Factor XIII A Is Synthesized and Expressed on the Surface of U937 Cells and Alveolar Macrophages

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Factor XIII A subunit was detected in U937 cells and human alveolar macrophages by immunohistochemistry and Western blotting. U937 cells synthesize factor XIII A subunit de novo under serum-free, platelet-free conditions, as indicated by 35S-methionine labeling and immunoprecipitation. Thrombin-dependent activity was demonstrated to account for 98% of the total transglutaminase activity in U937 cells (1.5 μg per 0.5 x 10^6 cells/mL). Intact U937 cells and alveolar macrophages and homogenates from these cells cross-linked fibrin to form γ-γ and α-polymers. Factor XIII A was detected on the surface of intact U937 cells and macrophages by flow cytometry and 125I-labeling and immunoprecipitation. Cell surface expression of factor XIII A was augmented in the presence of several soluble macrophage activators; however, no concurrent increase in its biosynthesis was observed. The presence and cell surface expression of factor XIII A subunit within macrophages suggest new pathways by which these cells may function in clotting and in the remodeling of the extracellular matrix during inflammation and wound healing.

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FACTOR XIIIa: SYNTHESIS IN U937 CELLS

several changes of phosphate buffered saline (PBS) and 0.05% Tween-20 for two hours and stained for one hour with 125I-protein A (New England Nuclear, Boston). After washing, the blots were dried, and autoradiography was performed for variable intervals at −80 °C with Kodak X-OMAT-AR film (Eastman Kodak, Rochester, NY).

Crosslinking of fibrin. Fibrinogen was purified from human plasma and iodinated with Na125I by a lactoperoxidase method.11 For the fibrin crosslinking experiments, 125I-fibrinogen (1 mg/mL) was crosslinked with purified placental factor XIII (20 μg/mL), CaCl2 (6 mmol/L), and α-thrombin (10 U/mL) (a gift from Dr John Fenton, NY State Department of Health). U937 cells, U937 cell extracts (2 to 3 x 106), or alveolar macrophages (7.8 x 105 to 1 x 106) were incubated in the presence of 125I-fibrinogen (~150,000 cpm/sample), which had been pretreated with p-hydroxymercuribenzoate (PCMB) to inactive traces of factor XIII in the fibrinogen, and factor XIII deficient plasma, CaCl2 (20 mmol/L) and human α-thrombin (0 to 10 U/mL) for 60 to 120 minutes at 37 °C. Controls of 125I-fibrinogen clotted in a 0, 1/10, or 1/100 dilution of normal plasma in HEPES-glycine buffer were also analyzed. Clots were removed and washed six times in PBS with 1mmol/L phenylmethylsulfonyl fluoride followed by additional washes in HEPES-glycine buffer, pH 7.5. The washed clot was suspended in reducing buffer, dissolved by boiling for ten minutes, and analyzed by 5% or 10% SDS-PAGE. Gels were dried and developed at −80 °C for autoradiography as above.

U937 culture and stimulatory conditions. U937 cells were maintained in stationary suspension cultures at a density of 1 to 2 x 105 cells/mL in RPMI 1640 (GIBCO, Grand Island, NY) in the presence of heat-inactivated, 5% fetal bovine serum (FBS) and gentamicin (50 μg/mL) in an atmosphere of 95% air and 5% CO2. Cells were stimulated in log-phase growth by recombinant IFN-γ (100 U/mL; Genentech, San Francisco), lipopolysaccharide (LPS; S. Typhimurium, Ortho Diagnostic Systems, Westwood, MA) after gating on the desired population utilizing their right and forward angle light scatter characteristics. Viable U937 cells form a cluster on the scatter plot in the same region as T cell blasts. The fluorescence amplifier gains were set at 520 to 600 units. These settings yielded a peak of fluorescence for glutaraldehyde-fixed erythrocytes at channel 100 using a range of 0 to 255 linear units. The median fluorescence intensity was used as a quantitative measure of the amount of binding of antibodies.

Biosynthetic labeling of U937 cells. U937 cells (6 x 106) were washed five times in methionine-free RPMI and plated in 24 well plates at 6 x 105 cells/mL for one hour. L-15 S-methionine (120 μCi) were added to each well, and the cells were incubated for four hours at 37 °C. The cells were washed three times in PBS, with 0.4% NaN3 at 4 °C. The cell pellets were washed in Tris buffer, pH 7.3, centrifuged at 4,000 x g, lysed, and extracted in 1 mL of Tris/ NP-40 with 1 mmol/L phenylmethylsulfonyl fluoride; lysing was continued on ice in the presence of 3.5 mol/L NaCl. The lysate was precleared with Staph A (New England Enzyme Center, Boston), immunoprecipitated with rabbit antihuman factor XIII A or anti-ovalbumin for 60 minutes at 37 °C, and antigen-antibody mixture was incubated with Staph A overnight. The immunoprecipitate was washed in buffer, subjected to three cycles of freezing and thawing, boiled in reducing sample buffer for ten minutes, and analyzed by 10% SDS-PAGE. The resulting gels were dried and developed in cassettes for two to four weeks with Ultralfilm (LK Broma, Sweden).

Surface membrane labeling of U937 cells. U937 cells (1 x 106) were washed four times in PBS with 0.4% NaN3 and once with 60 mmol/L sodium phosphate buffer. The cells were suspended in 200 μL of phosphate buffer in the presence of 200 μL of lactoperoxidase, Enzymobeads (Bio-Rad, Richmond, CA), 1% beta-D-glucose, and 2 mmol/L Na125I for 25 minutes. The reaction was quenched with iced Tris buffer, and the cell pellet was washed four times. Membranes were extracted in the presence of Lysis-EDTA buffer (0.5% SDS; 0.5% Triton; 0.23% DOC; 5 mmol/L EDTA) for 30 minutes on ice. The lysate concentrations were adjusted with 0.5 mol/L NaCl. The lysates were immunoprecipitated with rabbit antihuman factor XIII A antisera, rabbit antihuman XIII B antiserum, or anti-ovalbumin IgG; suspended and boiled in reducing buffer; and analyzed on 10% SDS-PAGE. Gels were dried and developed for one to two weeks in cassettes with Kodak-X-OMat film.

RESULTS

Extraction of the factor XIII A subunit from U937 cells. NP-40 extracts from U937 cells were analyzed for factor XIII subunits after SDS-PAGE and electrotransfer.
XIII A antigen was localized along the cell surface membrane. Intracytoplasmic staining was not detected. Staining and cytofluorographic analysis of paraformaldehyde-fixed cells confirmed the presence of factor XIII A antigen on the cell surface (Fig 3). To exclude the possibility that surface staining might be due to nonspecific binding by immunoglobulin to inadequately blocked surface F<sub>r</sub> receptors, cells were stained with other rabbit antisera, including antifactor XIII B, fibrinogen, and ovalbumin. These control antisera failed to stain the cells as judged by direct visual or cytofluorometric examination. Antifactor XIII A antibodies did not bind to other blood cells, including red cells, lymphocytes, T cell blasts, and polymorphonuclear granulocytes as judged by flow cytometry (not shown). When U937 cells were incubated in the presence of serial dilutions of purified factor XIII A (20 μg/mL) and antiserum to XIII A, a 40% maximal decrease in the intensity of baseline surface staining for this antigen was observed. However, complete inhibition of staining was not obtained in these experiments. An identical pattern of intracellular and cell surface staining of factor XIII A antigen was observed using mouse antihuman IgM monoclonal antibodies (MoAbs) that were raised against the purified factor XIII A subunit (unpublished observations, G. Lynch and J. McDonagh).

To confirm further the identity of factor XIII A on the U937 cell surface, iodination of factor XIII A and immunoprecipitation by rabbit antibody were performed. This procedure yielded a faint radiolabeled band in the autoradiogram at ~70,000 to 80,000, consistent with the observed relative mobility of factor XIII A subunit under these electrophoretic conditions (Fig 4). This band is not more distinct because factor XIII A antibodies are conformationally dependent; and detergent, which is used for cellular extraction, can interfere with the antigen-antibody reaction. Control rabbit anti-ovalbumin did not precipitate specific radiolabeled bands. Attempts at immunoprecipitating surface factor XIII with MoAbs were unsuccessful, probably because of the IgM nature of these antibodies and conformational dependency of the antigen. Specific cell surface staining of factor XIII A has also been observed in mouse WEHI-3 and J774.2 macrophage cell lines (unpublished results, G. Lynch and J. Oliver).

Synthesis of factor XIII by U937 cells. Biosynthetic labeling of U937 cells and immunoprecipitation were performed in serum-free medium. This produced a distinct band (Mr = 71,000) that is consistent with the observed electrophoretic mobility of the factor XIII A subunit (Fig 5). This effectively rules out the possibility that factor XIII A expression by the cells was due exclusively to factor XIII A that had been absorbed to or internalized by macrophages from trace levels in serum. Lanes examined using the immunoprecipitated products of control antisera, ovalbumin, and anti-XIII B (not shown) displayed no specific banding.

Modulation of cell surface factor XIII A on U937 cells. Cells were examined to test if expression of factor XIII A surface-related antigen could be modulated. The intensity of U937 cell surface staining for factor XIII A antigen increased after 48 to 72 hours of incubation in media containing PMA, LPS, or IFN-γ but was unaffected by the...
presence of PHA or α-thrombin (Table 1). Expression of factor XIII B subunit was not detected under these conditions.

**Factor XIII activity in macrophages.** Thrombin-dependent factor XIII activity was detected in extracts of U937 cells and alveolar macrophages. Lysates of 0.5 × 10^6 U937 cells contained 1.4 ± 0.1 μg/mL of thrombin-activatable transglutaminase activity. This represented ~89% of the total transglutaminase activity of the cells. Thrombin-independent activity in turn accounted for ~10 ± 4% of the total transglutaminase activity in U937 cells. However, if U937 cells were lysed in the presence of the serine esterase inhibitor, tosylarginylmethyl ester (TAME), thrombin-independent transglutaminase activity was reduced to >2% of the total activity.

U937 cells cultured in the presence of IFN-γ (10 to 100
Fig 3. Flow cytometric analysis of U937 cells stained with antibodies to factor XIII A, factor XIII B, and fibrinogen. U937 cells (0.5 x 10^6) were washed free of medium and incubated with aggregated IgG (100 µg/mL) before staining with antisera specific for: (A) antifactor XIII A subunit; (B) antifactor XIII B subunit; or (C) antifibrinogen for 30 minutes at 4°C. Cells were washed and stained with F(ab')2 goat antirabbit-IgG-FITC. The cells were analyzed on an Ortho Spectrum III cytofluorometer at standardized fluorescent gains. Staining histograms from all three conditions are represented. Vertical axis - cell number; horizontal axis - fluorescence intensity. Note the positive fluorescence for the factor XIII A subunit (filled stars). Curves representing the staining results for factor XIII B subunit and fibrinogen are superimposed (open stars) and failed to stain above the level for the second antibody control (F(ab')2 goat antirabbit-IgG-FITC) alone.

Fig 4. 125I surface labeling of U937 and immunoprecipitation of factor XIII A. U937 cells (1 x 10^6) were labeled with Na^{125I} by a solid phase lactoperoxidase technique. The labeled membranes were extracted with detergent, immunoprecipitated with antifactor XIII A and anti-ovalbumin, and analyzed by 10% SDS-PAGE. Lane 1 anti-human ovalbumin; lane 2, antifactor XIII A.

Fig 5. 35S-methionine biosynthetic labeling of U937 cells. U937 cells (6 x 10^6) were labeled in vitro by 35S-methionine in methionine-free, serum-free media. Supernatants and extracts were preclarified with Staphylococcus A and immunoprecipitated. The samples were boiled in reducing sample buffer and analyzed by 10% SDS-PAGE. Autoradiographs of the dried gels were produced on Kodak Ultrofilm. Lane 1: mol wt markers; lane 2: antifactor XIII A; lane 3: anti-ovalbumin. Antifactor XIII B was also negative.

U/Ml) for 18 to 48 hours showed no measurable increase in cell-related transglutaminase activity, nor was enzymatic activity detected in the external conditioned medium (Table 2). Factor XIII activity in U937 did not increase after similar times in culture in the presence of concanavalin A (Con A), PHA-induced human T cell lymphokine, LPS, and α-thrombin. It appears, therefore, that factors that can increase the surface expression of factor XIII A antigen (Table 1) may not increase the total factor XIII enzymatic activity.

Table 1. Modulation of Surface Expression of Factor XIII A Antigen

<table>
<thead>
<tr>
<th>Activator/Antigen</th>
<th>T3</th>
<th>Median Fluorescence Intensity</th>
<th>C3bR</th>
<th>XIII A</th>
<th>XIII B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>23</td>
<td>54</td>
<td>58</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>LPS (5 µg/mL)</td>
<td>28</td>
<td>61</td>
<td>96</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>PMA (20 ng/mL)</td>
<td>23</td>
<td>92</td>
<td>96</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>IFN-γ (10 U/mL)</td>
<td>24</td>
<td>90</td>
<td>90</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>PHA (5 µg/mL)</td>
<td>24</td>
<td>78</td>
<td>58</td>
<td>45</td>
<td></td>
</tr>
</tbody>
</table>

U937 cells were maintained in stationary culture at 1 to 2 x 10^6 cells/mL for 24 to 72 hours in the presence of various activators. Cells were washed, Fc receptors were blocked with aggregated IgG (100 µg/mL), and stained in suspension by an indirect fluorescence technique. T3 and C3bi receptor antigens were stained with F(ab')2 goat antihuman IgG-FITC. Factor XIII A and factor XIII B subunits were stained with F(ab')2 goat antirabbit-IgG-FITC. Cell staining, after 72 hours of incubation, was analyzed by flow cytometry. Data represent the median fluorescence intensity of the gated cell population for each condition. Note the increase in expression of factor XIII A antigen after stimulation with LPS, PMA, γ-IFN but not PHA. The C3bi receptor antigen increases with exposure to PMA, γ-IFN, and PHA but is minimally affected by LPS.
activity of the cell. Alternatively, increased synthesis of factor XIII A may be offset by its increased intracellular degradation.

**Macrophage-induced crosslinking of fibrin.** U937 and alveolar macrophage cellular extracts or nonextracted U937 cells and alveolar macrophages incubated in the presence of factor XIII-deficient plasma, PCMB-treated 125I-fibrinogen, thrombin, and calcium were capable of producing γ-γ cross-links and α-polymers in fibrin, but lanes analyzed in the absence of cells or their extracts failed to display crosslinking (Fig 6). Lanes containing cell extracts displayed degradation of the α-chain of fibrin. This phenomenon of α-chain degradation and γ-γ crosslinking has also been observed in systems in which platelets were the only source of factor XIII.24

Intact U937 cells and alveolar macrophages were pre-treated with human α-thrombin (0.1 to 10 U/mL), washed, and exposed to PCMB-treated 125I-fibrinogen in the presence of calcium, without additional thrombin. Supernatants analyzed by SDS-PAGE revealed the presence of labeled γ-γ dimers. Analysis of the gels by laser densitometry showed a progressive increase (0.5% to 14% of total protein per lane) in γ-γ dimer formation with the concentration of α-thrombin used to treat the intact U937 cells. Similar results were obtained for human alveolar macrophages (data not shown).

**DISCUSSION**

These studies show that macrophages synthesize factor XIII A subunit de novo and are a source of factor XIII activity. With the U937 cell as a model, biosynthesis of factor XIII A occurs in serum-free, platelet-free conditions. Furthermore, macrophage stimulation may be accompanied by translocation of factor XIII A from within the cytoplasm to the cell surface. These findings serve to expand the potential repertoire of macrophages in interactions with the clotting system and in the remodeling of the extracellular matrix that occurs following tissue injury.

Some previous studies have suggested that tissue transglutaminase is the dominant, if not sole source, of transglutaminase activity in macrophages.25-27 While other studies find factor XIII in macrophages.6,7 One reason for this discrepancy may be that factor XIII zymogen can only be isolated from cells in the presence of EDTA and without reducing conditions. Factor XIII is distinct from tissue transglutaminase by virtue of its immunohistochemistry, dependence on thrombin for activation, and its mode of crosslinking fibrin and other substances.12,28,29 In this study thrombin-dependent, factor XIII-like activity, assayed in the presence of TAME, accounted for ~98% of the transglutaminase activity within the U937 cell line. This decrease in non-thrombin-related activity in the presence of TAME (from 10% to >2%) may result from the presence of cathepsin-like acid proteases in macrophages, which can activate factor XIII in platelets and are inhibitable by TAME (unpublished observation, G. Lynch). U937 cells are free of surface contamination by platelets. This fact serves effectively to exclude platelets as the source of factor XIII in these experiments and further supports previous observations that factor XIII is present in macrophages.6,7

Factor XIII A antigen was detected on the cell surface membrane of macrophages. Most cysteine metalloenzymes are located within the cytoplasm of cells, making the presence of factor XIII on the surface of macrophages apparently unique. Only translocation of factor XIII A appeared to be affected by macrophage stimulation; factor XIII A secretion was not affected. The expression of cell surface factor XIII A was augmented under conditions that favor expression of other macrophage-associated clotting proteins. LPS is the prime stimulus to the production of procoagulant activity by
macrophages. PMA and IFN-γ display a broad array of macrophage activating properties, including promotion of cellular attachment and spreading on most surfaces, superoxide release, cytotoxicity for tumor targets, and increased expression of macrophage fibronectin receptors. Both PMA and IFN-γ promote an increase in the intracytoplasmic concentration of protein kinase-C. PMA has also been shown to produce translocation of intracytoplasmic protein kinase-C into the cell surface membrane.

Crosslinking of fibrin is a principal role of plasma factor XIII in hemostasis. This is an orderly process that proceeds stepwise via thrombin and calcium-dependent activation of the active site on the factor XIII A subunit and results in the formation of γ and α-crosslinkages with fibrin. This reaction, which occurs readily for fibrin, also takes place at slower rates, when fibrinogen is used as substrate, with α-chain polymerization being faster than γ-chain dimerization. With fibrin as a substrate, γ-chain dimers form more rapidly than α-polymers. Crosslinking of fibrin renders the clot highly insoluble and alters the elastic modulus of the clot. Also, by covalently incorporating α₂-plasmin inhibitor into the clot, factor XIII renders fibrin resistant to plasmin-mediated proteolysis.

In addition to its role in crosslinking fibrin, factor XIII can crosslink several other matrix proteins that are present in vivo in the inflammatory reaction. After fibrin, fibronectin constitutes the next most abundant protein in the plasma clot. Crosslinking of fibrin to fibronectin occurs between the amino-terminus of fibronectin and the α-chain of fibrin. Fibronectin can be crosslinked to most types of collagen except type IV. Factor XIII also crosslinks fibrin to von Willebrand factor (vWF) and vWF to collagen. Thrombospondin is a substrate for factor XIII crosslinking. Gelatin has recently been shown to be a substrate for factor XIII, and it is also incorporated by crosslinking into plasma clots.

The potential physiologic consequences of these reactions for macrophages are profound. Macrophages bind fibrin and fibronectin to their surface and secrete thrombospondin. They display increased adherence and spreading on surfaces coated with fibrin, fibronectin, and collagens. Macrophages increase their expression of F₄ and C3b receptors, secrete proteases, interleukin-1, and growth factors following adherence to matrix proteins that are substrates for factor XIII.

The expression of factor XIII represents a potential mode by which macrophages may interact directly with the extracellular matrix. Macrophages and exudative monocytes are likely to provide a local source of factor XIII in inflamed tissues. By stabilizing the extravascular clot to plasmin digestion, factor XIII may favor the persistence of the fibrin network at sites of injury. This clot matrix may provide an attachment site for other macrophages, fibroblasts, and endothelium during inflammation. Clark and Colvin and Covin have shown that fibrin and fibronectin provide a provisional matrix for cell attachment and migration following wounding and prior to the appearance of laminin and Type IV collagen. The capacity of factor XIII to crosslink fibronectin and vWF to fibrin and to collagens suggests that macrophages may play an important role in mediating the order of the remodeling process. A role for macrophages in wound healing is supported by the observation that healing is delayed in their absence. In addition to suffering from hemorrhagic diatheses and spontaneous abortions, ~14% of factor XIII-deficient individuals display defective wound healing. However, it is not yet known whether inflammation or the immune response in these patients is altered or whether they are specifically deficient in macrophage-related factor XIII. Clearly, further studies are indicated to define the role of macrophage-associated factor XIII in vivo.

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REFERENCES

14. Curtis CG, Brown KL, Credo RB, Dominik RA, Gray A,
Stenberg P, Lorand L: Calcium-dependent unmasking of active center cysteine during activation of fibrin stabilizing factor. Biochemistry 13:3774, 1974
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