The coagulation protease Factor Xa (Xa) triggers a variety of cellular responses that may be important for inflammatory reactions to tissue injury. Protease-activated receptors (PAR1, PAR2, and PAR4) can mediate Xa signaling in heterologous expression systems. However, other candidate Xa receptors have been described, and the extent to which one or more PARs account for Xa signaling in relevant differentiated cells is unknown. We examined Xa signaling in endothelial cells from wild-type and PAR-deficient mice. Wild-type endothelial cells responded to agonists for PAR1, PAR2, and PAR4. Relative to wild-type, Xa-triggered phosphoinositide hydrolysis was reduced by 60–75% in PAR2−/− endothelial cells, by 20–30% in PAR1−/− endothelial cells, and by ~90% in PAR2−/− endothelial cells treated with a PAR1 antagonist. Similar results were obtained when ERK1/2 phosphorylation was used to assess Xa signaling. Thus PAR2 is the main endogenous Xa receptor in these endothelial cell preparations and, together, PAR2 and PAR1 appear to account for ~90% of endothelial Xa signaling. By contrast, in fibroblasts, PAR1 by itself accounted for virtually all Xa-induced phosphoinositide hydrolysis. This information is critical for the design and interpretation of knockout mouse studies to probe the possible roles of Xa signaling in vivo.

The coagulation protease factor Xa (Xa) is generated at sites of vascular injury and inflammation by the actions of the tissue factor/VIIa (TF/VIIa) and VIIIa-Xa complexes (1, 2). The formation of Xa is localized to the surfaces of cells and membrane vesicles, and in addition to binding Va to form the prothrombinase complex, Xa can directly regulate cellular behavior. For example, Xa can cause endothelial cells to release cytokines (3, 4), display adhesion molecules (4), proliferate (5), and trigger endothelial-dependent vasorelaxation (6). Importantly, studies in animal models of septic shock and the recent clinical trial of activated protein C strongly suggest that coagulation proteases contribute to organ damage and death in this syndrome (7–12). Such studies have also raised the possibility that coagulation proteases upstream of thrombin might contribute to the pathogenesis of septic shock via activities unrelated to thrombin generation (11). These considerations motivate efforts to identify the receptors that mediate Xa responses in endothelial cells and other cell types.

Protease-activated receptors (PARs) are G protein-coupled receptors that mediate signaling to thrombin and other proteases by a mechanism that requires proteolytic cleavage of the receptor (13). Because inhibitors that block the proteolytic activity of Xa also ablate its ability to trigger cellular responses, PARs are candidates for mediating Xa signaling (14, 15). Indeed, there is substantial evidence that Xa can signal via PARs. For example, expression of PAR1, PAR2, and PAR4 in Xenopus oocytes confers calcium signaling in response to Xa (16). TF-VIIa complex can also activate PAR2 expressed in oocytes and fibroblasts, and when tissue factor and PAR2 are co-expressed in fibroblasts in the presence of zymogen factor X, picomolar VIIa is sufficient to trigger robust signaling (16). The TF-VIIa-Xa ternary complex may be especially effective for activating PAR2 (17). Thus PARs can mediate Xa signaling in heterologous expression systems. Are PARs the major endogenous mediators of Xa signaling in untransfected cells and tissues? Several pharmacological studies are consistent with this notion. For example, in HeLa cells, a PAR1-blocking antibody inhibited Xa-induced ERK phosphorylation and gene induction (18), and desensitization of PAR2 abolished Xa-induced relaxation of rat aortic rings (19). To further define the relative contribution of PARs to Xa signaling and to lay groundwork for studies to define the importance of Xa signaling in vivo, we utilized cells from PAR-deficient mice. We focused on endothelial cells because they express the three PARs that are candidate Xa receptors in mouse, because they are probably exposed to Xa at sites of tissue injury, and because their responses to Xa, such as cytokine release and surface expression of adhesion molecules (4), may contribute to the link between tissue injury and inflammatory responses.

**EXPERIMENTAL PROCEDURES**

Materials—The agonist peptides TFLLRNPNNDK (PAR1), SLIGRL (PAR2), SFLLRN (PAR1 and PAR2), and AYPFQK (PAR4) as well as the competitive PAR1 antagonist BMS (BMS-200261, N-trans-cinnamoyl-p-fluoroFpGuFLLR) (20) were synthesized as carboxyl amides and purified by reverse-phase high performance liquid chromatography. Hirudin, hiraparin, and anti-FGL antibody were from Sigma; antibodies to ICAM-2, PECAM, and Flk-1 from BD PharMingen; horseradish peroxidase-conjugated goat anti-rabbit immunoglobulins from Bio-Rad; rabbit anti-ERK1/2 and phospho-ERK1/2 from Cell Signaling; magnetic beads from Dynal; collagenase and dispase from Roche Molecular Biochemicals; endothelial cell growth supplement from BTI; trypsin and cell culture medium from Invitrogen; human plasma-derived Xa and thrombin from Enzyme Research Laboratories and Hematologic Technologies. 1 Unit/ml Xa corresponds to 174 nM.

This paper is available on line at http://www.jbc.org
**Isolation of Endothelial Cells and Fibroblasts from Wild-type and PAR-deficient Mice**—Generation of mice deficient in PAR1 and PAR2 has been described elsewhere (21, 22). Mice used for cell preparations had been bred five or more generations into the C57BL/6J strain. Endothelial cells were isolated by a modification of published techniques (23). For each experiment, skin from 3-month-old mice was excised, minced, digested with collagenase B and dispase II at 37 °C for 6 days after birth, respectively. Tissues were minced, digested with collagenase B and dispase II at 37 °C for 1 h with shaking, disrupted by passage through a 14-gauge cannula, and filtered through a stainless steel wire mesh. Cells were collected by centrifugation at 100 × g for 5 min, plated onto gelatin-coated culture dishes, and cultured in endothelial cell growth medium (50% Dulbecco’s modified Eagle’s medium (DMEM), 50% Ham’s F-12 with non-essential amino acids, 20% serum, 100 units/ml penicillin, and 100 μg/ml streptomycin, 0.1 mg/ml heparin, and 50 μg/ml endothelial cell growth supplement). On day 2, cell cultures were incubated with magnetic beads coated with rat anti-mouse ICAM-2. Cells were then released from plates by trypsinization and washed, and ICAM-2-expressing cells (endothelial cells) were isolated using a magnet, washed three times in DME, 0.1% bovine serum albumin and plated into gelatin-coated culture dishes. A second round of purification was performed 3–4 days later. Experiments were done 8–12 days after the initial tissue digest; each experiment used cells from a separate cell isolation. Purity of the endothelial cell preparations was characterized by immunostaining for PECAM, ICAM-2, and Flk-1, and by parallel isolation and 5-bromo-4-chloro-3-indolyl-β-D-galactosidase transfection and screening hygromycin B-resistant clones for cell surface PAR expression by cell surface enzyme-linked immunosorbent assay (26).

**Phosphoinositide Hydrolysis**—Cells in 24-well plates were loaded overnight with 2 μCi/ml myo-[3H]inositol in DMEM/bovine serum albumin (0.2%) without fetal bovine serum. Cells were washed in phosphate-buffered saline, and fresh DMEM/bovine serum albumin without myo-inositol for 2 h, treated with 20 mM LiCl in DMEM/bovine serum albumin with or without agonist for 90 min, then extracted with formic acid. Released total [3H]inositol phosphates were quantitated (26). Basal [3H]inositol phosphate release was ~350 cpm in dermal and lung endothelial cell cultures and ~500 cpm in lung fibroblast cultures, regardless of PAR genotype. Experiments comparing Xa responses in wild-type versus Par −/− cells were repeated 3–6 times; other comparisons were in most cases repeated three times. Within each experiment, there were four replicates for each condition. The data shown in Fig. 3 are the average of the pooled experiments.

**MAP Kinase (ERK) Phosphorylation**—Cells in 12-well plates were incubated overnight in serum-free medium, washed in phosphate-buffered saline, and changed to fresh serum-free medium 2 h prior to agonist addition. The time course of ERK phosphorylation after addition of Xa to wild-type skin endothelial cell cultures was characterized by an initial spike of ERK phosphorylation that peaked at ~5 min and declined to a plateau that lasted more than 60 min. Two sets of experiments were done: one in which cells were incubated with agonists for 5 min to sample the peak, and one in which cells were incubated with agonists for 30 min to sample the plateau. After incubation with agonists, cells were lysed directly in a reducing SDS sample buffer. Lysates were sheared with an insulin syringe then resolved by SDS-PAGE and transferred to polyvinylidene difluoride membranes. Immunoblotting with anti-phospho-ERK1/2 or anti-ERK1/2 antibodies followed by an horseradish peroxidase-conjugated goat anti-rabbit secondary antibody was done at 4 °C. After repeated washes with Triton-buffered saline with 0.1% Tween, the membranes were developed with ECL plus (Amerham Bioscience), and visualized using either film or a PhosphorImager (Molecular Dynamics).

**β-Galactosidase Staining for PAR2 Expression**—PAR2-lacZ knockin mice were generated using a strategy analogous to that used for the PAR2-lacZ knockin mouse. In this mouse, the β-galactosidase gene was inserted into PAR2 exon 1 such that the lacZ start codon supplanted that of PAR2. Tissues from mice homozygous or heterozygous for the PAR2-lacZ allele were stained for β-galactosidase as described (24). Tissue sampling was preceded by saline perfusion when tissues were to be used for sectioning.

### RESULTS

**Xa Triggers Cellular Responses via PAR1, PAR2, and PAR4 in Transfected Fibroblasts**—In previous studies, we showed that overexpression of human PAR1, PAR2, or PAR4 in Xenopus oocytes conferred Xa-dependent calcium mobilization (16), which reflects phosphoinositide hydrolysis in those cells. PAR3 did not confer Xa responsiveness (16). Because PAR1 and PAR4 can be activated by thrombin while PAR2 cannot and because it seemed likely that thrombin would be generated in most situations in which Xa was present, we focused on PAR2 signaling in response to Xa in that study. Xa did trigger phosphoinositide hydrolysis in KOLF cells (KOLFs) stably expressing PAR2 (16).

For the present study, we were interested in Xa activation of all candidate PARs. Accordingly, we compared Xa signaling in KOLFs stably expressing similar levels of PAR1, PAR2, or PAR4. Xa triggered phosphoinositide hydrolysis (Fig. 1a) and ERK1/2 phosphorylation (Fig. 1b) in all three PAR-transfected cell lines (in PAR4 expressing cells, ERK phosphorylation in response to Xa was seen at 40 min only). Untransfected KOLFs showed no responses to Xa. In each of three experiments with KOLFs expressing PAR1 or PAR2, the threshold concentration of Xa for detecting Xa-induced increases in phosphoinositide hydrolysis was ~7 nM; for PAR4-expressing KOLFs, the threshold was ~35 nM (Fig. 1a and additional data not shown). The concentration response curves to Xa did not clearly saturate in the transfected KOLFs, even at 870 nM Xa. In PAR1-transfected cells, the response to 870 nM Xa was only ~25% of that triggered by a saturating concentration of PAR1-activating peptide. In PAR2- and PAR4-transfected cells, the response at 870 nM Xa was ~70% of the maximal peptide response. Xa was used at 1 unit/ml (174 nM) in most subsequent studies. This corresponds to the concentration of Xa that would be achieved if all zymogen X in plasma were converted to active Xa. We chose this highest practical concentration of Xa because our studies in PAR-deficient cells would look for loss of Xa signaling. The increase in phosphoinositide hydrolysis in response to 1 unit/ml Xa in PAR2-expressing cells was consistently ~50% of the maximal response to PAR2-activating peptide, while the response to Xa in cells expressing PAR1 or PAR4 was 20–30% of the maximal response to PAR1- or PAR4-activating peptides (Fig. 1a and data not shown). Thus Xa can signal via PAR1, PAR2, or PAR4 heterologously expressed in KOLFs, and PAR2 appears to be a relatively better receptor for Xa than PAR1 or PAR4 in the context of this expression system. To determine which of these PARs, if any, are the major endogenous Xa receptors in a biologically interesting differentiated cell type, we next examined Xa signaling in endothelial cells from mice deficient in individual PARs.

**Characterization of Cells Cultured from Wild-type and Knockout Mice**—Endothelial cells were isolated from lung or skin from wild-type and PAR-deficient neonatal mice using ICAM2 antibodies bound to magnetic beads. Over 90% of the cells in each preparation expressed endothelium-specific mark-

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C. T. Griffin and S. R. Coughlin, manuscript in preparation.
ers at the time of the experiments (see “Experimental Procedures”).

Response Patterns in Wild-type Endothelial Cells—We first characterized agonist responses in wild-type endothelial cells using phosphoinositide hydrolysis and ERK phosphorylation as end points. Endothelial cells from lung and skin responded to both Xa and thrombin (Figs. 2 and 3). The thrombin inhibitor hirudin blocked responses to thrombin but not Xa, thus the Xa responses seen in these studies were unlikely to be due to contaminating thrombin or to activation of any prothrombin remaining in the cultures (Figs. 2 and 3 and not shown). The threshold Xa concentration for detectable increases in phosphoinositide hydrolysis in wild-type dermal and lung endothelial cell preparations was $7 \, \text{nm}$ (Fig. 2a and not shown). The $\text{ED}_{50}$ was $25 \, \mu\text{M}$ and the maximal response to Xa was similar to that seen in response to $100 \, \mu\text{M}$ PAR2-activating peptide and greater that than seen with $10 \, \text{nm}$ thrombin (Fig. 2a). For ERK phosphorylation in endothelial cell preparations from wild-type mice, the threshold Xa concentration for detectable increases was $1.5-7 \, \text{nm} (0.008-0.04 \, \text{units/ml})$ and the $\text{EC}_{50}$ was $30 \, \text{nm}$ (Fig. 2b). The time courses for ERK phosphorylation in endothelial cells after addition of Xa, SLIGRL, and thrombin were similar, with a peak at $5 \, \text{min}$ followed by a plateau lasting $40-60 \, \text{min}$ (Fig. 2c).

In endothelial cells from both skin and lung of wild-type mice, the average fold increase in phosphoinositide hydrolysis triggered by a saturating concentration of PAR2 agonist (SLIGRL) was greater than that for PAR1 (TFLLRNPDNK), which was in turn greater than that for PAR4 (AYPGKF) Fig. 3, a and b, and not shown). These pharmacological data suggested that PAR2, PAR1, and PAR4 were all functionally expressed in endothelial cells from mouse skin and lung and that any of these receptors might contribute to endothelial Xa signaling. The relative robustness of PAR2 versus PAR1 and PAR4 as a Xa receptor in the KOLF studies (Fig. 1), the relative robustness of responses to PAR2- versus PAR1- and PAR4-activating peptides in endothelial cells, and the observation that maximal responses to Xa were greater than those seen to thrombin in endothelial cells (Figs. 2 and 3) were consistent with PAR2 being the major Xa receptor in these cells. By contrast, in wild-type lung fibroblasts, PAR2-activating peptide triggered
virtually no response and the maximum response to Xa and thrombin were similar (Fig. 3d and not shown). Thus PAR1 appeared to be the major Xa receptor in fibroblasts (see below).

Xa Responses in Wild-type Versus PAR-deficient Endothelial Cells—Xa signaling was substantially reduced in endothelial cells derived from Par2−/− mice relative to wild-type mice (Fig. 3). In endothelial cells from wild-type mouse skin, Xa-stimulated a 3.7-fold increase in phosphoinositide hydrolysis versus an only 1.7-fold increase in cells from Par2−/− mice (p < 0.01; Fig. 3a). Similarly, in lung endothelial cells, Xa stimulated a 3.3-fold increase in phosphoinositide hydrolysis in cells from wild-type mice versus a 2.0-fold increase in cells from Par2−/− mice (p < 0.05; Fig. 3b). ERK phosphorylation in response to Xa was also substantially reduced in Par2−/− endothelial cells relative to wild-type (Fig. 3c). In five independent experiments, ERK phosphorylation in response to 1 unit/ml Xa in PAR2−/− skin endothelial cells ranged from absent to less than 2-fold over unstimulated, but was uniformly greater than 5-fold increased in wild-type. In contrast to Xa responses, responses to thrombin were not decreased in cells from Par2−/− mice (Fig. 3, a–c). These data strongly suggest that PAR2 is an endogenous Xa receptor in these endothelial cell preparations. Indeed, PAR2 appears to account for more than half of Xa-triggered phosphoinositide hydrolysis and most of the ERK activation in these cells.

Xa signaling was only moderately reduced in endothelial cells from Par1−/− mice compared with wild-type (Fig. 3). In wild-type skin endothelial cells, Xa triggered a 3.7-fold increase in phosphoinositide hydrolysis versus 2.8-fold in Par1−/− cells (Fig. 3a). In wild-type lung endothelial cells, Xa stimulated a 3.3-fold increase in phosphoinositide hydrolysis versus a 2.9-fold increase in Par1−/− cells (Fig. 3b). These differences in Xa signaling in wild-type versus Par1−/− endothelial cells did not reach statistical significance. To better probe the role of PAR1 in Xa signaling in endothelial cells in the absence of the contribution made by PAR2, we utilized PAR2-deficient endothelial cells and the PAR1 antagonist BMS200261 (BMS) (20). In addition to its activity as a PAR1 antagonist, BMS is a partial agonist for PAR2 (not shown). Therefore, BMS could only be used informatively in Par2−/− cells. Treatment of endothelial cells from Par2−/− mice with BMS decreased Xa-induced phosphoinositide hydrolysis and ERK1/2 phosphorylation to near basal levels (Fig. 3 and not shown). In Par2−/− endothelial cells from skin, BMS decreased Xa-stimulated phosphoinositide hydrolysis from 1.7- to 1.2-fold basal (Fig. 3a). In Par2−/− endothelial cells from lung, BMS decreased Xa-triggered phosphoinositide hydrolysis from 2.0-fold basal to 1.2-fold basal (p < 0.05) (Fig. 3b). BMS did not decrease serum- and lysophosphatidic acid-stimulated phosphoinositide hydrolysis nor did it cause cell loss (data not shown). Moreover, BMS reduced the thrombin responses in Par2−/− cells to a level similar to that of the AYPGKF response (Fig. 3, a and b), consistent with inhibition of PAR1 but not PAR4 and concordant with previous studies (28). These data suggest that PAR1 is an endogenous Xa receptor and may account for 20–30% of the response to Xa in the endothelial cell preparations studied.

The PAR4 agonist AYPGKF stimulated an increase in phosphoinositide hydrolysis in endothelial cells (Fig. 3) and responses to AYPGKF were absent in Par4−/− endothelial cells; these results suggest that PAR4 is expressed in microvascular endothelial cells at levels sufficient to mediate signaling. PAR4-mediated phosphoinositide hydrolysis in response to Xa when expressed in KOLF (Fig. 1) or Xenopus oocytes (16). The...
residual Xa signal in the BMS-inhibited Par2 −/− cells (Fig. 3) was less than that elicited by PAR4 activation. Thus our data are consistent with the notion that the residual Xa signal in the BMS-inhibited Par2 −/− cells may be mediated by PAR4, but certainly not probed. Taken together, these experiments suggest that Xa activates microvascular endothelial cells through PAR2, PAR1, and possibly PAR4.

Studies of fibroblast preparations, cultures derived from cells left behind after immunopurifying endothelial cells, were done in parallel with the endothelial cell studies described above. Fibroblasts derived from wild-type mouse lungs responded to agonists for PAR1 but showed little or no increase in phosphoinositide hydrolysis in response to PAR2- and PAR4-activating peptides (Fig. 3d and not shown). In the mouse, PAR3 serves a cofactor function rather than itself mediating transmembrane signaling (27, 29). Thus, of the four known PARs, PAR1 was the major candidate for mediating Xa signaling in our lung fibroblast preparations, and Xa-induced phosphoinositide hydrolysis was indeed absent in lung fibroblasts from Par1 −/− mice (Fig. 3d). Skin fibroblasts derived from wild-type mice showed little response to any PAR agonist but did show robust phosphoinositide hydrolysis in response to serum and lysophosphatidic acid (not shown); they were not studied further. These experiments suggest that Xa activates fibroblasts from lung predominantly through PAR1 and support the hypothesis that PAR1 is an endogenous Xa receptor in some cell types. The observation that PAR2 is the main Xa receptor in endothelial cells but plays little or no role in fibroblasts shows that different PARs mediate Xa signaling in different cell types and also suggests that fibroblast contamination did not confound signaling results obtained with endothelial cell preparations.

**PAR2 Is Expressed in Skin Endothelial Cells in Vivo**—The data presented above suggest that PAR2 is the major mediator of Xa signaling in early passage endothelial cells from skin and lung. PAR2 expression in endothelial cells can be regulated (30, 31). To determine whether PAR2 is expressed in mouse microvascular endothelial cells in vivo, we utilized a PAR2-lacZ knockin (“Experimental Procedures”). Strong β-galactosidase staining was apparent in skin from mice heterozygous or homozygous for the PAR2-lacZ allele. No staining was seen in wild-type mice. Intriguingly, only a fraction of vessels showed strong staining (Fig. 4, a and b) compared with the ubiquitous vascular staining seen in mice with an endothelial-specific Tie2-lacZ transgene (Fig. 4c). Moreover, paired vessels, one with strong staining and one without detectable staining, were often seen in the PAR2-lacZ mice (Fig. 4b). Based on vessel diameter, it appeared that a subset of arterioles stained strongly while staining in venules was difficult to detect in the gross.

Analysis of histological sections confirmed that β-galactosidase staining in PAR2-lacZ knockin mice labeled endothelial cells. At the level of individual vessels, staining in PAR2-lacZ knockin and Tie2-lacZ transgenics was indistinguishable; in both cases the innermost juxtalumenal layer of cells was stained (Fig. 4f versus g). As noted in the gross, β-galactosidase staining was detected in only a subset of vessels in the PAR2-lacZ knockin (Fig. 4f). Arterioles, particularly those deep in the dermis, stained robustly in PAR2-lacZ mice (Fig. 4d and not shown) compared with more ubiquitous robust vascular staining in Tie2-lacZ transgenics (Fig. 4e). Lighter staining was also noted in some capillary and venous endothelial cells and in some vascular smooth muscle in PAR2-lacZ mice (Fig. 4f and not shown), thus the selectivity of PAR2 expression for arterio-
cular endothelium was relative rather than absolute. These data strongly suggest that PAR2 is expressed in vivo in skin microvascular endothelial cells, particularly in arterioles. Similar results were obtained with staining of viscera. These results are in accord with the observation that the PAR2 agonist S1L-GIR causes nitric-oxide synthase-dependent and presumably endothelial-dependent vasodilatation and hypotension in mice (32) and with detection of PAR2 by immunostaining in endothelial cells in some but not all vessels in man (33, 34).

DISCUSSION

Heterologous expression of PARs conferred Xa signaling in two systems, *Xenopus* oocytes and the mammalian fibroblast line KOLF (16) (Fig. 1). The EC_{50} values for Xa signaling observed in PAR-transfected fibroblasts and in wild-type endothelial cells were of similar magnitude and, indeed, were similar to those obtained in most studies of Xa-induced biological responses (4–6, 15, 35, 36). Xa signaling was not seen in cell preparations that were not responsive to at least one PAR agonist, and Xa signaling was markedly reduced in previously responsive cell types when PAR function was inhibited by gene deletion and/or drug. Thus our results provide strong genetic evidence that PARs are endogenous Xa receptors and necessary for most if not all of Xa-triggered phosphoinositide hydrolysis and ERK phosphorylation in endothelial cells and fibroblasts. These results do not exclude a role for other Xa-binding sites, whether specialized lipid surfaces or proteins like TF/VIIa or factor Va, that might help localize Xa to the cell surface and promote its interaction with PARs. Indeed, Xa lacking a Gla-domain is less potent than intact Xa for activating PAR2 (16), and Ruf and colleagues (17) have recently shown that TF/VIIa can promote PAR activation by serving as a Xa-binding site. EPR-1 has also been put forward as a Xa receptor or binding site (35, 37), but its importance as such is unclear (19, 38).

Regardless, our data suggest that PARs are required for most if not all Xa signaling and that PAR knockout mice will provide a valuable strategy for defining the importance of Xa signaling in vivo.

The markedly decreased responsiveness to Xa in cells derived from Par2^{-/-} mice suggests that PAR2 is the major mediator of Xa signaling in endothelial cells, at least under the conditions for isolating and culturing endothelial cells employed in this study. This result is concordant with the observation that desensitization with a PAR2 agonist inhibited Xa-induced vasorelaxation of rat aortic rings (19). A contribution from PAR1 to endothelial Xa signaling was also apparent, especially in Par2^{+/−} cells. A small residual response to Xa was seen in the absence of both PAR2 and PAR1 function.

Because Xa can activate PAR4 and because our pharmacological studies suggest that PAR4 is functionally expressed in these endothelial cell preparations, it is possible that PAR4 mediates this small residual response. The possibility that PARs account for all Xa signaling might be formally tested if not all Xa signaling might be formally tested, and PAR2-deficient embryos show no apparent lethality in this same background.) Evidence for a genetic interaction between PAR2 and PAR1 would strongly suggest that they play related roles in vivo and respond to proteases generated in the same setting(s), consistent with the notion that PAR2 and PAR1 respond to Xa and thrombin, respectively, in vivo. Such information would also provide an additional rationale for the use of inhibitors of the coagulation cascade in disease states like sepsis in which endothelial responses to coagulation proteases may play a role (7, 8, 39, 41).

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Additions and Corrections

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Regulation of enhanced vacuolar \( H^+ \)-ATPase expression in macrophages.

Shui-Ping Wang, Irina Krits, Yan Lu, Shuting Bai, and Beth S. Lee

Yan Lu’s name was inadvertently omitted from the list of authors. The corrected list is shown above.

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ERK activation mediates cell cycle arrest and apoptosis after DNA damage independently of p53.

Damu Tang, Dongcheng Wu, Atsushi Hirao, Jill M. Lahti, Lieqi Liu, Brie Mazza, Vincent J. Kidd, Tak W. Mak, and Alistair J. Ingram

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Genetic evidence that protease-activated receptors mediate factor Xa signaling in endothelial cells.

Eric Camerer, Hiroshi Kataoka, Yao-Wu Zheng, Mark Kahn, Katy Lease, and Shaun R. Coughlin

Yao-Wu Zheng was inadvertently omitted from the author list. The corrected list is shown above.

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MECHANISMS OF SIGNAL TRANSDUCTION:
Genetic Evidence That Protease-activated Receptors Mediate Factor Xa Signaling in Endothelial Cells

Eric Camerer, Hiroshi Kataoka, Mark Kahn, Katy Lease and Shaun R. Coughlin
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