Expression of Factor VIII by Murine Liver Sinusoidal Endothelial Cells*

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(Received for publication, March 11, 1999, and in revised form, May 3, 1999)

Factor VIII (fVIII) is the procoagulant plasma glycoprotein that is missing or decreased in hemophilia A. The cellular origin of fVIII synthesis is controversial. Liver transplantation cures hemophilia A, demonstrating that the liver is a major site of fVIII synthesis. We detected fVIII mRNA in purified populations of murine liver sinusoidal endothelial cells (LSECs) and hepatocytes, but not Kupffer cells. LSECs and hepatocytes contained comparable numbers of fVIII mRNA (40 and 70 transcripts per cell, respectively) by quantitative competitive reverse transcriptase-polymerase chain reaction analysis. There was no detectable mRNA for factor IX, a hepatocyte marker, in the LSEC preparation, nor was there detectable mRNA for von Willebrand factor, an endothelial cell marker, in the hepatocyte preparation. This excludes the possibility that detectable fVIII mRNA is due to cross-contamination in the hepatocyte or LSEC preparations. Primary cultures of LSECs were established in which fVIII mRNA levels were indistinguishable from purified LSECs. LSECs secreted active fVIII into the culture medium. This finding represents the first demonstration of homologous expression of fVIII mRNA and protein in cell culture and should facilitate studies of fVIII gene regulation. Additionally, LSECs potentially are targets for a fVIII transgene during gene therapy of hemophilia A.

The site of the cellular origin for the biosynthesis of fVIII remains controversial despite studies that date back nearly 50 years (see Refs. 1 and 2, for reviews). Human and canine hemophilia A are cured by liver transplantation (3–6), which demonstrates that the liver contributes significantly to fVIII synthesis. Hepatocytes (7, 8), liver sinusoidal endothelial cells (LSECs) (9–11), or both (12), have been proposed as sites of fVIII synthesis. In this study, we isolated hepatocytes, LSECs, and Kupffer cells and measured steady-state levels of fVIII mRNA in these preparations. Our results indicate that both LSECs and hepatocytes synthesize fVIII mRNA. Additionally, LSECs in culture secrete active fVIII, providing a model for studies of the regulation of homologous fVIII gene expression.

* This work was supported by National Institutes of Health Grant R01 HL40921. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
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1 The abbreviations used are: fVIII, factor VIII; LSEC, liver sinusoidal endothelial cell; vWF, von Willebrand factor; ICAM-1, intercellular cell adhesion molecule-1 (CD54); VCAM-1, vascular cell adhesion molecule-1 (CD106); PECAM-1, platelet endothelial cell adhesion molecule-1 (CD31); PBS, fetal bovine serum; PBS, phosphate-buffered saline; HBSS, Hank's balanced salt solution; FITC, fluorescein isothiocyanate; PE, phycoerythrin; RT, reverse transcription; bp, base pairs; DMEM, Dulbecco's modified Eagle's medium.

EXPERIMENTAL PROCEDURES

Materials—Gey's balanced salt solution, Hank's balanced salt solution (HBSS), Dulbecco's phosphate-buffered saline (PBS), Liver Digest Medium, DMEM/F-12 medium, and AIM-V medium were purchased from Life Technologies, Inc. (Gaithersburg, MD). Penicillin (50 units/ml) and streptomycin (50 μg/ml) were added to DMEM/F-12 medium. Collagenase (type IV), gelatin, and dibutyryl cAMP were purchased from Sigma. DNase I was purchased from Roche Molecular Biochemicals (Indianapolis, IN). The following murine monoclonal IgG, antibody was purchased from Pharmingen (San Diego, CA): FITC-conjugated anti-PECAM-1 (anti-CD31), FITC-conjugated anti-VCAM-1 (anti-CD106), PE-conjugated anti-ICAM-1 (anti-CD54), the corresponding FITC-conjugated- and PE-conjugated isotype-specific control antibodies, and biotinylated anti-ICAM-1. FITC-conjugated wheat germ agglutinin was purchased from Molecular Probes (Eugene, OR). Streptavidin-conjugated and anti-CD11b-conjugated magnetic beads were purchased from Miltenyi, Inc. (Auburn, CA). Liver Cell Isolation—Single cell suspensions were prepared from livers of five or six 10–12-week-old Balb/c female mice (Harlan Sprague-Dawley Laboratories). Mice were anesthetized intraperitoneally with sodium pentobarbital. Livers were perfused in situ via the portal vein with 15–20 ml of Gey's balanced salt solution with drainage through a severed inferior vena cava. Livers were perfused with 60 ml of digestion medium at a flow rate of 5 ml/min and then removed and submerged in DMEM/F-12 medium on ice. Subsequent steps were carried out at 4 °C unless indicated otherwise. To purify hepatocytes, liver capsules were gently disrupted in DMEM/F-12 medium using two 1-ml serological pipettes. The hepatocytes were allowed to settle by gravity for 6 min and then washed five times with DMEM/F-12 medium by gravity sedimentation.

To obtain sinusoidal cells (LSECs and Kupffer cells), gall bladders were removed and the livers were minced with a sterile razor blade. The livers were digested with 0.02% (w/v) collagenase and 0.0005% (w/v) DNase I in 50 ml of HBSS containing 20 μM HEPES, pH 7.4, at 37 °C for 30 min with occasional shaking. The resulting cell suspension was filtered through a 75-μm nylon mesh and the filtrate was centrifuged at 200 × g for 10 min to pellet the hepatocytes. The supernatant was removed and pelleted by centrifugation at 600 × g for 10 min. The cells were washed once with HBSS, layered over a 1.037 g/ml solution of Percoll (Pharmacia) and centrifuged at 400 × g for 20 min. The resulting upper layer and interface, containing Ito cells, dead cells, and debris, were removed. The lower layer was diluted 3-fold with Dulbecco's PBS and centrifuged at 600 × g for 10 min to pellet the cells.

Kupffer cells and LSECs were isolated by anti-CD11b-conjugated and biotinylated ICAM-1/streptavidin-conjugated magnetic bead cell sorting, respectively, over MS™ MiniMACS separation columns (Miltenyi, Inc.) according to instructions supplied by the manufacturer. Briefly, the cell pellet from the Percoll centrifugation step was suspended in 0.45 ml of Dulbecco's PBS, 0.5% bixin-free bovine serum albumin, 2 μM EDTA, followed by addition of anti-CD11b-conjugated magnetic beads and elution of anti-CD11b-positive cells. Biotinylated anti-ICAM-1 antibodies (50 μg/ml) were added to the anti-CD11b and non-adherent fraction for 15 min, followed by isolation of LSECs using streptavidin-conjugated magnetic beads. Both preparations were eluted with DMEM/F-12 medium, 15% FBS, dibutyryl cAMP (250 μg/ml) and stored at concentration of 1–2 × 10^6 cells per ml. Approximately 3 × 10^6 LSECs were obtained from six livers.

Cell Culture—Cells were cultured at 37 °C in a humidified atmosphere containing 5% CO2. Freshly isolated LSECs were plated onto 0.1% (w/v) gelatin-coated 12-well Falcon 3043 plates (401 mm² per well) at a density of 1 × 10^5 cells per well. The cells were incubated for 2 h, non-adherent cells were washed off with HBSS and fresh medium.
containing DMEM/F-12, 15% FBS, 250 μg/ml dibutyryl cAMP was added. Initially, the cells were 30–40% confluent and grew to confluence in 1 or 2 days. Each well contained approximately 1 × 10^5 cells at confluence. Confluent cultures of human umbilical vein endothelial cells and human dermal microvascular endothelial cells were purchased from the Emory Skin Cell Center and were also maintained in DMEM/F-12, 15% FBS, 250 μg/ml dibutyryl cAMP. For studies of FVIII secretion, confluent monolayers were washed three times with HBSS and then maintained in AIM-V, a serum-free cell culture medium for 2 days.

Freshly isolated hepatocytes were plated onto collagen-coated Costar six-well plates (962 mm² per well) at 6 × 10^4 cells per well for 30 min in DMEM/F-12, 10% FBS. Nonadherent cells were washed off with HBSS. Hepatocytes were maintained in medium containing DMEM/F-12 plus 10% FBS for 2 days prior to assay for FVIII secretion.

Characterization of Cells—Purified cells were prepared for differential staining and light microscopy by centrifugation onto microscope slides using a Cytospin 3 Cell Preparation System (Shandon Scientific, Cheshire, United Kingdom). Cells were stained using a modified Wright-Giemsa stain (Diff-Quik, Baxter, McGaw Park, IL).

Freshly isolated LSECs and trypsin/EDTA-solubilized cultured LSECs were characterized further by flow cytometry. Cells were diluted to 1 × 10^6 cells/ml with Dulbecco’s PBS, 3% FBS and incubated with saturating concentrations of dye-labeled specific antibody: isotype control antibody, or whole germ agglutinin. The cells were washed once and resuspended in Dulbecco’s PBS, 3% FBS. Data collection and analysis were done using a Becton Dickinson FACSort flow cytometer and CellQuest software.

RT-PCR Reactions—Total RNA from hepatocyte, Kupffer cell, and LSEC preparations and from cultured LSECs was isolated using a RNeasy Mini Kit (Qiagen, Santa Clarita, CA). RNA was quantitated spectrophotometrically at 260 nm using an extinction coefficient of 25 ml/mg/cm. Reverse transcriptase (RT) reactions were conducted using a First-Strand cDNA Synthesis Kit (Pharmacia), 0.2 μg of total RNA template and primers specific for FVIII, von Willebrand factor (vWF) or factor IX (Table I).

The resulting cDNA fragments were amplified by PCR using Taq DNA polymerase (Promega, Madison, WI) and the primers listed in Table I. Samples were denatured for 2 min at 94 °C, followed by 28 cycles of denaturation for 1 min at 94 °C, annealing for 2 min at 55 °C, and elongation for 2 min at 72 °C. Reactions were completed by a final elongation step for 5 min at 72 °C. The products were subjected to 1.5% agarose gel electrophoresis and visualized using ethidium bromide.

FVIII mRNA levels in LSECs and hepatocytes were quantitated by a modification of published competitive RT-PCR procedures (13–15). In this method, a known concentration of FVIII-specific cRNA is added to the RT reaction, which produces a cDNA that competes for the native FVIII cDNA during PCR amplification. The point of equivalence, where cRNA-derived and mRNA-derived cDNA products are equal, determines the number of mRNA molecules in the sample.

To initially test the method, two regions of FVIII were amplified using cRNA As and PCR primers corresponding to the A3 and C2 domains of murine FVIII. The PCR products contained intron-spanning sequences to avoid potential signals from contaminating genomic DNA. The cRNAs were identical to endogenous FVIII mRNA except for an internal 50-bp deletion so that the resulting cDNA-derived PCR product could be distinguished from the endogenous FVIII PCR product electrophoretically. This produced 500/450- and 400/350-bp products for the A3-specific and C2-specific PCR reactions, respectively.

The first step in the cRNA constructions was synthesis of cDNAs containing 50-bp loop-outs. The cDNAs were synthesized by PCR amplification of RT products obtained from total liver RNA using the A3-specific and C2-specific primers shown in Table I. The A3 sense loop-out primer, 5’-AAAACTCAGGGTTCTACTCTTATTTCTAGG CGTCAGCTGGAAAGATACCATCG-3’, corresponded to nucleotides 5713–5819 with the deletion of nucleotides 5737–5736. The A3 antisense primer, 5’-TTTCTGCAAGCC CATGCTCGAGATCTACGTC-3’, corresponded to nucleotides 6189–6212. The C2 sense loop-out primer, 5’-AAAACTCGAGCTGGT AGGGCTGATGACAAAACTCGTCTCC-3’, corresponded to nucleotides 6858–6952. The C2 antisense primer, 5’-TCTCAGCTATTTCT GCTGGGCACTACCATCTAG-3’, corresponded to nucleotides 7341–7364.

The loop-out cDNA products were cloned into pBluescript II phagemid (Stratagene, La Jolla, CA) using PsiI restriction sites that had been incorporated into the PCR primers (underlined). The sequence of the cDNA products was verified by dideoxy sequencing. The cRNA was produced by T3 RNA polymerase-catalyzed in vitro transcription off pBluescript using a Ribomax Transcription Kit (Promega) and purified using a RNeasy Mini Kit. The molar concentration of cRNA was calculated from the absorbance at 260 nm and the molecular weight.

The cRNA-dependent inhibition of endogenous FVIII cDNA synthesis was quantitated by densitometry of ethidium bromide-stained products. Gel photographs were obtained using a Hewlett-Packard 6200C ScanJet scanner and were analyzed using digitization and quantitation software (UN-SCAN-IT, Silk Scientific, Orem, UT). Plots of the ratio of mRNA-derived and cRNA-derived products as a function of cRNA concentration were linear. The point of equivalence was determined by linear regression analysis.

FVIII Activity Assay in Endothelial Cell Culture Supernatants—The measurement of FVIII in cell culture supernatants was measured using a plasma-free chromogenic assay that measures the thrombin-activated FVIII-dependent rate of factor X activation by factor IXa as described previously (16, 17). The reaction components included limited-activated factor VIII, 0.5 nm porcine factor IXa, 425 nm porcine factor X, and 50 μm phospholipid. The initial rate of factor Xa formation was measured using the chromogenic substrate Spectrozyme Xa (American Diagnostica, Greenwich, CT). The results were compared with a standard curve prepared using recombinant VIII of known coagulant activity (provided by Hyland-Immuno Div., Baxter Healthcare, Duarte, CA).

RESULTS

Isolation of Liver Cell Populations—Hepatocytes were isolated from liver cell suspensions by low speed centrifugation. They were identified as large (20–25 μm), frequently binucleate cells with basophilic cytoplasm. The preparation was less than 5% contaminated by other cell types. Kupffer cell preparations were obtained using anti-CD11b magnetic bead cell sorting and identified by their relatively large size (10–12 μm), eccentric nuclei, and numerous vacuoles. CD11b-positive preparations contained approximately 80% Kupffer cells/monocytes and 20% granulocytes. LSECs were isolated from the CD11b-negative population by anti-ICAM-1 magnetic bead cell sorting. They were identified by their relatively small size (8–10 μm), clear cytoplasm, and oval nuclei (Fig. 1A). LSEC preparations typically were 90% pure and contained Kupffer cells (2%) and red blood cells (8%) as contaminants.

Analysis of the LSEC preparation by flow cytometry demonstrated that the cells expressed cell surface markers ICAM-1 and PECAM-1 and contained a subpopulation of the cells that bound wheat germ agglutinin (Fig. 2). LSECs did not stain with the monocyte/macrophage-specific marker CD14 or the endothelial marker VCAM-1 (data not shown). These findings are similar to previously described phenotypic characteristics of murine LSECs, which were ICAM-1+/PECAM-1−/VCAM-1− and contained two subpopulations that differentially bound wheat germ agglutinin (18, 19). The reason for the lack of VCAM-1 staining in our LSEC preparation is not known, although the cells became VCAM-1− after cell culture.

Quantitation of FVIII mRNA by Competitive RT-PCR—Ini-
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Fig. 2. Analysis of purified LSECs by flow cytometry. Staining of LSECs using fluorophore-conjugated antibodies specific for ICAM-1 (CD54) (A) and PECAM-1 (CD31) (B). The isotype control is represented by the black profile for each histogram plot. C, staining of LSECs with FITC-conjugated wheat germ agglutinin.

|=|TABLE I
|**Oligonucleotide primers used for RT-PCR**

| Gene                  | Oligonucleotide (5′→3′)       | Position |
|-----------------------|-------------------------------|----------|
| RT primers            |                               |          |
| FVIII A3 sense        | GCCCATGCTGAGAAGATACCATCG      | 6189–6212|
| FVIII C2 sense        | GTATTGGCTGAGGCTCCATCC         | 7341–7364|
| Factor IX sense       | TTTTCCCCAGCCACCTGACATAGCCA    | 1044–1068|
| vWF sense             | GGCAGTTGCAGACCCTCCTTGT        | 5124–5144|
| PCR primers           |                               |          |
| FVIII A3 sense        | GTCCCTACTCTTCTATCTGACCCA      | 5713–5736|
| FVIII A3 antisense    | GCCCATGCTGAGAAGATACCATCG      | 6189–6212|
| FVIII C2 sense        | CTTCCGGATTGGAGTTGAGGCTGTC     | 6852–6875|
| FVIII C2 antisense    | TACATCAGGTTGAGTGGCTCTG        | 7227–7251|
| Factor IX sense       | CTGCAGTTGGATGAGGAAACCG        | 668–691  |
| Factor IX antisense   | TTTTCCCCAGCCACCTGACATAGCCA    | 1044–1068|
| vWF sense             | ATGATGGGAGGTACATC             | 4015–4035|
| vWF antisense         | GGCAGTTGCAGGACCCTCCTTGT       | 5124–5144|

*: Numbering is based on the cDNA sequences of murine FVIII (GenBank accession number L05573) and murine factor IX (GenBank accession number M23109). vWf numbering is by homology to the human vWf cDNA sequence (47).

Experimental Procedure. In this method, the concentration of cRNA that produces equal amounts of fVIII mRNA can be determined by finding the concentration of added competitor cRNA, which produces a 450-bp product. Regression analysis of the band intensities yielded a value of 3.3 × 10^6 FVIII mRNA transcripts/μg of total cellular RNA. Similar experiments were done using a hepatocyte preparation. The results of several experiments with LSECs and hepatocytes are shown in Table I. Purified LSECs contain approximately 5-fold more FVIII transcripts/μg of total cellular RNA than hepatocytes. Because hepatocytes contain more total RNA per cell than LSECs, hepatocytes contain approximately twice as many FVIII transcripts per cell than LSECs.

In parallel RT-PCR reactions, purified LSECs were positive for vWF mRNA, a marker for endothelial cells (Fig. 3, lane 10). Factor IX mRNA, a hepatocyte marker, was not detected in LSECs (Fig. 3, lane 8). Conversely, Factor IX mRNA was detected in hepatocytes (Fig. 3, lane 9), but vWF mRNA was not (data not shown.).

**FVIII Expression in Cultured LSECs**—Primary monolayer cultures of LSECs were established by growing cells on gelatin in the presence of DMEM/F-12, 15% FBS, dibutylryl cAMP. LSECs were spindle-shaped and displayed long cytoplasmic extensions prior to confluence (data not shown). As they approached confluence, they formed a polygonal, flat “cobblestone” monolayer (Fig. 1B) that is characteristic of cultured endothelial cells (20). Cultured LSECs were positive by flow cytometry for ICAM-1, PECAM-1, VCAM-1, and bound wheat germ agglutinin (data not shown). Comparative RT-PCR analysis of FVIII mRNA of cultured LSECs (Fig. 4) yielded levels that were indistinguishable from purified LSECs (Table I). Additionally, like purified LSECs, cultured LSECs expressed vWF, but not factor IX (Fig. 4). Cultured LSECs maintained their phenotypic characteristics when trypsinized, split 1:1, and regrown to confluence. They did not survive a second passage under the growth conditions described under “Experimen-
The FVIII-dependent rate of factor Xa formation in LSEC supernatants was significantly greater than medium alone (Fig. 5). The measured rate corresponds to $1.43 \times 10^2$ units/ml of coagulant activity using human FVIII as a standard. In contrast, rates of factor Xa formation due to human umbilical vein endothelial cells or dermal microvascular endothelial cells were not significantly above background. FVIII activity was not detected in primary cultures of hepatocytes (data not shown).

**DISCUSSION**

Hepatic FVIII gene expression was studied by RT-PCR using purified populations of murine LSECs, hepatocytes, and Kupffer cells. We detected FVIII mRNA in LSECs and hepatocytes, but not Kupffer cells. The absence of factor IX mRNA, a hepatocyte marker, in purified LSECs (Fig. 3) excluded the possibility that the FVIII mRNA was due to contaminating hepatocytes. Conversely, the absence of vWF mRNA, an endothelial cell marker, in the hepatocyte preparation excluded the possibility of a false positive FVIII signal due to contaminating LSECs.

We also identified FVIII mRNA in cultured LSECs (Fig. 4). This represents the first demonstration of homologous expression of FVIII mRNA in primary cell culture. In contrast, previous studies of FVIII expression in cell culture have been conducted by transfecting FVIII gene fragments into liver cell-derived cell lines (21, 22) or Chinese hamster ovary cells (23, 24). Our results should facilitate studies of FVIII gene regulation under more physiological conditions.

There has been considerable controversy regarding which type of liver cell synthesizes FVIII. FVIII mRNA was identified in a human hepatocyte preparation by RNase protection assay (7). However, the preparation was contaminated with LSECs and Kupffer cells. In the same study, FVIII mRNA was not detected in a liver sinusoidal cell preparation. FVIII protein was localized by immune electron microscopy to the rough endoplasmic reticulum of both human hepatocytes and LSECs.
(12). In contrast, FVIII was localized immunohistochemically to LSECs but not hepatocytes (9, 10, 25, 26). FVIII activity was detected in rat LSECs, but not hepatocytes (11). FVIII mRNA levels were not determined in that study.

Our results show that LSECs and hepatocytes make similar amounts of FVIII mRNA (40 versus 70 transcripts per cell, respectively, Table II). The ratio of hepatocyte to LSECs in liver is approximately 3 to 1 (27, 28). Thus, the ratio of hepatocyte to LSEC steady-state FVIII mRNA transcripts in liver is approximately 5 to 1, suggesting that LSECs may contribute 15–20% of the normal hepatic synthesis of FVIII. FVIII levels rise to normal after liver transplantation in patients with hemophilia A, during which there can be no extrahepatic synthesis of FVIII (3). This indicates that LSECs potentially synthesize hemostatically significant amounts because FVIII levels in the 15–20% range substantially ameliorate the hemostatic defect in hemophilia A. In fact, FVIII levels actually are increased in fulminant hepatic failure (29–31), which is associated with a profound loss of protein synthesis by hepatocytes. In this setting, levels of all other hepatic coagulation and fibrinolytic factors, including fibrinogen, prothrombin, factors V, VII, IX, X, XI, XII, XIII, prekallikrein, high molecular weight kinogen, protein C, plasminogen, antithrombin III, and α2-antiplasmin are decreased. Up-regulation of FVIII synthesis by LSECs may occur under these circumstances.

The amount of FVIII that is secreted by cultured LSECs is consistent with significant synthesis in vivo. There are approximately $8 \times 10^{10}$ LSECs in adult human liver. Synthesis of $1.4 \times 10^{-2}$ units of FVIII per 10$^6$ cells over 48 h (Fig. 5) would correspond to total LSEC expression of 5,600 units/day in vivo. By comparison, the estimated daily synthesis of FVIII in adults is roughly 3000 units because total circulating FVIII is approximately 3000 units and turnover occurs approximately daily.

In contrast, we did not detect FVIII activity in cultured hepatocytes. The expected levels are relatively low ($1.4 \times 10^{-2}$ units/ml observed in LSECs corresponds to 12 pmol). Cellular uptake, which occurs during heterologous expression of FVIII by Chinese hamster ovary cells (23), or degradation by a protease secreted by hepatocytes, could account for the lack of detectable activity.

The identification of FVIII in LSECs raises the question of whether endothelial cells from other tissues contribute significantly to FVIII synthesis. FVIII mRNA has been detected in spleen, lymph node, heart, brain, lung, kidney, testes, muscle, and placenta (7, 16, 32), which is consistent with a common endothelial cell origin. However, FVIII has not been identified in cultured endothelial cells from human umbilical vein and other tissues (7, 33). This is consistent with our finding that human umbilical vein or dermal microvascular endothelium does not contain detectable FVIII mRNA or activity (Fig. 5). Furthermore, hemophilia A is not cured by kidney transplantation (34, 35), which further indicates that significant FVIII synthesis is not a general property of endothelium. The inability of bone marrow transplantation to cure hemophilia A (36) also excludes cells of the monocyte/macrophage system as a source of FVIII synthesis, which is consistent with our finding that Kupffer cells do not contain detectable FVIII mRNA.

However, several observations suggest that the extrahepatic synthesis of FVIII can be clinically significant. The most compelling finding is that liver transplantation from hemophilia A dogs to normal dogs does not produce hemophilia A (6). Additionally, spleen transplantation has been reported to produce increased FVIII levels in human (36–39) and canine hemophilia A (40), although other investigators have not observed this in the canine system (5, 6). Overall, these findings, combined with the unequivocal demonstration of endothelial synthesis of FVIII in the present study, are most consistent with the hypothesis that both LSECs and nonhepatic endothelial cells contribute significantly to FVIII synthesis.

FVIII circulates bound noncovalently to vWF. In contrast to FVIII, vWF has been identified throughout the vascular endothelium and is widely used as an endothelial cell marker. Interestingly, vWF mRNA levels in liver are low relative to other tissues (41). Circulating FVIII protein levels are regulated by vWF (see Refs. 1 and 2, for reviews). Infusion of vWF into patients with severe von Willebrand disease leads to a rapid increase in circulating FVIII levels (42, 43). This increase occurs without an increase in synthesis of FVIII mRNA (44). This indicates that vWF increases secretion of stored FVIII in this condition. Whether vWF influences FVIII mRNA and/or protein secretion under normal conditions is unknown. The identification of FVIII and vWF synthesis in the same cell type (Figs. 3 and 4) raises the possibility of coordinate gene regulation in vivo. The availability of cultured LSECs that synthesize both FVIII and vWF should facilitate studies in this area.

Hemophilia A is an attractive target for gene therapy. Our finding that the LSEC can support substantial synthesis of FVIII make it a potentially attractive host for FVIII synthesis. Portal vein infusion of a suitable vector could deliver FVIII directly to LSECs. Alternatively, FVIII could be introduced into cultured LSECs ex vivo, followed by autologous transplantation. Subsequent expression of FVIII by transduced LSECs under physiological conditions, particularly with respect to regulatory control by vWF, could offer a superior approach to the management of hemophilia A.

Acknowledgments—We thank Dr. Thomas D. Boyer, Emory University, for interesting discussions leading up to the origination of this project and Dr. Susan Voss and Dr. Richard Whalen, Emory University, for advice on performing liver perfusions. We thank Dirk Hunt, Emory University, for help with gel scanning software. We thank Jose Cardier, Centro de Medicina Experimental, Caracas, Venezuela, for advice regarding the LSEC preparation.

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J. Biol. Chem. 1999, 274:19587-19592.
doi: 10.1074/jbc.274.28.19587

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