl of a reaction containing the vector without insert or 2 μl of a reaction containing the vector with insert were loaded on the gel, respectively.

Isolation of Endomucin and CD34

Human endomucin and CD34 were isolated by homogenizing 1 g of human tonsils in an Ultraturrax in 2.5 ml of lysis buffer (50 mmol/L Tris/HCl, pH 7.4, 150 mmol/L NaCl, 0.02% NaN_3, 0.1 U/ml α_2-macroglobulin, 1 mmol/L phenylmethyl sulfonyl fluoride, 1 mmol/L benzamidine). After homogenization the same volume of lysis buffer containing 2% Triton X-100 was added, followed by incubation for 1.5 hours on ice and 20 minutes of centrifugation at 3000 rpm in a tabletop centrifuge, and a high-speed spin at 55,000 rpm in a Beckman ultracentrifuge (TLA 100.4 rotor) for 20 minutes. The supernatant was passed through a 0.45-μm filter. For the isolation of mouse endomucin and CD34, 800 μl of peripheral lymph nodes (from ~35 mice) were homogenized by sonication in 2 ml of lysis buffer followed by the addition of the same volume of lysis buffer containing 2% Triton X-100 and incubation on ice for 1 hour. Extracts were centrifuged first for 10 minutes at 3000 rpm in a tabletop centrifuge, second at 55,000 rpm for 20 minutes in a tabletop ultracentrifuge (Beckmann Instruments GmbH, München, Germany). For isolation of endomucin and CD34 from bEnd.3 mouse endothelioma cells, 3×10^7 bEnd.3 cells were lysed in 5 ml of lysis buffer containing 1% Triton X-100 for 15 minutes on ice and centrifuged for 15 minutes at 14,000 rpm in a tabletop centrifuge. Each affinity isolation was done with 20% of the tissue or cell extract incubated with 40 μl of antibody-loaded protein A Sepharose beads. The beads contained either 10 μg of mouse anti-human CD34 mAb (clone QBEnd10, DAKO), rat anti-human endomucin mAb L6H10 from 1 ml of culture supernatant, 10 μg of rat anti-mouse CD34 mAb (clone RAM34, Pharmingen), 20 μg of affinity-purified rabbit anti-mouse endomucin antibodies. For negative control, immunoisolations 10 μg of affinity-purified rabbit anti-mouse VE-cadherin[32] was used with mouse lymph node extracts and 10 μg of mAb V.7C7 against mouse endomucin (not reactive with human endomucin) was used with human tonsil extracts. Rat and mouse mAbs were conjugated to the beads with either rabbit anti-rat or with rabbit anti-mouse IgG (Dianova), respectively. Tissue extracts were incubated with the beads overnight on ice, followed by washing three times with washing buffer (50 mmol/L Tris/HCl, pH 8.4, 400 mmol/L NaCl, 1 mmol/L $CaCl_2$, 0.1 U/ml α2-macroglobulin, 1 mmol/L phenylmethyl sulfonyl fluoride, 1 mmol/L benzamidine, 0.1% Triton X-100) and elution of bound proteins by boiling the beads in sodium dodecyl sulfate-polyacrylamide gel electrophoresis sample buffer. Eluted proteins were electrophoresed, transferred to nitrocellulose filters, and probed with MECA-79 hybridoma superna-

tant followed by incubation with peroxidase-conjugated goat anti-rat IgM, μ-chain-specific (Becton Dickinson). Immunoreactive proteins were detected as described above.

Results

Generation and Binding Characteristics of a Panel of mAbs against Human Endomucin

To identify and analyze human endomucin we searched the National Center for Biotechnology Information gene data base for human ESTs homologous to the mouse cDNA. Several clones were found with high homology to the 3′-part of endomucin covering the sequence for the cytoplasmic tail, the transmembrane region, and the membrane proximal 19 amino acids of the extracellular part of mouse endomucin. No ESTs were found with homology to regions upstream of this part. A human endomucin cDNA coding for an open reading frame of 783 bp (261 amino acids) was isolated by 5′-RACE PCR using a commercially available heart cDNA library and a 5′ oligonucleotide annealing to the 5′-adaptor sequence of the library and a 3′ oligonucleotide corresponding to the 3′-noncoding sequence of the human ESTs. A second round of reverse transcriptase-PCR using total RNA from HUVECs allowed the verification of the sequence and to clone another cDNA fragment coding for an additional 108 bp of 5′-untranslated sequence and another clone coding for 584 bp of the 3′-untranslated region. Although the 5′ sequence upstream of the start codon of the 783-bp open reading frame did not contain an in-frame stop codon, the start codon was preceded by a consensus for translational initiation[33] (Figure 1). The deduced protein sequence had a 13-amino acids longer extracellular domain than mouse endomucin.[24] The overall homology between the human and mouse amino acid sequence was 53%, with only 32.5% for the extracellular part, 87.5% for the putative transmembrane region, and 96% for the cytoplasmic domain, with only two exchanges among 48 amino acids. Similar to the mouse cDNA the human sequence coded for a 20- and 23-residue-long signal peptide and transmembrane region, respectively.

While our study was in progress, sequences for human and mouse endomucin were published.[26] The mouse sequence (called mouse endomucin-1) represented a slightly longer splicing variant of our sequence,[24] with a 13-amino acid insertion behind amino acid K128. Correlates of both splicing variants seem also to exist for human endomucin, because the longer mouse variant is of identical length as human endomucin (Figure 1B, isoform a) and a 13-amino acid smaller human variant is suggested by EST AW391174 (Figure 1B, isoform b). Analyzing the exon/intron structure by comparing our cDNA sequence with the genomic sequence in the National Center for Biotechnology Information data base revealed, that the 13-amino acid insertion is represented by one exon (Figure 1B). A third potential splicing variant is suggested by EST BG536532 that could be generated

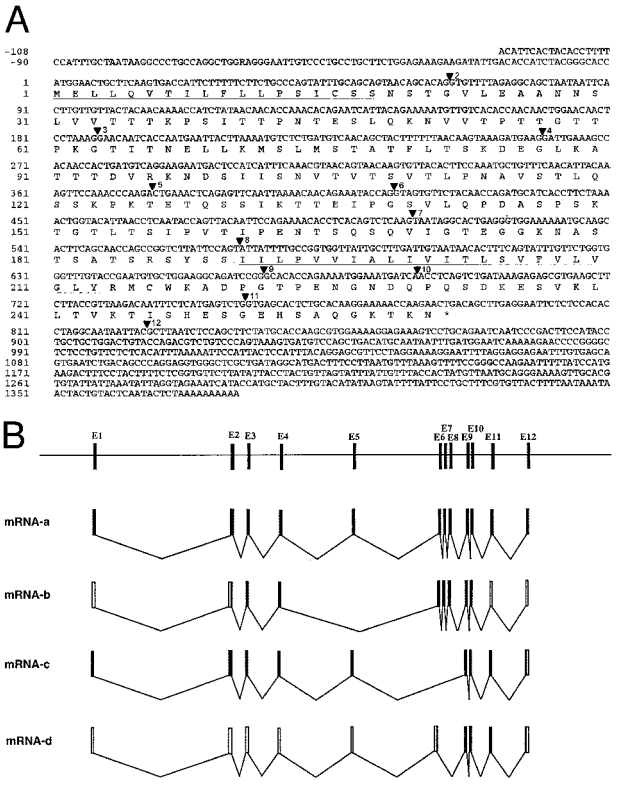

Figure 1. Sequence of human endomucin and putative exon/intron structure. **A:** Nucleotide and amino acid sequence of human endomucin. The putative signal sequence and single transmembrane region are **underlined**. The exon boundaries are delineated by **arrowheads** followed by the number of the beginning exon. **B:** Exon/intron structure of human endomucin based on the comparison of the full-length cDNA sequence depicted in **A** (accession no. AF205940, mRNA-a) and the genomic sequence given under accession no. AC011580. Three shorter splicing variants are depicted below as based on EST AW391174 lacking exon 5 (mRNA-b); BG536532 lacking exons 6, 7, and 8 (mRNA-c); and AF121324 lacking exons 7 and 8 (mRNA-d). **Filled boxes** indicate sequences included in the ESTs, **open boxes** show putative exons.

by removal of exons 6, 7, and 8 coding for the transmembrane region, 52 amino acids of the membrane proximal extracellular part and 8 amino acids of the membrane proximal intracellular part. If this potential splicing variant is indeed translated it would result in a soluble secreted form of endomucin having an identical C-terminus for the last 40 amino acids. A fourth splicing variant suggested by EST AF121324 lacks exons 7 and 8, resulting in a similar soluble protein without a transmembrane region.

Kinoshita and colleagues[26] reported on two human cDNA sequences of identical length (261 amino acids) called human endomucin 1 and 2. The latter had been cloned from a human heart cDNA library and was identical with the human cDNA that we cloned. However, the former had only been identified as human EST AA085169, representing a sequence that is 100% identical with the mouse sequence. Based on this sequence identity, the designation of EST AA085169 as a human sequence needs to be corrected, especially because the gene bank entry already cautioned that the human cDNA library that had been used to obtain this EST was contaminated with mouse sequences.

Table 1. Characterization of Eight mAbs against Human Endomucin: Reactivity with an Endomucin-IgG Fusion Protein (ELISA hEM-IgG), Binding to Intact HUVECs (ELISA HUVEC), Immunoprecipitation of Endomucin from Surface Biotinylated HUVECs (IP HUVEC), Binding to Endomucin from HUVECs in Western Blots (WB HUVEC). Immunoprecipitation of *in Vitro* Translated Endomucin (IP *in Vitro* Translated Protein), Determination of the Ig-Subclass (ELISA Subclass), Determination of the Type of Light Chain by Binding to Protein L Agarose.

Hybridome	ELISA hEM-IgG	ELISA HUVEC	IP HUVEC	WB HUVEC	IP *in vitro*-translated protein	ELISA Ig-subclass	Protein L-agarose-IP (light chain)
L10B5	+++	++	+	nd	++	Rat IgG_{2b}	− (λ)
L5F12	+++	++	+	+	nd	Rat IgG_1	− (λ)
L3F12	+++	(+)	−	−	+	Rat IgG_{2a}	+ (κ)
L4B1	+++	++	+	+	++	Rat IgG_{2a}	− (λ)
L6H10	+++	++	+	+	++	Rat IgG_{2a}	− (λ)
L6H3	++	(+)	+	−	+	Rat IgG_{2a}	+ (κ)
L9H8	+++	(+)	−	+	+	Rat IgG_{2a}	−(λ)
L10F12	+++	(+)	+	+	−	Rat IgG_1	− (λ)

−, no binding; +, binding; nd, not determined.

To generate mAbs against human endomucin we immunized rats with a human endomucin-IgG fusion protein containing the complete extracellular domain of the protein and purified from transfected Chinese hamster ovary cells. Antibodies were screened for binding to the fusion protein in enzyme-linked immunosorbent assays, and in a second screen for binding to HUVEC monolayers in a cell-surface enzyme-linked immunosorbent assay. Thirteen mAbs were identified that recognized endomucin IgG, but not a control IgG fusion protein. Of these, four mAbs stained HUVECs strongly (L6H10, L4B1, L5F12, L10B5), four only weakly (L3F12, L6H3, L9H8, L10F12), and five did not stain the cells (not further mentioned). The isotypes of these antibodies are given in Table 1. None of the antibodies recognized mouse endomucin on mouse bEnd.3 cells (not shown). Six of the mAbs recognized endomucin in immunoprecipitations with cell-surface biotinylated HUVECs (Figure 2A), whereas only four of them recognized endomucin in Western blots that had been immunoprecipitated from HUVECs (Figure 2B). To test, whether the epitopes of the antibodies were dependent on the carbohydrate modifications of the extensively glycosylated antigen, we synthesized the protein backbone without carbohydrate side chains in a coupled *in vitro* transcription/translation reaction. As shown in Figure 2C, six mAbs recognized the unglycosylated protein in immunoprecipitations, whereas the mAb L10F12 did not bind, suggesting that only this antibody requires native glycosylation of endomucin for binding. To obtain anti-endomucin antibodies against glycosylation-independent epitopes, ie, epitopes that cannot be affected by any change of glycosylation of the antigen, we generated polyclonal rabbit antibodies against a peptide covering the 15 C-terminal amino acids of human and mouse endomucin (identical sequence) and affinity-purified them on the peptide. These antibodies specifically detected human endomucin in immunoprecipitations as shown in Figure 2, A and C, as well as mouse endomucin from bEnd.3 mouse endothelioma cells (not shown).

Tissue Distribution of Human Endomucin

To analyze the distribution pattern of human endomucin, paraffin-embedded sections were stained with mAbs. Of our eight mAbs five mAbs stained endothelium in paraffin sections, of which one only stained very weakly, whereas four mAbs gave strong signals. In each analyzed tissue, staining was exclusively detected on endothelium of capillaries and venules, as is illustrated for kidney, suprarenal gland, ileum, skin, and brain (Figure 3). Endothelial-specific staining was also observed in stomach, liver, jejunum, appendix, colon, pancreas, endometrium, uterus, placenta, skin, tonsils, lymph nodes of the mesocolon, thyroid gland, lung, and heart.

Although the mAb L6H10 and several others did not recognize carbohydrate residues and instead directly bound to the protein backbone of the antigen as shown above (Figure 2C), we could not exclude that additional glycosylation, extending that on venules and capillaries in most organs, would possibly mask endomucin epitopes on other cells. Therefore, we evaluated the tissue distribution with our polyclonal antibodies directed against the cytoplasmic tail of endomucin. We could confirm the exclusive endothelial expression of endomucin in all analyzed tissues with one exception. In the skin, the epithelium of the epidermis as well as the epithelium in eccrine glands and myoepithelial cells in apocrine glands were at least as strongly stained as blood capillaries (Figure 4). This staining was highly specific for the peptide that had been used as immunogen. Depleting the affinity-purified antibodies with the endomucin peptide completely abolished epithelial staining, whereas mock depletion with an irrelevant peptide had no effect (not shown). Re-evaluating this staining with the mAbs revealed that none of our mAbs stained these epithelia under conditions that were sufficient to strongly stain endothelium. However, mAbs L6H10, L10B5, L5F12, and L9H8 stained epidermis and eccrine and apocrine glands, when an amplification detection kit was used (not shown). Similar staining of epidermal epithelial cells was seen with the polyclonal antibodies in the mouse (not shown). Our results suggest that skin epithelial cells express differentially glycosylated forms of endomucin and that the protein epitopes of our mAbs are primarily masked on these endomucin glycoforms. Importantly, no other epithelia in other tissues than the skin were stained by the polyclonal antibodies.

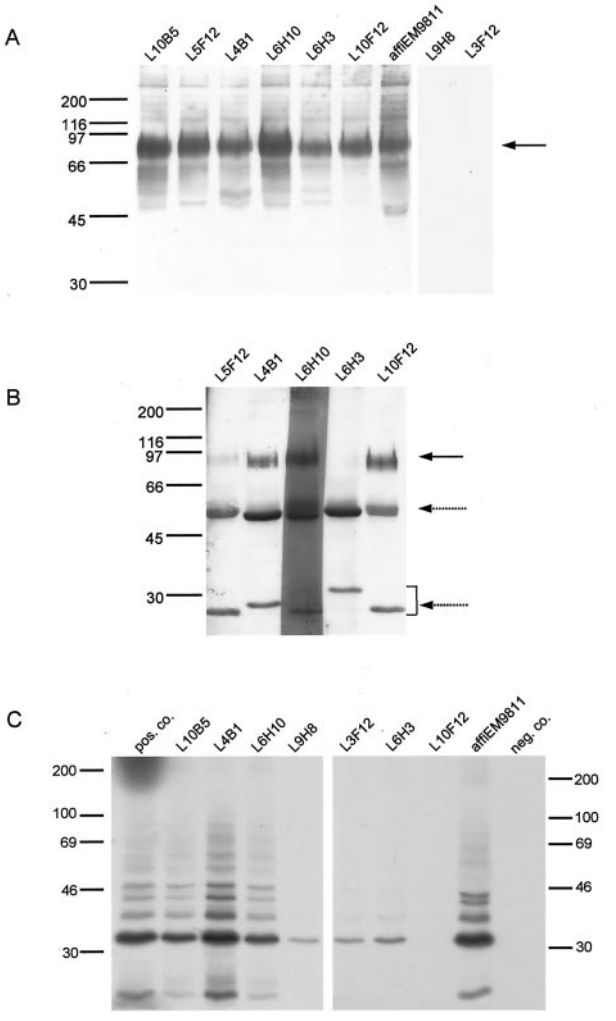

Figure 2. Reactivity of various mAbs with human endomucin. **A:** HUVECs were cell-surface biotinylated and subjected to immunoprecipitations with the indicated mAbs against human endomucin and with affinity-purified polyclonal rabbit antibodies against a C-terminal peptide of the antigen (affiEM9811). Immunoprecipitates were electrophoresed, transferred to nitrocellulose, and detected with peroxidase-conjugated streptavidin. For negative controls, the last two lanes show immunoprecipitates with two mAbs generated in this screen that did not react with endomucin in immunoprecipitations. **B:** Endomucin from HUVECs was first immunoprecipitated and then immunoblotted with the same anti-endomucin mAbs or polyclonal antibodies, as indicated. **Dashed arrows** mark the heavy and light chains of IgG, the **solid arrow** marks endomucin. **C:** Human endomucin was synthesized in a coupled *in vitro* transcription/translation reaction and either electrophoresed directly (pos. co.), or subjected to immunoprecipitations with mAbs or polyclonal antibodies against endomucin, as indicated, or with a negative control antibody (neg. co.). No translation product was observed if the endomucin cDNA was omitted (not shown). Molecular mass markers (in kd) are indicated.

We had recently found that arterioles were negative for staining with the mAbs against mouse endomucin in various tissues.[24] Analyzing several human tissues, we found that the mAb L6H10 can weakly stain arterioles, as shown for the kidney and the skin in Figure 5, A and B. However, staining was patchy and focal and usually more difficult to detect than on venules and capillaries. No stronger staining was detected with the polyclonal antibodies (not shown). This suggests that endomucin is indeed less strongly expressed on arteriolar endothelium and that the weak staining is not because of differences in glycosylation.

Endothelium of lymphatic vessels was well stained with mAb L6H10 (Figure 5C), indicating that endomucin is as well expressed in lymphatic vessels as in venules and capillaries. Class-matched irrelevant negative control antibodies gave consistently no staining (not shown). Thus, except for epithelial cells of the skin, endothelial cells along the whole vascular tree including lymphatic vessels are the predominant cell type expressing endomucin.

Endomucin Is Not Expressed on Most Leukocytes and Only Weakly on Monocytes

Expression of endomucin on human peripheral blood leukocytes was analyzed with mAb L6H10 by flow cytometry. Granulocytes were separated from mononuclear cells by gradient centrifugation, B cells, T cells, natural killer cells, and monocytes were defined by cell-surface markers. Endomucin was undetectable if L6H10 binding was analyzed with a phycoerythrin-conjugated second stage antibody. Even if a triple-stage analysis was performed with a biotinylated second stage antibody and peroxidase-conjugated streptavidin as third stage reagent, most leukocytes were negative, except for monocytes that were weakly positive (Figure 6). For comparison, HUVECs were stained with L6H10 by double-stage analysis, revealing bright signals (Figure 6). Expression levels on monocytes were too weak to detect endomucin by immunoblot analysis with our polyclonal antibodies (not shown). In addition, monocytes of some donors did not yield any endomucin-specific FACS signal (not shown). We conclude, that most leukocytes are negative for endomucin except monocytes for which weak expression cannot be ruled out completely.

Endomucin Is Found on HEVs in Human and Mice Lymphatic Organs

Using our three mAbs against mouse endomucin we had been unable to detect the antigen on HEVs of lymph nodes.[24] Therefore, we tested HEV staining in tonsils with mAb L6H10 and compared this with the staining of HEVs in mouse mesenteric lymph nodes with the anti-mouse endomucin mAb V.5C7. As shown in Figure 7, D and E, HEVs in mouse lymph nodes were not significantly stained with V.5C7, although HEVs were clearly visible as shown by staining with the HEV-specific mAb MECA-79 (Figure 7F). This was primarily confirmed with mAb L6H10 on human peripheral lymph nodes, although areas were found where the bulk structures of HEVs were negative, whereas the area where the HEV was leading into a smaller vessel was sometimes stained (Figure 7, A and B). To elucidate whether HEVs indeed do not express endomucin or whether the endomucin epitopes of our anti-mouse and anti-human mAbs would be masked on HEVs, we performed fluorescence staining experiments on serial sections with the HEV marker MECA-79 and the mAb V.5C7 or double staining with our affinity-

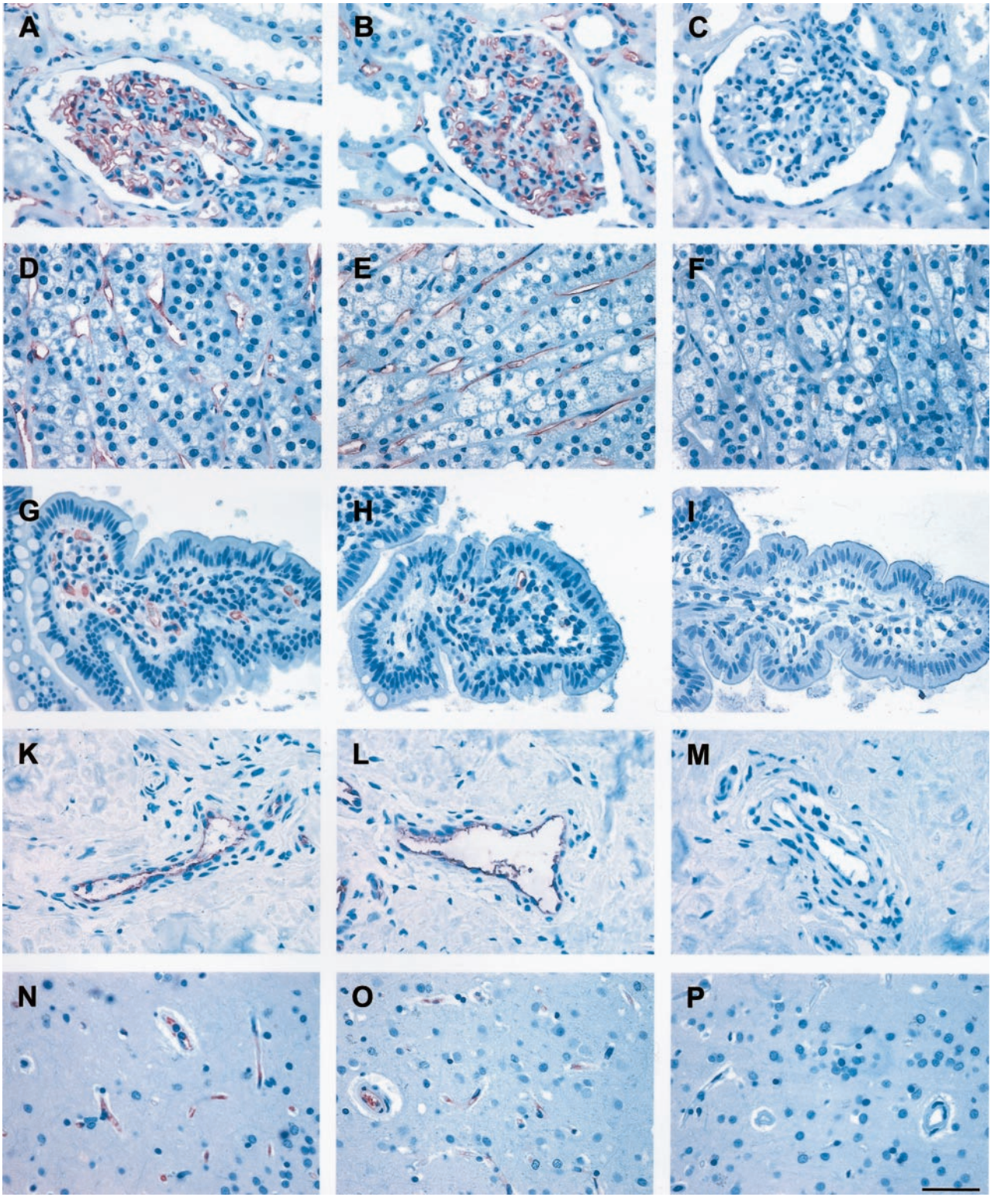

Figure 3. Distribution pattern of endomucin in five different human tissues. Immunohistochemistry of paraffin sections of human kidney (**A–C**), suprarenal gland (**D–F**), ileum (**G–I**), skin (**K–M**), and brain (**N–P**) with mAb L6H10 against endomucin (**A**, **D**, **G**, **K**, **N**), a mAb against CD34 (**B**, **E**, **H**, **L**, **O**) and an irrelevant rat control mAb (**C**, **F**, **I**, **M**, **P**). First antibodies were detected by indirect immunoperoxidase staining. Scale bar, 50 μm.

purified polyclonal antibodies on cryostat sections of mouse lymph nodes. As shown in Figure 7I, V.5C7 stained endothelium of small blood vessels efficiently, whereas the vessel that was defined as an HEV by MECA-79 staining in the adjacent section (Figure 7K) was primarily negative. By contrast, our polyclonal anti-endomucin antibodies stained HEVs efficiently (Figure 7L). The identity of the stained blood vessel was documented

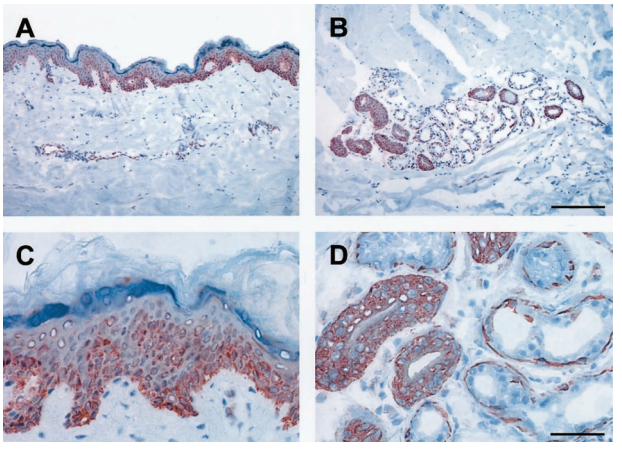

Figure 4. Endomucin is expressed on epithelium of the skin. Immunohistochemistry of paraffin sections of human skin with affinity-purified polyclonal antibodies against a C-terminal peptide of human endomucin. **C** and **D** show similar areas as **A** and **B**, respectively, at higher magnifications. First antibodies were detected by indirect immunoperoxidase staining. Scale bars, 200 μm (**A, B**) and 50 μm (**C, D**).

by double staining of the same section with MECA-79 (Figure 7M, merge illustrated in Figure 7N). Importantly, MECA-79 staining was also detected in more basal areas, whereas endomucin staining was restricted to the more luminal areas of the sectioned HEV. Similar results were obtained with human lymph nodes (not shown). The specificity of the polyclonal antibodies was controlled by depleting specific antibodies with the endomucin peptide. Depleted antibodies showed no staining (Figure 7G), whereas antibodies mock-depleted with a control peptide still specifically stained HEVs (Figure 7H). We conclude that endomucin is indeed expressed on HEVs and that HEV-specific glycosylation most likely masks the accessibility of the mAb epitopes in both species.

Endomucin on HEVs Is Decorated with the PNAd Epitope MECA-79

Expression of endomucin on HEVs prompted us to test whether it would indeed be modified with the MECA-79 epitope. To this end, we isolated endomucin from mouse peripheral and mesenteric lymph nodes (Figure 8A) or from human tonsils (Figure 8B) by affinity isolation with polyclonal antibodies against the C-terminus of endomucin or mAb L6H10, respectively, and tested whether the isolated antigens would be recognized in immunoblots by MECA-79. For comparison, we isolated CD34 from the same tissue samples as a positive control. For negative controls, we immunoprecipitated VE-cadherin from mouse lymph nodes and used a class-matched mAb against mouse endomucin (not cross-reactive with human endomucin) for immunoprecipitations with human tonsils. As shown in Figure 8, mouse as well as human, endomucins were recognized by MECA-79, similarly to CD34, whereas a control IgM gave no immunoblot signal (Figure 8, right panel). Endomucin and CD34 immunoprecipitated from cultured mouse endothelioma cells (bEnd.3) did not react with MECA-79 in immunoblots (not shown). This demonstrates that endomucin, similar to CD34, is specifically decorated with the carbohydrate epitope MECA-79.

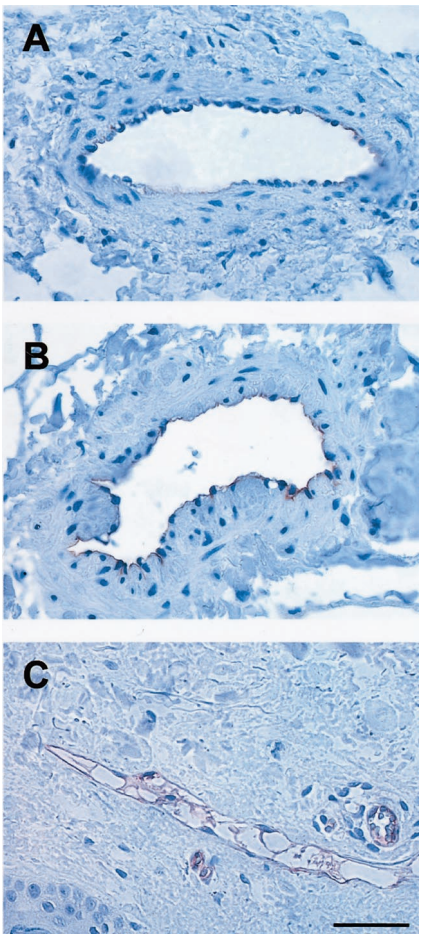

Figure 5. Expression of endomucin on arteriolar and lymphatic endothelium. Immunohistochemistry of paraffin sections of human skin (**A**, **C**) and kidney (**B**) with mAb L6H10 (**A–C**). Endomucin staining in arterioles (**A**, **B**) is patchy and confined to focal areas whereas endothelium of lymphatic vessels (**C**) is continuously stained with L6H10. Scale bar, 50 μm.

Discussion

Endomucin was originally identified as an endothelial sialomucin in the mouse.[24] Based on immunohistochemical analysis of a large panel of mouse organs with three mAbs we had found that endothelia were exclusively stained in all organs. In contrast to venules and capillaries, arterioles had not readily been detected and especially HEVs in lymph nodes, the sites of L-selectin-mediated lymphocyte homing, had not been stained with any of these mAbs.[24] Using a panel of novel monoclonal and polyclonal antibodies against the human orthologue of endomucin, we demonstrate here the primarily endothelial nature of the antigen, but identify epithelia of the epidermis and of apocrine and eccrine glands in the skin as additional sites of endomucin expression. Furthermore, we show that endomucin is expressed on endothelium all along the vascular tree, including lymphatic vessels and arterioles, although staining of arterioles is weaker and more patchy than in venules. Most impor-

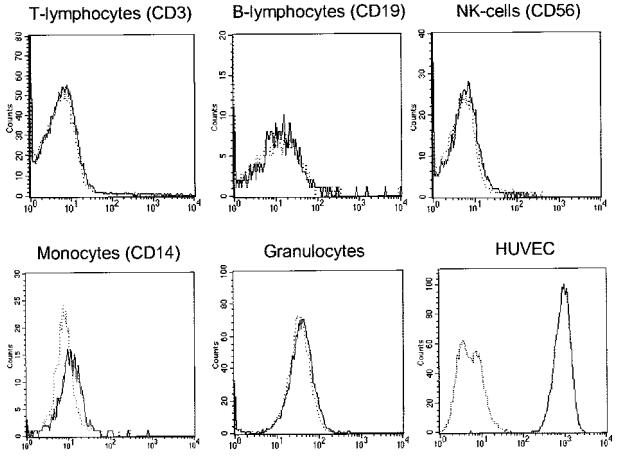

Figure 6. Analysis of the expression of endomucin on human peripheral blood leukocytes. Human granulocytes and mononuclear cells were isolated by density gradient centrifugation and analyzed by flow cytometry. HUVECs were used as second passage culture. Cells were incubated with purified L6H10, followed by incubation with a biotinylated anti-rat IgG and peroxidase-conjugated streptavidin (**solid line**), for negative controls the first antibody was replaced by a rat IgG of the same subclass (**dotted line**). T lymphocytes, B lymphocytes, NK cells, and monocytes were gated based on the staining with directly labeled antibodies against the indicated CD surface markers, granulocytes had been analyzed as separated cell fraction.

tantly, our antibodies against glycosylation-independent epitopes allowed us to identify endomucin on HEVs of human and mouse lymphoid organs. In addition, we detected endomucin as a carrier of the MECA-79 epitope, a denominator of PNAds.

None of our three mAbs against mouse and eight mAbs against human endomucin are able to stain HEVs efficiently, although each binds well to endomucin across a large variety of endothelia and two of the anti-mouse mAbs[25] and six of the anti-human mAbs recognize polypeptide epitopes, because they bind to nonglycosylated endomucin, synthesized by *in vitro* translation. Thus, their epitopes neither depend on, nor are affected by, the type of glycosylation of endomucin across a large variety of endothelia. This raises the question, whether endomucin that we recognize on HEVs with polyclonal antibodies against the cytoplasmic protein domain, differs from endomucin on other endothelia by glycosylation or whether the HEV form represents a splicing variant, lacking all polypeptide epitopes recognized by the mAbs. Indeed, human, as well as mouse, endomucins exist each as two splicing variants coding for a shorter protein of 248 amino acids and a longer version of 261 amino acids, with the longer version containing a 13-amino acid insertion behind position 128 in the mouse and position 126 of human endomucin. Because all three of our anti-mouse endomucin mAbs recognize the smaller splice product,[24] lack of binding to HEVs cannot be explained by a HEV-specific expression of this splice form. A third splice product of mouse endomucin that was detected by reverse transcriptase-PCR in various embryonal and adult tissues[27] lacks amino acids 91 to 128. This splice variant, if HEV-specific and if lacking the mAb epitopes, could theoretically explain the lack of mAb staining of HEVs in the mouse. However, a corresponding splice variant has not yet been documented with human ESTs. Two further human splice products, (called c and d in Figure 1B), if existing as proteins, would represent soluble secreted forms of endomucin, sharing the last 39 C-terminal amino acids with the transmembrane form and lacking the transmembrane domain and some sequences upstream and downstream of this region. Although we have tried, we failed to detect these forms in human serum with our polyclonal antibodies (not shown). Further splicing variants have not yet been described.

A more likely explanation for the lack of all mAb epitopes on HEV-type endomucin in both species is a HEV-specific glycosylation that leads to masking of the epitopes. Indeed, we could demonstrate that endomucin is specifically glycosylated on HEVs. The HEV-specific carbohydrate epitope MECA-79 was only found on endomucin isolated from lymph nodes, but not on endomucin expressed in other endothelia. Whether this HEV-specific glycosylation is directly linked to the differential antibody reactivity is unknown. However, another indication for epitope masking on HEV-type endomucin, is based on the fact that we could immunoprecipitate endomucin efficiently from tonsil extracts with one of our mAbs (L6H10), although staining of HEVs with this antibody was extremely weak. Because the purified endomucin carried the HEV-specific epitope MECA-79, we conclude that L6H10 can readily bind its epitope on HEV-derived endomucin, if the antigen is solubilized in a detergent extract. Thus, a combination of HEV-specific glycosylation and certain spatial arrangements of endomucin in the context of surrounding cell-surface molecules might lead to the strongly reduced accessibility of several epitopes on endomucin in HEVs.

The mAb MECA-79 blocks L-selectin-mediated lymphocyte binding to HEVs and defines a glycoprotein fraction on HEVs designated as PNAds. Four major proteins belonging to this fraction can be affinity-isolated from human tonsils.[21–23] Two of the proteins were identified as CD34 and podocalyxin. We found that affinity-isolated endomucin and CD34 from human tonsils were labeled with MECA-79 in immunoblots with similar intensities. Although this comparison was not strictly quantitative, immunoblots had been performed under nonsaturating conditions and affinity isolations had been done with identical amounts of tissue and under identical conditions. Our results suggest that endomucin is a novel member of the PNAd protein fraction, that migrates very closely to CD34 in polyacrylamide gels. Indeed such a second protein, almost co-migrating with CD34 was reported in CD34-depleted PNAd fractions isolated from human tonsils.[22]

The detection of endomucin with polyclonal antibodies on epithelial cells of the epidermis and of apocrine and eccrine glands in the skin was unexpected, because none of the mAbs showed specific staining of these cells under routine conditions that allowed bright staining of endothelium. Although we cannot formally rule out that these epithelial cells express different splice variants of endomucin, it is more likely that, as in the case of HEVs, the difficulties to detect endomucin in skin epithelial cells are again because of masking by differential glycosylation. In agreement with this assumption, we found that

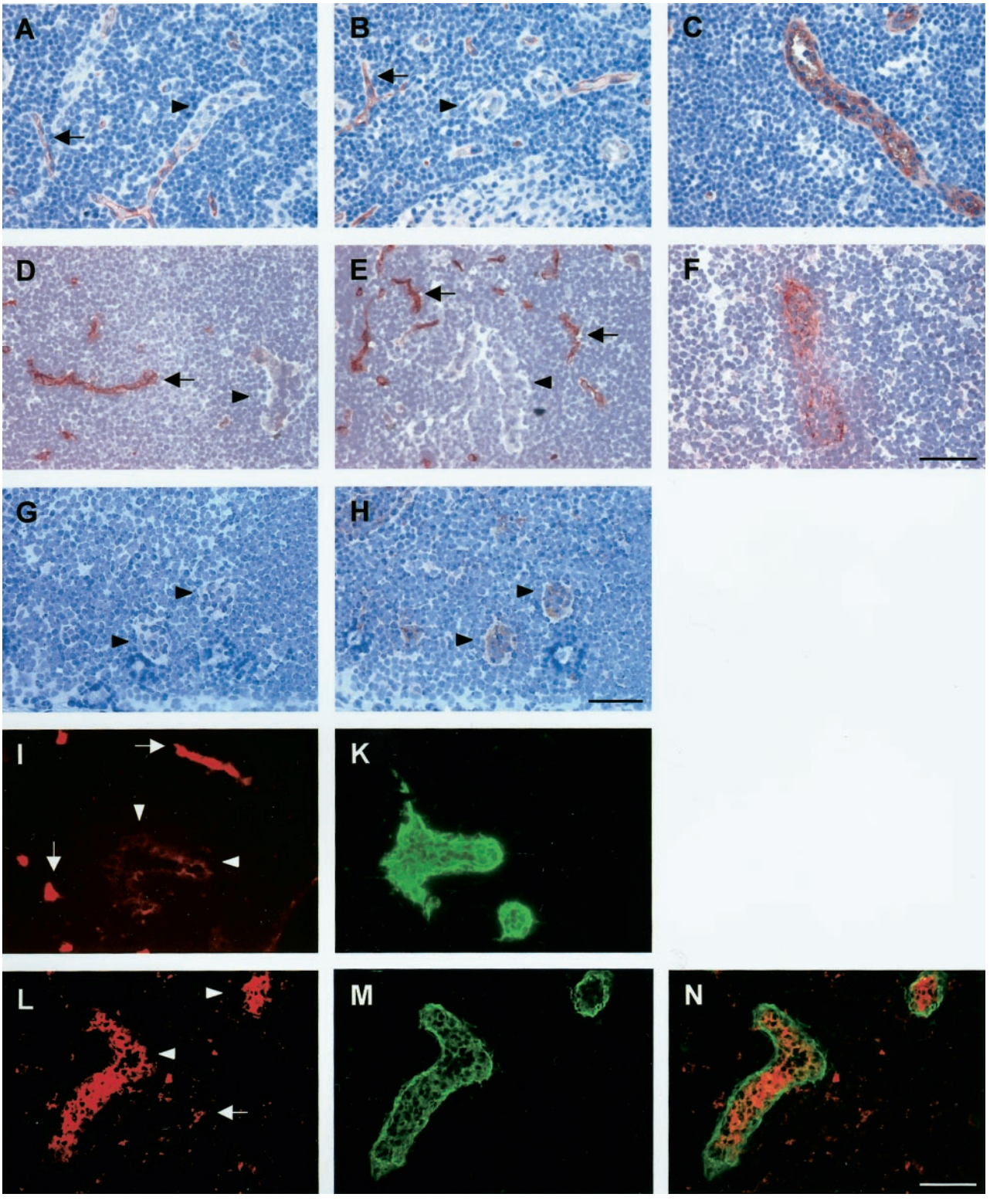

Figure 7. Endomucin is expressed on HEVs, but epitopes for mAbs are masked. Indirect immunoperoxidase staining of paraffin sections of human lymph nodes (**A–C**) and cryostat sections of mouse mesenteric lymph nodes (**D–H**) with anti-human endomucin L6H10 (**A**, **B**), anti-PNAd MECA-79 (**C**, **F**), anti-mouse endomucin V.5C7 (**D**, **E**) and polyclonal antibodies against a C-terminal peptide of endomucin, either depleted for specific antibodies by the peptide (**G**) or mock depleted (**H**). Note that capillaries are well stained by mAbs against endomucin whereas HEV structures are not significantly detectable. **I–N:** Indirect immunofluorescence staining of cryostat sections of mouse mesenteric lymph nodes. The sections depicted in **I** and **K** are directly adjacent and are stained with mAb V.5C7 (**I**) and anti-PNAd MECA-79 (**K**). Micrographs depicted in **L–N** show the same section double stained with polyclonal antibodies against a C-terminal peptide of endomucin (**L**) and MECA-79 (**M**), the merge is shown in (**N**). Capillaries are marked by **arrows**, HEVs are marked by **arrowheads**. Scale bars, 50 μm.

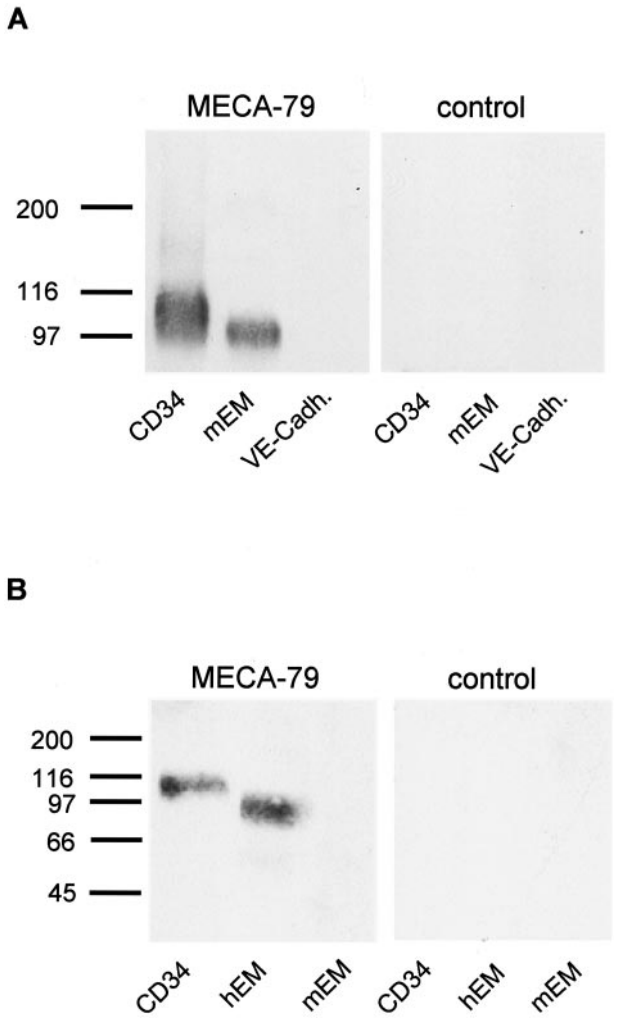

Figure 8. HEV-specific decoration of endomucin with the carbohydrate epitope MECA-79. Lysates of mouse mesenteric lymph nodes (**A**) and of human tonsils (**B**) were subjected to immunoprecipitations with the following antibodies: mAbs against mouse CD34 (CD34), polyclonal rabbit antibodies against the C-terminus of endomucin (mEM), or polyclonal rabbit antibodies against mouse VE-cadherin (VE-Cadh.) (**A**); and mAb against human CD34 (CD34), mAb L6H10 against human endomucin (hEM), or mAb V.7C7 against mouse endomucin (mEM) (**B**). V.7C7 does not cross-react with human endomucin. Immunoprecipitates were immunoblotted either with mAb MECA-79 (**left**) or with a negative control IgM (**right**). Molecular mass markers (in kd) are indicated on the **left**.

amplifying the staining procedure allowed the staining of skin epithelial cells specifically with several of the mAbs. Differential glycosylation of sialomucins in different cell types affecting the detectability with various antibodies has also been found for other sialomucins. A striking example is CD164, which functions as an adhesion receptor on $CD34^+$ hematopoetic progenitor cells mediating contact to bone marrow stromal cells and thought to play a role as negative regulator of progenitor cell proliferation.[34] Different epitopes on this sialomucin show different distribution patterns. Although all epitopes defined by four different antibodies are commonly distributed on hematopoetic cells, some of the epitopes are differentially and some even reciprocally expressed on lymphoid cells, endothelia, and epithelia of various tissues.[35] Most recently it was shown that these different epitopes vary in their sensitivity to various glycosidases, indicating that the differential expression pattern of these epitopes is indeed because of differential posttranslational modifications.[36] A similar classification of epitopes was reported for CD34, and these epitopes are also differentially expressed on different cells among hematopoetic progenitors and different types of endothelial cells.[37] Although the difficulties to detect endomucin with mAb on HEVs and on skin epithelium are most likely because of differential glycosylation, weak detection on arteriolar endothelium was because of weak expression, because even with our polyclonal antibodies against cytosolic epitopes only weak signals were obtained.

Dependent on the type of cell that expresses endomucin and on the type of glycosylation, endomucin may serve different functions. We found recently that during ontogeny endomucin is already expressed on endothelial cells of the E8.0 mouse embryo and that it seems to be present on putative clusters of hematopoetic precursors in the dorsal aorta at stage E9.0 to E11.5.[25] Because endomucin was brightly stained on endothelium of the aorta at stage 11.5, but only very weakly stained at stage E15.5 and also in the adult, it would be conceivable that endomucin has anti-adhesive activities that might help dislodging early hematopoetic cells from the dorsal aorta. Indeed, ectopic expression of endomucin in human embryonal kidney cells weakens the association of these cells with the cell-culture substratum[26] and inhibits cell-cell aggregation of these cells.[27] It is possible that this anti-adhesive activity is not directly linked to carbohydrate-mediated repulsive activities, but rather functions indirectly through signaling activities, because staurosporine, a kinase inhibitor known to enhance integrin-mediated adherence to cell substratum via focal contacts, was shown to overcome the endomucin effect.[26] The strong conservation of 46 of the 48 amino acids of the cytoplasmic domain between mouse and human endomucin indeed argues for a function of endomucin as a signal-transducing receptor.

Our results demonstrate that endomucin is primarily restricted to endothelium along the vascular tree in the mouse and human, with the only exception of expression on epithelium in the skin, but no other organs. Among peripheral leukocytes, endomucin is only weakly expressed on subpopulations of monocytes and B cells. The strong expression of endomucin on HEVs in mouse and human lymphoid organs suggests for the first time that endomucin could serve together with other sialomucins as a ligand for L-selectin. This hypothesis is strongly supported by the decoration of endomucin with the PNAd epitope MECA-79 and by the fact that staining of endomucin on HEVs was restricted to the luminal domain of HEVs in contrast to the staining with MECA-79 that was more widely distributed extending into the more basal areas. The wide distribution of endomucin on venules of all tissues would also provide endomucin as a potential carrier of the MECA-79 epitope at sites of chronic inflammation, where this epitope is inducible. In conclusion, our results allow the consideration of a proadhesive function for endomucin as a possible ligand for L-selectin on HEVs.

Acknowledgments

We thank Dr. Britta Engelhardt for valuable help and advice with the FACS analysis, Frank Kurth and Gabi Krull for excellent technical assistance, and Dr. Dieter Metze (Department of Dermatology, University of Münster) for providing human tissue samples and for valuable help in the histological analysis.

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