Binding of Factor VIIa to Tissue Factor on Keratinocytes
Induces Gene Expression

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The abbreviations used are: TF, tissue factor; AR, amphiregulin; BTEB2, basic transcription element binding protein 2; CTGF, connective tissue growth factor; ETR101, eukaryotic transcription factor 101; FGF-5, fibroblast growth factor-5; DEGRck, 1,5-dansyl-Glu-Gly-Arg chloromethyl ketone; FVIIa, Factor VIIa; FVIIai, DEGRck-inhibited FVIIa; Fxa, Factor Xa; Fxai, DEGRck-inhibited Fxa; hbEGF, heparin-binding epidermal growth factor-like growth factor; IL, interleukin; LIF, Leukemia inhibitory factor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MIP2α, macrophage inflammatory protein 2α; MAPK, mitogen-activated protein kinase; PAI, plasminogen activator inhibitor; PGE2R, prostaglandin E2 receptor; TTP, tristetraproline; uPAR, uPA receptor.

This work was supported by grants from the Research Council of Norway, the Norwegian Cancer Society, the Norwegian Council on Cardiovascular Diseases, and the Owren and Jahre foundations (to H. P.). 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 1754 solely to indicate this fact.

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Binding of the zymogen serine protease Factor VII (FVII) to its cellular cofactor tissue factor (TF) triggers blood coagulation. Several recent reports have suggested that the formation of this complex may serve additional functions. We have used cDNA arrays to study differential gene expression in response to the interaction of activated FVII (FVIIa) with TF on a human keratinocyte cell line. Of 931 mRNA species observed up to 6 h after FVIIa (10 nM) addition, 24 were significantly up-regulated in what may resemble a wound-type response. Responders included mRNA species coding for transcription regulators (c-fos, egr-1, ETR101, BTEB2, c-myc, fra-1, and tristetraprolines), growth factors (amphiregulin, hbEGF, CTGF, and FGF-5), proinflammatory cytokines (IL-1β, IL-8, LIF, and MIP2α), proteins involved in cellular reorganization/migration (RhoE, uPAR, and collagenases 1 and 3), and others (PAI-2, cyclopulin, GADD45, Jagged1, and prostaglandin E₂ receptor). The transcriptional response to FVIIa was abrogated by antibodies to TF and left unaffected by hirudin. The pattern of genes induced suggests that the FVIIa-TF complex may play an active role in early wound repair as well as hemostasis. The former is a novel function ascribed to the complex that may also be contributing to the pathophysiology of unwarranted TF expression.

In this study we have addressed the physiological role of FVIIa-TF signaling by looking for transcriptional consequences of FVIIa-induced activation of HaCaT cells (7), a spontaneously transformed keratinocyte line much used as a keratinocyte model. These cells respond to regulatory signals in a similar manner to normal keratinocytes (8), express a range of epidermal differentiation markers (8), and are able to support epidermal differentiation and basement membrane formation in surface transplants (9). The epidermis is a rich source of TF (1), and in a wound situation, a site where FVIIa-TF signaling might be functioning. We have recently shown (6) that FVIIa binding to TF on HaCaT cells induces dose-dependent calcium responses, phosphorylation of MAPKs, and mitogen-activated protein kinase/extracellular signal-regulated kinase kinase 1/2-dependent up-regulation of the immediate early gene egr-1. Here we report the use of cDNA expression arrays to look for further differential gene expression resulting from FVIIa binding to TF on HaCaT cells. Importantly, the concentration of FVIIa used (10 nM) corresponds to the plasma concentration of zymogen FVII (10). We have recently shown that the FVIIa-TF complex induces signaling via three different MAPKs (6), and we show here that at least two of these (extracellular signal-regulated kinase 1/2 and p38) lead to differential induction of a series of immediate early genes. Several of these genes are involved in the earliest steps of the wound reaction and may also mediate other effects ascribed to TF on tumor development and metastasis.

EXPERIMENTAL PROCEDURES

Materials—Hirudin was obtained from Sigma; PD98059 and SB203580 were from Calbiochem; keratinocyte serum-free medium with additives, Dulbecco’s modified Eagle’s medium, and trypsin-EDTA were from Life Technologies, Inc.; recombinant human FVIIa- and DEGRck-inactivated human FVIIa (FVIIai) were from Novo-Nordisk (Bagsvaerd, Denmark); monoclonal mouse anti-human TF antibodies were purchased from American Diagnostica (Greenwich, CT) or were kindly donated by Dr. S. Carson (hTF; Omaha, NE); oligo(dT)-conjugated magnetic beads were from Dynal (Oslo, Norway); and the Atlas™ cDNA expression array kit and ExpressHyb™ hybridization solution were purchased from Clontech (Burlingame, CA).

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were from CLONTECH (Palo Alto, CA). A complete list of the cDNAs on the Atlas™ membranes is given at the CLONTECH web site.

**Cell Culture**—The constitutively TF-expressing keratinocyte line HaCaT was kindly provided by Dr. U. Birg Jensen (University of Aarhus, Aarhus, Denmark). HaCaT cells were cultured in keratinocyte serum-free medium supplemented with recombinant epidermal growth factor (0.5 ng/ml) and bovine pituitary extract (25 μg/ml). Medium was changed to Dulbecco’s modified Eagle’s medium without additives for 2 h prior to addition of agonists to the cells. Pretreatment with inhibitor or vehicle was done in this period.

**Isolation of RNA and cDNA Array Hybridization**—Cells pretreated with various agonists (0.5 ng/ml) or vehicle 2 h prior to stimulation with FVIIa (10 nM) were harvested in ice-cold phosphate-buffered saline at 30, 90, 180, or 360 min, pelleted, and immediately frozen at −70 °C. RNA isolation and generation of 32P-labeled cDNA probes were as recommended by CLONTECH. In brief, total RNA was isolated from the frozen cell pellets and DNase treated. mRNA was isolated from total RNA using oligo(dT)-conjugated magnetic beads. Radiolabeled cDNA probes were generated by reverse transcription of 0.5–1 μg of mRNA (the same amount for stimulated and control in each case) using Moloney murine leukemia virus polymerase in the presence of [α-32P]dATP. Hybridization of the cDNA probes to Atlas™ membranes (human or human cancer) was done overnight at 68 °C in ExpressHyb™. For the cancer array, this was done twice at each time point using mRNA isolated from independent experiments on two different filter sets. The results were analyzed using a PhosphorImage™ (Molecular Dynamics). Differential expression was determined as the percent deviation from control after normalizing all spots according to the average relative intensities of the nine housekeeping genes on stimulated and control membranes. cDNA spots that showed substantial deviation from control membranes were selected. The threshold was set around 60–100% for up-regulation and 40–80% for down-regulation (much less frequent), but it would also depend on visual impression of factors such as intensity, background, or overshadowing signals from neighboring spots.

**Northern Blot Analysis**—Isolation of mRNA, Northern blotting, labeling of probes, and membrane hybridization were as described previously (6). For reprobing, the membranes were stripped in 0.5% SDS at 100 °C for 5–10 min. Most of the following probes were made from IMAGE consortium clones (IMAGE identity in parentheses): amphi-regulin (AR; 1174235), connective tissue growth factor (CTGF; 625522), fibroblast growth factor-5 (FGF-5; 796724), GADD45 (591683), macrophage inflammatory protein 2α (MIP2α; 841361), interleukin-1β (IL-1β; 324655), interleukin-8 (IL-8; 328846), Jagged1 (141815), and RhoE (1270730), tristetraproline (TTP; 380713), basic transcription element box 1 (BTEB1; 1161000), c-fos (ATCC number 6581), collagenase 3 (ATCC number 841361), interleukin-1β (IL-1β; 324655), interleukin-8 (IL-8; 328846), Jagged1 (141815), and RhoE (1270730), tristetraproline (TTP; 380713), basic transcription element box (BTEB2; 587776), cyclophilin (1961397), and TTP (380713).

**Results**

**Identification of Differentially Expressed Gene Products**—Atlas™ cDNA expression membranes were used to identify genes induced in response to binding of FVIIa to TP on HaCaT cells. Each of the membrane sets used (human and cancer) represented 588 cDNA species in addition to controls. Correcting for overlap between the two membrane sets left 931 different cDNA species to be included in this study. Messenger RNA was isolated from HaCaT cells stimulated with FVIIa (10 nM) or from control cells pretreated with FVIIa prior to addition of FVIIa. We have previously shown that such pretreatment inhibits FVIIa signaling in HaCaT, presumably by blocking the binding of FVIIa to TP (6). Such mRNA obtained 30, 60, 90, 180, and 360 min after FVIIa addition) was used to generate 32P-labeled cDNA probes that were hybridized to the Atlas™ membranes. Positive genes were selected as described under “Experimental Procedures.”

Fig. 1 shows an example of a quadrant from duplicate membranes hybridized with cDNA probes generated from mRNA isolated 90 min after FVIIa addition. All of the cDNA spots indicated with arrows were selected for further examination. Five of these were confirmed to be differentially regulated (see below). In total, we selected 61 potentially regulated genes for further investigation. One of the strongest responder genes identified was egr-1, an immediate early gene whose induction by FVIIa in HaCaT we have recently described in detail (6).

**Confirmation of Differential Expression**—Genes identified by the cDNA array screen were further examined for differential expression by Northern analysis. Messenger RNA was isolated from HaCaT up to 6 h after stimulation with FVIIa (10 nM) or vehicle. We could confirm up-regulation of mRNA (Fig. 2) coding for several transcription regulators (c-fos, ETR101, BTEB2, fra-1, e-cmyc, egr-1 and TTP), growth factors (CTGF, AR, hbEGF, and FGF-5), proinflammatory cytokines (IL-8, IL-1β, LIF, and MIP2α), and others (RhoE, uPAR, collagenases 1 and 3, PAI-2, Jagged1, cyclophilin, GADD45, and PGE2R), several of which are associated with cellular reorganization or migration. All these genes were significantly up-regulated in three or more independent experiments (Table I). 37 of the genes selected for further study from the cDNA arrays, including all those selected for down-regulation, were not confirmed to be differentially regulated following FVIIa stimulation. Most appeared unchanged after stimulation, and some were not detected because of low expression levels.

**Further Analysis of Selected Gene Products**—Six gene products (IL-8, PGE2R, CTGF, LIF, e-cmyc, and Jagged1) were further investigated 90 min after FVIIa stimulation (Fig. 3). Inhibition of all six was substantially inhibited by pretreatment with FVIIai or either of two different anti-TP antibodies (htF1 or 4505). None of these gene products were induced upon addition of FVIIai (Fig. 3). Concentrations of hirudin shown to abolish up-regulation of egr-1 mRNA by thrombin in the same setting (6) were without effect (Fig. 3). This confirms specificity, TP dependence, and activity requirements and excludes downstream activation of thrombin as a source of the signals. The absence of serum in the medium used for cell culture, the
use of recombinant FVIIa with no detectable FX activity, and the left-shifted dose-response curve for induction of egr-1 by FVIIa relative to FXa in HaCaT cells render activation of contaminating FX an unlikely source for the signals (6). TF dependence was confirmed for all 24 responders (including egr-1) by inhibition of their up-regulation using anti-TF antibodies (results not shown), excluding a TF-independent pathway responsible for any of them. We have recently shown that FVIIa induces phosphorylation of all of the three main MAPKs; extracellular signal-regulated kinase 1 and 2, p38 MAPK, and c-Jun N-terminal kinase in HaCaT cells (6). In the same study, egr-1 induction by FVIIa was inhibited >90% by pretreating with the mitogen-activated protein kinase/extracellular signal-regulated kinase kinase 1/2 inhibitor, PD98059, whereas the p38 MAPK inhibitor, SB203580, showed no inhibition of this particular gene (6). It was therefore of interest to investigate several genes for induction by signaling through extracellular signal-regulated kinase 1/2 and p38 MAPK. Using the same two inhibitors at the same concentrations as in the previous study, the effects were observed to range from no effect of either inhibitor (PGE2R) through a partial (CTGF) or complete (egr-1) effect of PD98059 only to strong effect of both inhibitors (IL-8 and Jagged1 (Fig. 3) and collagenase 3 (not shown)). This shows that signaling through at least these two MAPK pathways may be important for different end points. Additional signaling cascades may also be triggered by FVIIa. A number of the genes were induced as early as 30 min after agonist addition (Table I). Up-regulation of mRNA was independent of prior protein synthesis as demonstrated by superinduction in the presence of cycloheximide. In all cases actinomycin D was inhibitory.

**DISCUSSION**

Cellular activation by FVIIa binding to TF was first described in the form of Ca$^{2+}$ signaling in endothelial cells and Madin-Darby canine kidney cells (3, 4), later supported by reports of various changes in cell signaling (5, 6), gene regulation (6, 11–13), and cell motility (14). The amount of FVIIa needed to trigger signaling has been high compared with circulating plasma levels of FVII. We have recently found the EC$_{50}$ for FVIIa-induced up-regulation of the immediate early gene egr-1 (3 nM) to be an order of magnitude lower than for triggering a Ca$^{2+}$ response in the same cells (6), well below the plasma concentration and very close to the $K_{d}$ for FVIIa binding to cell surface TF (15). Signaling requires a yet unidentified, transducing component and does not occur via TF itself. It is clearly independent of downstream formation of thrombin (6). Studies examining effects of FVIIa TF signaling on gene transcription reported induction of four different genes in three different cell types (fibroblast, colon cancer, and keratinocyte). Three of these genes (vascular endothelial growth factor (11), uPAR (12), and egr-1 (6)) were identified studying candidate genes, and a more general differential display approach led to the report of only one gene product (poly(A) polymerase) (13). These reports did not reveal any pattern that might suggest a physiological function of FVIIa TF signaling. We have taken an expanded candidate approach to identify gene targets for FVIIa-induced signaling in cells known to be responsive to the protease. All 931 genes were studied over a period of 6 h, and all strong responders were included in further analyses. Supporting the value of this screen was the fact that we picked up two of the four genes previously reported to be induced by FVIIa (uPAR and egr-1). The genes induced were all classifiable as immediate early response genes based on the effect of actinomycin D and cycloheximide. The pattern of gene induction observed shows great likeness to a wound-type response (16). This may provide a good starting point for looking at FVIIa TF signaling in vivo.

Not much is known about regulation of transcription in response to epithelial wounding. Egr-1 and c-Fos are two factors suggested to be active (16–18). We show up-regulation of c-fos, egr-1, and uPAR, all of which are known to enhance cell proliferation. c-fos is primarily associated with cell growth and proliferation (19), and c-Fos has been shown to synergize with egr-1 (20). Our data show that both genes are induced in the presence of cycloheximide, indicating that actinomycin D is effective in blocking protein synthesis. It is possible that c-fos and c-Fos might be involved in the regulation of gene expression during wound repair.
**TABLE I**

**FVIIa-induced up-regulation of mRNA species in HaCaT**

Changes in mRNA levels with time after addition of FVIIa (10 nM) to HaCaT. Cells were harvested for mRNA isolation at the given time after addition of FVIIa or vehicle. Intensity values from Northern blots hybridized with 32P-labeled probes were determined using a PhosphorImager™ and were normalized to GAPDH intensities of the same samples, prior to further calculation. Fold induction is the ratio between these normalized intensities of corresponding samples from cells exposed to FVIIa or vehicle only. *p < 0.05; **p < 0.01.

| time/gene product | 0.5 | 1 | 1.5 | 3 | 6 | n |
|-------------------|-----|---|-----|---|---|---|
| **egr-1**         | 4.8 ± 0.7* | 5.8 ± 0.8* | k | 1.4 ± 0.1 | 0.5 ± 0.1** | 0.5 ± 0.2 | 3 |
| c-fos            | 4.3 ± 0.9** | 6.1 ± 1.4* | 1.7 ± 0.6 | 2.0 ± 0.4 | 1.7 ± 0.4 | 4 |
| ETR101           | 2.7 ± 0.3** | 2.3 ± 0.1** | 1.2 ± 0.1 | 1.0 ± 0.0 | 1.3 ± 0.2 | 5 |
| BTEB2            | 1.1 ± 0.1 | 1.4 ± 0.1** | 2.3 ± 0.2** | 1.9 ± 0.2** | 1.2 ± 0.2 | 5 |
| c-myc            | 0.9 ± 0.1 | 2.1 ± 0.5** | 2.3 ± 0.5** | 2.0 ± 0.2** | 1.8 ± 0.2* | 6 |
| fra-1            | 1.2 ± 0.1 | 1.9 ± 0.2* | 2.5 ± 0.4* | 1.8 ± 0.3 | 2.0 ± 0.2** | 4 |
| TTP              | 2.4 ± 0.3** | 2.8 ± 0.3** | 1.9 ± 0.1** | 1.4 ± 0.0** | 1.3 ± 0.1 | 5 |

| time/gene product | 0.5 | 1 | 1.5 | 3 | 6 | n |
|-------------------|-----|---|-----|---|---|---|
| **IL-8**          | 2.3 ± 0.3* | 18.8 ± 2.2** | 14.3 ± 2.4* | 4.5 ± 0.9* | 2.6 ± 0.3** | 4 |
| LIF               | 3.0 ± 0.6* | 4.8 ± 1.6 | 9.6 ± 1.7** | 2.6 ± 0.4* | 1.1 ± 0.5 | 5 |
| MIP2α             | 3.1 ± 0.9 | 15.4 ± 4.8* | 5.0 ± 1.3* | 4.5 ± 1.3* | 3.7 ± 1.0* | 6 |
| IL-1β             | 1.1 ± 0.1 | 2.4 ± 0.1** | 2.4 ± 0.1** | 1.4 ± 0.1 | 1.7 ± 0.4 | 4 |

| time/gene product | 0.5 | 1 | 1.5 | 3 | 6 | n |
|-------------------|-----|---|-----|---|---|---|
| RhoE              | 1.3 ± 0.0* | 3.3 ± 0.3** | 2.6 ± 0.2** | 1.4 ± 0.1 | 1.5 ± 0.2 | 4 |
| uPAR              | 1.3 ± 0.1 | 1.5 ± 0.2 | 2.9 ± 0.5* | 2.1 ± 0.2* | 2.1 ± 0.3* | 4 |
| collagenase 1     | 0.8 ± 0.1 | 1.4 ± 0.2* | 1.7 ± 0.3* | 1.9 ± 0.1** | 1.7 ± 0.1** | 7 |
| collagenase 3     | 1.3 ± 0.0** | 4.8 ± 1.2 | 7.0 ± 1.5* | 7.0 ± 1.4* | 2.9 ± 0.8 | 4 |

| time/gene product | 0.5 | 1 | 1.5 | 3 | 6 | n |
|-------------------|-----|---|-----|---|---|---|
| Jagged1           | 1.5 ± 0.1* | 2.4 ± 0.4* | 2.7 ± 0.2** | 1.9 ± 0.2* | 1.4 ± 0.1 | 6 |
| PAI-2             | 1.1 ± 0.1 | 2.7 ± 0.4* | 6.3 ± 1.1* | 7.0 ± 0.5** | 3.1 ± 0.4 | 4 |
| cyclophilin       | 1.2 ± 0.0** | 1.9 ± 0.1** | 2.2 ± 0.2** | 1.7 ± 0.2** | 1.2 ± 0.1 | 4 |
| GAPDH             | 1.3 ± 0.0** | 2.0 ± 0.5* | 1.7 ± 0.1* | 0.9 ± 0.1 | 1.0 ± 0.1 | 4 |
| PGE_R             | 2.5 ± 0.1** | 4.1 ± 0.4* | 3.2 ± 0.4* | 1.0 ± 0.0 | 1.7 ± 0.1* | 3 |

**ETR101**, and TTP mRNA in response to FVIIa, with rapid and transient kinetics almost identical to those recently reported for the egr-1 gene product (6). Three other transcription regulator genes, c-myc, BTEB2, and fra-1, were induced somewhat later, peaking at 1.5 h after FVIIa addition. c-myc is induced in migrating renal epithelial cells after wounding (17), and the c-Myc protein has been implicated in growth factor-induced migrating renal epithelial cells after wounding (17), and the c-Myc later, peaking at 1.5 h after FVIIa addition. ***c-myc*** intensities of corresponding samples from cells exposed to FVIIa or vehicle only. **neuropoetic cytokines** that exert similar effects on a variety of wound repair (17, 30). LIF is a member of the IL-6 family of mitogen FGF-5 promote growth of connective tissue and angiogenesis (26–29). Like the EGF ligands, CTGF is induced during wound repair (17, 30). LIF is a member of the IL-6 family of neuroepoetic cytokines that exert similar effects on a variety of tissues through binding to receptor complexes that all contain the transducing subunit gp130 (31). IL-8 and MIP2α are two closely related CXC chemokines that both act on IL-8 receptors (32). Together with IL-1β these are major proinflammatory cytokines commonly induced during trauma, which may act on a wide variety of cells.

Several of the genes induced by FVIIa code for proteins related to cellular reorganization or migration. RhoE is a member of the Rho GTPase family, which is known to be involved in regulation of the actin cytoskeleton. RhoE induces cell spreading when injected into macrophages or Madin-Darby canine kidney cells, suggesting a role in cell migration (33). Jagged1 is a membrane-bound ligand for the developmentally important Notch transmembrane receptor, which acts in intercellular communication (34). The uPAR is expressed at the leading edge during keratinocyte re-epithelialization of mouse skin wounds (35). The uPAR-uPA complex activates plasminogen. The resulting plasmin may both activate various metalloproteases and lyse the fibrin clot. It is indispensable for wound healing (36). In addition to controlling plasmin-mediated proteolysis, the uPA-uPA complex has been suggested to act directly in cell adhesion to vitronectin (37). Also a member of the fibrinolytic system, and probably the main regulator of pericellular uPA activity in keratinocytes (38), is PAI-2, which was strongly induced by formation of the FVIIa/TF complex. PAI-2 can associate with fibrin to inhibit uPA-mediated fibrin clot lysis (39).

To induce PAI-2 at the same time as uPAR is probably essential to prevent lysis of the fibrin clot in the initial phase of wound healing, prior to closure of the epithelial layer. Collagenases 1 and 3 preferentially degrade collagen type III and II, respectively (40, 41). Collagenase 1 also supports migration of kera-
Factor VIIa–Tissue Factor–Induced Gene Expression

6584

PGE2R
GAPDH
CTGF
IL-8
GAPDH

A

B

FIG. 3. Specificity of the FVIIa-induced responses and effect of inhibitors. HaCaT cells were pretreated (for 2 h, as shown in panels A and B) with vehicle (-), FVIIai (100 nM), anti-TF antibodies hTF1 (25 µg/ml) or 4505 (25 µg/ml), hirudin (5 units/ml), PD98059 (50 µM), or SB203580 (25 µM) prior to treatment with vehicle (-, column 1, open bars), FVIIai (10 nM; columns 2, 3, and 5–9, closed bars), or FVIIai (100 nM; column 4, hatched bars). Cells were harvested for mRNA isolation 90 min after addition of FVIIai. A, example of Northern membranes hybridized with 32P-labeled probes to PGE2R, CTGF, and IL-8, and glyceraldehyde-3-phosphate dehydrogenase (CTGF and IL-8 were from the same blot). B, sum of hybridizations with PGE2R, CTGF, IL-8, LIF, c-myc, and Jagged1. Intensity values for the different mRNA species obtained using a PhosphorImager™ were normalized to intensity values obtained for glyceraldehyde-3-phosphate dehydrogenase mRNA.

Alder binding protein for cyclosporin A (45), and is possibly also involved in mRNA processing. GADD45 is one of five growth arrest and stress/damage-inducible genes (46, 47).

In an epidermal wound scenario (16), the genes we have identified that respond to formation of the FVIIa-TF complex on HaCaT cells all appear to fit different parts of a reparative response. Moreover, a number of the genes or their respective protein products have been demonstrated to be up-regulated in various models of epithelial and endothelial wounding (see above). The FVIIa-TF interaction would occur immediately upon wounding, and FVIIa-TF might thus provide a very early signal in this process.

A potentially important consequence of our findings is the possible contribution of a FVIIa-TF-induced transcriptional response to disease states associated with aberrant TF expression (48–50). In vivo studies of FVIIa-TF signaling and its contribution to such conditions are complicated by the fact that TF expression will usually be coupled to both thrombin generation and platelet activation, where release of growth factors and cytokines from the platelets and direct actions of thrombin are hard to distinguish from the direct actions of FVIIa-TF. Moreover, yet another function for TF has recently been described in cell adhesion (51). Perhaps the most compelling observation suggesting that FVIIa-TF signaling may be active in vivo comes from studies of the effects of specific inhibitors of different components of the coagulation cascade on the outcome of Escherichia coli-induced sepsis in baboons (48). Inhibition downstream of the FVIIa-TF complex would inhibit the coagulopathic response to E. coli without inhibiting lethality, whereas direct inhibition of FVIIa-TF would inhibit both, suggesting a direct role for FVIIa-TF in the lethal response (48).

This hypothesis was supported by the cytokine release patterns, which showed a late response in IL-6 and IL-8, apparently attributable to a direct effect of TF and/or FVIIa (48). It is thus intriguing to observe that FVIIa-TF signaling can lead to induction of LIF, IL-8, and IL-1β, which are three of the main cytokines induced during sepsis. We are currently looking into whether these are also induced by FVIIa in endotoxin-treated endothelial cells or monocytes. TF protein is accumulated in a wound. TTP is a zinc finger protein that binds AU-rich elements and is involved in RNA destabilization (44), and cyclophilin is a peptidyl-prolyl cis-trans-isomerase, an intracellular protein that binds AU-rich elements and is involved in ribosome-mRNA translation (45). As above, TF has been shown in vitro to induce vascular smooth muscle cell migration (53). TF is expressed in various malignant tumors (54) and may promote tumor angiogenesis and hematogeneous metastasis (50, 55–57). Induction of growth factors, several of which have been implicated in angiogenesis (FGF-5, CTGF, and VEGF) and associated with atherosclerosis (CTGF and VEGF), cytokines, metalloproteases, and other proteins (such as uPAR and c-myc) by FVIIa-TF signaling, may be important in some or all of these settings.

In conclusion, we describe in this study the induction of a number of genes secondary to FVIIa binding to TF on keratinocytes. At least two of the three MAPK pathways activated (6) by the formation of this complex are involved in the signaling. The pattern of genes induced may suggest a role for FVIIa-TF signaling in wound repair, and the ability of FVIIa to induce these genes adds a level of complexity to many of the physiological and pathophysiological settings where FVIIa will be allowed to interact with TF. The studies need to be expanded to other cells and in vivo models to understand the importance and extent of FVIIa-TF signaling.

Acknowledgments—We thank J.-A. Røttingen for kind assistance in preparing the draft manuscript.
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J. Biol. Chem. 2000, 275:6580-6585.
doi: 10.1074/jbc.275.9.6580

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