CONCISE REPORT

Identification of Normal Human Peripheral Blood Monocytes and Liver as Sites of Synthesis of Coagulation Factor XIII a-Chain

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Factor XIII is the fibrin-stabilizing factor that covalently cross-links fibrin monomers into a stable fibrin clot, a tetrameric complex (a2b2), consisting of two a-chains and two b-chains. The a-chain, an enzyme that catalyzes the formation of stable cross-links in fibrin, contains a transglutaminase domain. To address questions regarding sites of synthesis of factor XIII a-chain, an EcoRI restriction fragment from the protein-coding region of the factor XIII a-chain cDNA was used as a probe for Northern blot analysis. The cDNA probe showed hybridization with a single ~4.0-kilobase (kb) message in poly (A)+ mRNA prepared from normal human peripheral blood monocytes and normal human liver. The results demonstrate conclusively that factor XIII a-chains are actively synthesized in circulating monocytes and in liver. To our knowledge, these data represent the first demonstration of synthesis of any blood coagulation factor in primary uncultured and unstimulated monocytes or macrophage cells.

MATERIALS AND METHODS

Monocyte isolation. Human peripheral blood monocytes were isolated from a normal donor plateletpheresis residue bag. Mononuclear cells were separated on Ficoll-Hypaque gradients (Ficoll-Paque, Pharmacia, Piscataway, NJ) and washed in phosphate-buffered saline (PBS) + 10 mmol/L of EDTA. Monocytes were separated from lymphocytes by discontinuous Percoll (Pharmacia) density gradient sedimentation, and again washed in PBS + 10 mmol/L of EDTA. The monocytes were incubated twice in pooled human serum + 10 mmol/L of EDTA at 37°C for 15 minutes to remove platelets. After further washing in PBS + 10 mmol/L of EDTA, Wright's stain and esterase stain showed 96% monocytes, 4% lymphocytes, and no visible platelets. The cells were washed in 45% Dulbecco's modified Eagle's medium (DMEM, GIBCO, Grand Island, NY) 40% heat-inactivated fetal bovine serum, and 15% dimethylsulfoxide. They were slowly frozen in this medium and stored at < -100°C in liquid nitrogen vapor. Immediately before RNA preparation, the monocytes were thawed by incubation in a 37°C water bath for 2 minutes, and were washed three times in prewarmed PBS.

Tissue preparation. Fresh normal human liver, obtained at the time of an open liver biopsy procedure performed for other reasons, was homogenized in the presence of 5 mol/L of guanidine thiocyanate lysis buffer in a Poltron blander. Frozen placental tissue was similarly homogenized in a Poltron blander; no thawing took place before guanidine thiocyanate was added to the specimen. Monocytes, after thawing as described above, were homogenized by vigorous vortexing in the presence of guanidine thiocyanate.

RNA preparation. The guanidine thiocyanate/tissue homogenates were precipitated in 4 mol/L of LiCl, and RNA was prepared according to the method of Cathala and colleagues. The poly (A)+ mRNA fraction was selected by elution from an oligo (dT)-cellulose column.

Northern blot analysis. Poly (A)+ mRNA was electrophoresed on a 1% agarose 2.2 mol/L formaldehyde gel and blotted to nitrocellulose. The filters were hybridized overnight at 42°C in 50% formamide, 10% dextran sulphate, 3 x SSC, 50 mmol/L of NaH2PO4, 200 μg/mL of sheared salmon sperm DNA, 150 μg/mL of yeast RNA. The molecular probe was a 630-base pair (bp) EcoRI restriction fragment of the cDNA for factor XIII a-chain (Fig 1), labeled with [α-32P]dCTP (Amersham, Arlington Heights, IL) by calf thymus DNA priming. Following hybridization, the filters were rinsed once at room temperature in 2 x SSC, and then washed twice at 50°C in 0.1 x SSC, 0.1% sodium dodecyl sulfate (SDS). The hybridized filters were subjected to autoradiography at -70°C using Kodak XAR-2 film and DuPont Cronex intensifying screens.

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RESULTS

In the first experiment, RNA was isolated from human peripheral blood monocytes and, as a positive control, from frozen human placental tissue. One of our EcoRI restriction fragments from the cDNA for factor XIII, previously described, was 630 bp long (Fig 1) and was located within the protein-coding region. This fragment was 32P-radiolabeled and used as a probe for Northern blot analysis of the monocyte and placental RNA samples. The autoradiogram (Fig 2) shows that there is a single ~4.0-kb message for factor XIII a-chain in human peripheral blood monocytes and placenta. Poly (A)+ mRNA from K562 cells (human chronic myelogenous leukemia, ATCC CCL 243) showed no hybridization. K562 cells were chosen because they express platelet glycoproteins, suggesting that they might synthesize factor XIII a-chain, another platelet protein.

In the second experiment, the same 32P-radiolabeled probe was hybridized to poly (A)+ mRNA from fresh human liver, again using placental poly (A)+ mRNA as a positive control and K562 cell poly (A)+ mRNA as a negative control for Northern blot analysis. Autoradiography (Fig 3) demonstrates the presence of a single ~4.0-kb message for factor XIII a-chain in human liver and placenta.

DISCUSSION

Because monocytes and macrophages are phagocytic cells, identifying factor XIII a-chains intracellularly or on the cell surface would not exclude the possibilities that the a-chains were endocytosed or adsorbed from the plasma rather than synthesized directly. Moreover, a-chains in peripheral blood monocytes may represent protein previously synthesized in bone marrow monocyte precursors rather than in the mature cells themselves. The data of the present study (Fig 2) conclusively demonstrate primary peripheral blood monocytes as a site of synthesis of factor XIII a-chains. The intensity of hybridization of our 32P-radiolabeled cDNA probe is approximately equal for the monocyte (lane A) and placental (lane B) poly (A)+ mRNA samples; since the amount of RNA applied to the gel was 100 times as great from monocytes (1 μg) as from placenta (0.01 μg), we estimate that an ~100-fold greater relative abundance of message for factor XIII a-chain exists in placenta than in peripheral blood monocytes. Platelets have factor XIII a-chains, but synthesis of protein by platelets has not been convincingly demonstrated. Even if a small amount of megakaryocyte-derived mRNA persisted in circulating platelets, our monocyte preparation appeared to be entirely free of platelet contamination, thus eliminating this potential cause of false positive results.

To our knowledge, these data represent the first demonstration of synthesis of any blood coagulation factor in primary uncultured and unstimulated monocytes or macrophage cells. Although several studies report identification of factor XIII a-chain protein in such cells, demonstration of actual synthesis of the protein was performed on a tissue culture cell line (U937, a human monocytelike cell line).
Several studies have reported monocyte-macrophage synthesis of other coagulation factors. Synthesis of factor VII by human alveolar macrophages in vitro was demonstrated on cultured cell monolayers. Increased expression of factor VII protease activity, possibly representing synthesis, was demonstrated with bacterial lipopolysaccharide, an endotoxin, but not in freshly isolated cells or unstimulated cultured cells. Mouse peritoneal macrophages synthesized factors II, VII, IX, and X when grown in tissue culture. These previous studies were performed on mononcytic cell lines, fresh monocyte or macrophage cells grown in tissue culture, or stimulated cells, to demonstrate synthesis of the clotting factors. Based on our observation that normal, unstimulated monocytes synthesize factor XIII a-chains, this enzyme is likely to have an important function under physiologic conditions.

The function of factor XIII a-chains in circulating monocytes is uncertain. Factor XIII is a transglutaminase enzyme that covalently cross-links fibrin monomers and stabilizes the fibrin clot. In addition, it has several other substrate specificities and has been shown to cross-link collagen, fibronectin, thrombospondin, Factor V, and von Willebrand's factor. The factor XIII a-chains synthesized in monocytes might be a readily available source of transglutaminase activity in areas of vascular endothelial injury for processes such as clot retraction and wound healing. As monocytes exit the vascular spaces and enter tissues to become macrophages, they may be expected to carry with them the ability to synthesize factor XIII a-chains and to participate in similar processes in areas of tissue inflammation.

Our data demonstrate human liver as a site of synthesis of factor XIII a-chains (Fig 3). More than 100 times as much poly (A)* mRNA from liver (lane A) as from placenta (lane B) was applied to the gel (10 and 0.09 µg, respectively), yet hybridization was approximately five times more intense for the placental sample. This result indicates that there is an ~500-fold greater relative abundance of message for factor XIII a-chain in the placenta than in the liver. Thus, the results provide a possible explanation for the finding by Grundmann and colleagues, who reported hybridization of a factor XIII a-chain cDNA fragment to poly (A)* mRNA from human placenta but not to poly (A)* mRNA from human liver. In that study, equal quantities (4 µg) of poly (A)* mRNA from each tissue source were probed, and hybridization to the liver RNA appeared to be negative.

Some investigators have found factor XIII a-chain protein in human and rabbit hepatocytes and the hepatoma cell line Hep G2, but others did not detect the protein in human hepatocytes. A variety of techniques were used for these studies, including enzyme-labeled antibody staining, identification of protein by fluorescent antibody binding and measurement of synthesis by radioimmunoassay and [14C] leucine incorporation in isolated hepatocytes. Fear and associates did not find factor XIII a-chains in hepatocytes by an immunoperoxidase technique using rabbit anti-human factor XIII a-chain antibody on sections of paraffin-embedded human liver. They did, however, find a-chains in cells interpreted as inactive fibroblasts in the portal tracts. Our results indicate a very low abundance of message for factor XIII a-chains in liver tissue, most of which is comprised of hepatocytes. If the intracellular concentration of a-chain protein were also very low in hepatocytes, the discrepant findings in the previous studies might be due to different sensitivities of the various techniques used. In addition, since fibroblasts may be very difficult to distinguish from tissue macrophages, it is interesting to speculate that the positively stained cells in the study of Fear and colleagues were actually macrophages. Such cells, if derived from blood monocytes, might have a greater concentration of factor XIII a-chains than do hepatocytes, thus appearing positive by an immunoperoxidase technique while hepatocytes appear negative. To identify the specific cells in liver tissue that synthesize factor XIII a-chain, we have begun work on situ hybridization using cDNA probes.

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