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Adhesive receptor Mac-1 coordinates the activation of factor X on stimulated cells of monocytic and myeloid differentiation: An alternative initiation of the coagulation protease cascade

(leukocyte integrins/ADP/tissue factor/procoagulant response)

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ABSTRACT **Monocytes initiate coagulation through regulated surface expression of tissue factor and local assembly of a proteolytic enzymatic complex formed by tissue factor and factor VII/activated factor VII. We now show that, in the absence of these initiating molecules, monocytes and cell lines of monocytic/myeloid differentiation can alternatively initiate coagulation after exposure to ADP. The molecular basis for this procoagulant response consists of two distinct events. First, cell stimulation with ADP induces high-affinity binding of coagulation factor X to the surface-adhesive receptor Mac-1. Locally, Mac-1-concentrated factor X is then rapidly proteolytically cleaved to an active protease with size and activity characteristics of activated factor X, which supports the cell-associated formation of thrombin and the procoagulant response. We conclude that the monocytic/myeloid adhesive receptor Mac-1 has the unexpected, specifically inducible property to organize a molecular assembly culminating in rapid fibrin formation that is independently regulated from tissue factor and factor VII/activated factor VII.**

Cellular initiation of a coagulation protease cascade has been implicated in important homeostatic and pathologic responses including hemostasis, intravascular coagulation (1), inflammatory reactions (2), antigen-specific immune responses (3–5), and viral diseases (6, 7). The induced expression by monocytes (8, 9) and endothelial cells (10) of tissue factor (TF) (11), the high-affinity receptor for factor VII or activated factor VII (factor VIIa) (12, 13), results in the proteolytic cell surface complex TF:factor VII/VIIa (TF: VII/VIIa), the major pathway for initiating coagulation. This bimolecular complex cleaves the zymogen factor X to the active serine protease activated factor X (factor Xa), (14) followed by assembly of factor Xa into the cell surface prothrombinase complex (15), which results in thrombin formation. Other mechanisms of initiation of coagulation include an inducible cellular prothrombinase (16, 17) and possibly the direct activation of factor X (18).

We recently observed that ADP can perturb monocyte function and trigger specific cellular responses, including a membrane-associated procoagulant activity (PCA) (19). In more recent studies, we have observed that ADP, as well as the calcium ionophore ionomycin, induces specific and saturable high-affinity binding of factor X to monocytes and cell lines of this lineage (20). This receptor was identified as the leukocyte heterodimeric adhesive molecule Mac-1, a member of the integrin gene family (21, 22) expressed by cells of monocytic/myeloid differentiation (23). Mac-1 has been implicated as an active participant in binding and phagocytosis of opsonized (i.e., C3bi coated) particles (24). However, our recent finding that this receptor possesses an additional ligand specificity for factor X (20) suggests that Mac-1 exhibits the multifunctional receptor versatility typical of other, partially related, integrin receptors (21, 22).

These observations have now been extended to demonstrate that, in the absence of demonstrable TF or TF: VII/VIIa complex, ADP-stimulated monocytes and myeloid cells bearing Mac-1 directly convert surface-bound factor X to a proteolytically active derivative characteristic of factor Xa. This appears to represent an additional mechanism of initiating the coagulation protease cascade on the surface of cells.

MATERIALS AND METHODS

Cells. Monocytes were isolated from medication-free, informed normal volunteers (19) and were suspended at 1.5–2 $\times 10^7$ per ml in serum-free, low-endotoxin (<30 pg/ml) RPMI 1640 medium (M. A. Bioproducts, Walkersville, MD)/ 20 mM Hepes (Calbiochem–Behring)/gentamicin (100 μg/ml) (Geramycin, Schering). Polymorphonuclear leukocytes (PMN) were prepared by dextran sedimentation of acid citrate/dextrose-anticoagulated blood and suspended at 1.5–2 $\times 10^7$ per ml in RPMI 1640. The monoblast line U937 (25), monocytic line THP-1 (26), and HL-60 promyelocytic line (27) were maintained in RPMI 1640/10% fetal calf serum (HyClone, Logan, UT)/20 mM Hepes/gentamicin (100 μg/ml)/2 mM L-glutamine (Irvine Scientific)/10 μM 2-mercaptoethanol (Eastman, Rochester, NY). Granulocytic differentiation of HL-60 cells was induced by culture for 4 days in growth media containing 1.25% dimethyl sulfoxide (Me_2SO; Sigma) as described (27).

Monoclonal Antibodies and Binding Studies. Neutralizing monoclonal antibodies 11D12 and 5G9 bind different epitopes on TF [contained within residues 1–30 and 26–49 (28), respectively] and block binding of factor VII. Cell surface expression of TF was analyzed by indirect immunofluorescence with 5G9. Monocytes were stimulated with 10 μM ADP for 15 min at room temperature in the presence of 2.5 mM $CaCl_2$ or were incubated with bacterial lipopolysaccharide (LPS) at 1 μg/ml (Calbiochem) for 6 hr at 37°C. Cells (1 $\times 10^6$) were incubated in v-bottom microtiter plates (Costar, Cambridge, MA) with saturating concentrations of 5G9 for 30 min at 4°C, stained with fluorescein-conjugated goat F(ab')$_2$ anti-mouse IgG (heavy and light chain specific) antibody (Tago, Burlingame, CA), washed with fetal calf serum and RPMI 1640, and analyzed by flow cytometry on a Becton Dickinson FACS IV/40 fluorescence-activated cell sorter. The irrelevant antibody V82A6 and the anti-Mac-1 antibody

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Abbreviations: PCA, procoagulant activity; TF, tissue factor; factor Xa, activated factor X; factor VIIa, activated factor VII; LPS, bacterial lipopolysaccharide; PMN, polymorphonuclear leukocytes; ^{125}I-11D12, ^{125}I-labeled 11D12; ^{125}I-factor X, ^{125}I-labeled factor X.

OKM1 were used as negative and positive markers, respectively.

11D12 was iodinated by the Iodo-Gen (Pierce) method (29) to a specific activity of 0.55 μCi (1 Ci = 37 GBq) per μg of protein. The binding of ^{125}I-labeled 11D12 (^{125}I-11D12) to ADP- or LPS-stimulated monocytes was quantitated. Increasing concentrations of ^{125}I-11D12 (0.5–100 μg/ml) were incubated with monocytes pretreated with either ADP (10 μM for 15 min at room temperature) or with LPS (1 μg/ml for 6 hr at 37°C). After 30 min at room temperature, aliquots of the reaction mixtures were centrifuged through silicone oil (Dow Corning Specialty Lubricants, New Bedford, MA) at 12,000 × g for 2 min to recover cell-associated radioactivity. Nonspecific binding was assessed in the presence of a 100-fold excess of unlabeled 11D12. Binding was analyzed by the method of Scatchard by using the LIGAND program (G. A. McPherson, Elsevier Biosoft, 1985) on a VAX 11/750 computer. The experimental procedures for the isolation, purification, and labeling of human factor X have been reported previously (20). The ability of PMN or Me_2SO-differentiated HL-60 cells to bind ^{125}I-labeled factor X (^{125}I-factor X) after stimulation with ADP (10 μM) was analyzed as described previously for monocytes and U937 and THP-1 cells (20).

Analysis of PCA and Factor Xa Coagulant Activity. Assay and quantitation of cellular PCA expressed by ADP- or LPS-stimulated cells was as described (5, 19). Factor Xa coagulant activity was measured by a modification of the method of Miletich *et al.* (30). Cell suspensions were incubated at 20°C for various times with 10 μM ADP, 2.5 mM $CaCl_2$, and factor X at 10 μg/ml. At selected intervals between 1 and 30 min, 0.1 ml of the reaction mixture was transferred to 37°C with 0.1 ml of factor VII- and factor X-deficient bovine plasma (Sigma). The reaction was initiated with 0.1 ml of 0.025 M $CaCl_2$, and the clotting time was read. A standard curve with serial concentrations of factor Xa, isolated as described (31), was constructed to convert clotting times to ng of factor Xa coagulant activity per ml.

The ability of anti-TF antibodies 5G9 or 11D12 to inhibit the ADP-induced PCA and factor Xa coagulant activity was analyzed by incubating aliquots of ADP-stimulated cells with saturating amounts of these antibodies for 30 min at room temperature. In other experiments, stimulated cells were incubated with anti-Mac-1 antibodies TS1/18 and 60.3 under the same experimental conditions. At the end of the incubation, control cells or cells treated with each inhibitor were separately assayed for PCA and factor Xa coagulant activity as described above.

Sodium Dodecyl Sulfate (SDS)/PAGE. Aliquots (0.2 ml) of monocytes, THP-1 cells, or U937 cells were incubated with ^{125}I-factor X at 2.5 μg/ml in the presence of 10 μM ADP and 2.5 mM $CaCl_2$ for 20 min at room temperature. Cells were washed, centrifuged at 12,000 × g for 2 min, and lysed in 2% SDS buffer, pH 6.8. Samples were boiled for 5 min at 100°C, clarified at 12,000 × g for 2 min, and electrophoresed on 10% SDS/polyacrylamide slab gels (32). Gels were stained with 0.08% Coomassie brilliant blue R 250, destained in 5% acetic acid, dried, and exposed for autoradiography at −70°C by using intensifying screens (DuPont de Nemours) and Kodak X-Omat AR x-ray film.

Table 1. Effects of neutralizing anti-TF monoclonal antibodies (5G9 and 11D12) on ADP-induced monocyte PCA

Monocyte treatment	Clotting time, sec	PCA, milliunits per 10^6 cells
Unstimulated	66 ± 3.5	17 ± 4.3
ADP stimulated	28 ± 1.3	1165 ± 335
5G9 treated*	30.2 ± 1.9	835 ± 281
11D12 treated*	28.7 ± 1.5	1072 ± 282
Control IgG* treated	30 ± 1	829 ± 137

*The monocytes were stimulated with ADP prior to incubation with the antibody. Values are the means ± SEM.

RESULTS

After a relatively brief (10–20 min) exposure to 10 μM ADP, monocytes acquired the capacity to markedly accelerate the coagulation of recalcified plasma (Table 1). Similarly, monocytoid THP-1 cells expressed the procoagulant response after ADP stimulation, establishing that cells of monocyte lineage are responsible for this property rather than other blood-derived contaminating cells.

Though the rapidity of the response was inconsistent with synthesis and expression of TF, analyses for the presence of TF and TF:VII/VIIa complex on ADP-stimulated cells was essential to distinguish between this classic molecular mechanism of initiation (12, 13) and an independent mechanism. The participation of TF was analyzed functionally and immunochemically. Neutralizing anti-TF monoclonal antibodies were employed in an attempt to abolish the ADP-mediated monocyte procoagulant response. Concentrations of antibodies 5G9 or 11D12 that completely block TF-mediated monocyte PCA on LPS-induced cells did not diminish the ADP-induced monocyte PCA (Table 1). Similarly, polyclonal neutralizing anti-factor VII antibodies had no effect on the rapid, ADP-mediated monocyte PCA, indicating that TF-bound factor VII or VIIa were not responsible for the response (data not shown).

That ADP-stimulated monocytes might express TF responsible for the initiation of the coagulation cascade was considered further. Flow cytofluorometric analysis of the cell suspension with antibody 5G9 was consistently negative for the expression of TF on the surface of ADP-stimulated monocytes (Fig. 1*A*). In contrast, 6 hr after stimulation with LPS, virtually all monocytes were positively stained for cell surface expression of TF (Fig. 1*B*), although with heterogeneity in surface density.

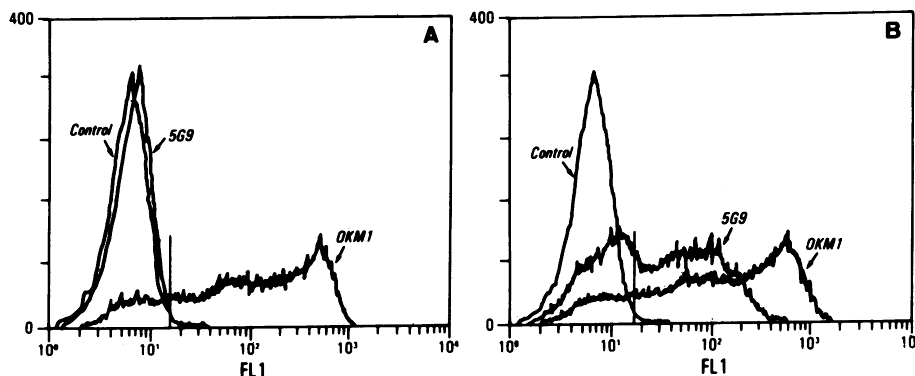

FIG. 1. Flow cytometry of monocytes with anti-TF antibody 5G9. Monocytes (1 × 10^6) were stimulated either with 10 μM ADP for 15 min at room temperature (*A*) or with LPS at 1 μg/ml for 6 hr at 37°C (*B*), incubated with saturating concentrations of 5G9, stained with fluorescein-conjugated goat anti-mouse IgG, washed, and analyzed by flow cytometry. V82A6 and OKM1 were the negative and positive controls, respectively. Fluorescence intensity (abscissa, FL1) quantifies the surface expression of the antigen, and the ordinate quantifies the cell number.

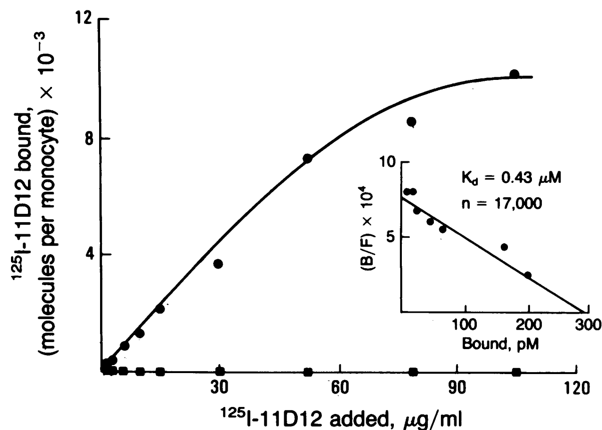

FIG. 2. Binding of ^{125}I-11D12 anti-TF monoclonal antibody to monocytes. Monocytes were stimulated with ADP or LPS as described above. ^{125}I-11D12 (0.5–100 μg/ml) was equilibrated with the cells, and the specific binding of ^{125}I-11D12 to ADP (■)- or LPS (●)-stimulated monocytes was determined. (*Inset*) Scatchard plot analysis for ^{125}I-11D12 binding to LPS-stimulated monocytes. Each point is the mean of four separate experiments.

To quantitate more precisely the expression of TF on the surface of LPS- or ADP-stimulated monocytes, we analyzed the direct interaction of ^{125}I-labeled anti-TF antibody 11D12 with the stimulated cells. The binding of ^{125}I-11D12 to LPS-stimulated monocytes was specific and concentration-dependent and was saturated at 10,100 molecules of antibody per cell surface (Fig. 2). In contrast, ADP-stimulated monocytes did not exhibit specific binding of ^{125}I-11D12 (Fig. 2). Scatchard plot analysis of ^{125}I-11D12 binding to LPS-stimulated monocytes was extrapolated to ≈17,000 binding sites per monocyte with a dissociation constant of 4.3×10^{-7} M, which is consistent with specific immunologic binding of a monoclonal antibody (Fig. 2 *Inset*).

Taken together, these data exclude the TF molecule and the TF:VII/VIIa complex as the molecular basis for the ADP-mediated monocyte procoagulant response. We have reported presumptive evidence that the ADP-mediated monocyte PCA requires factor X and factor V but not factor VII based on use of selected single-factor-deficient plasma (19). In a further study, we demonstrated that, after stimulation with ADP, monocytes bind factor X in a high-affinity reaction mediated by the adhesive receptor Mac-1 (20).

We therefore hypothesized that the ADP-induced binding of factor X to the monocyte receptor Mac-1 was a molecular prerequisite for the expression of the cellular procoagulant response. To test whether this postulated mechanism was a general property of cells of monocytic/myeloid differentiation, circulating PMN were analyzed for their ability to interact with factor X. After ADP (10 μM) stimulation, suspensions of PMN bound ^{125}I-factor X (Table 2) with the same quantitative characteristics previously observed for monocytes and related cell lines (20). Furthermore, to exclude possible contaminations with other blood-derived cells, the promyelocytic cell line HL-60 was cultured in the presence of Me_2SO to induce differentiation into mature granulocytes (27). Treatment of Me_2SO-differentiated HL-60 cells with ADP induced specific binding of ^{125}I-factor X in a reaction indistinguishable from that observed for freshly isolated PMN (Table 2).

Table 2. ^{125}I-factor X binding to ADP-stimulated PMN and Me_2SO-differentiated HL-60 cells

^{125}I-factor X added, nM	^{125}I-factor X bound, molecules per cell: PMN	Me_2SO-differentiated HL-60 cells
1.5	7,144 ± 1,609	6,618 ± 1,560
3	18,424 ± 5,958	13,924 ± 2,698
7.6	41,805 ± 6,763	42,473 ± 8,061
15	93,036 ± 16,395	84,158 ± 20,162
38	232,286 ± 23,134	195,497 ± 47,366
61	322,890 ± 58,871	284,610 ± 50,280

Suspensions of PMN or HL-60 cells treated with Me_2SO for 4 days were separately stimulated with 10 μM ADP in the presence of 2.5 mM $CaCl_2$. Increasing concentrations of ^{125}I-factor X were then added, and specific binding was quantitated after a 15-min incubation at room temperature. Values are the means ± SEM.

Moreover, under the same experimental conditions, when a fixed concentration of factor X was separately added to the ADP-stimulated suspensions of monocytes or Me_2SO-differentiated HL-60, a progressive, time-dependent shortening of the clotting times was recorded, which is consistent with a cell surface-associated generation of factor Xa coagulant activity (Table 3). This was not observed in suspensions of unstimulated cells, and, similar to the data presented in Table 1, it was not inhibited by neutralizing anti-TF antibody 5G9 or by a polyclonal neutralizing anti-factor VII antiserum (data not shown). In contrast, anti-Mac-1 antibodies TS1/18 and 60.3, which block factor X binding to Mac-1 (20) consistently abolished the ADP-mediated PCA, further confirming that the availability of Mac-1 in its receptor function for factor X is the obligatory prerequisite for the further expression of the procoagulant response. Supernatants from the ADP-stimulated cell suspensions contained no factor Xa coagulant activity as measured by the clotting assay or by monitoring the hydrolysis of the chromogenic substrate S2222 (Helena Laboratories, Beaumont, TX), which con-

Table 3. Generation of factor Xa coagulant activity by ADP-stimulated monocytes or Me_2SO-differentiated HL-60 cells

Incubation time, min	Monocytes: Clotting time, sec	Monocytes: Factor Xa coagulant activity, ng/ml	Me_2SO-differentiated HL-60 cells: Clotting time, sec	Me_2SO-differentiated HL-60 cells: Factor Xa coagulant activity, ng/ml
0	>120	<10	>120	<10
1	81 ± 7	22 ± 3.5	105.2 ± 1.6	15 ± 1.5
3	65.5 ± 4.5	31 ± 3.5	61.2 ± 3.9	33 ± 3.7
5	51 ± 6	45 ± 6.2	47.5 ± 2.6	50 ± 4
10	24.5 ± 1.5	130 ± 10	34.7 ± 0.6	80 ± 3.5
20	12 ± 2	350 ± 75	18.7 ± 1.5	180 ± 17.5

Suspensions of monocytes or Me_2SO-differentiated HL-60 cells were incubated with 10 μM ADP, 2.5 mM $CaCl_2$, and factor X (10 μg/ml) at room temperature. After various time intervals, aliquots of the incubation reaction mixture were mixed with factor VII- and factor X-deficient plasma at 37°C and were recalcified with 0.025 M $CaCl_2$. The mean clotting time for each experiment was converted to factor Xa coagulant activity (in ng/ml) from a standard curve constructed with serial dilutions of factor Xa. Values are the means ± SEM.

firmed the membrane-associated property of this procoagulant response (19).

Additional experiments were addressed to determine whether the state of cell differentiation influences the ability of monocytic/myeloid cells to initiate coagulation after the ADP-induced cellular binding of factor X to Mac-1. Untreated U937 were assumed as the prototype of the monoblast with the least degree of differentiation (25), whereas THP-1 cells have a more monocyte-like phenotype (26). The expression of TF and TF-dependent initiation of coagulation was absent from untreated U937 or THP-1 cells as for unstimulated monocytes. In contrast, following stimulation with ADP, both cell lines specifically bound factor X (20) and generated factor Xa coagulant activity. This procoagulant response requires selective cell stimulation with ADP since the ionophore ionomycin, which induces factor X binding to Mac-1 in all cells tested, does not initiate the cell-associated coagulation process (20). In Fig. 3*A*, the efficiency of ADP-stimulated monocytes, THP-1 cells, or U937 cells to generate factor Xa coagulant activity is compared. Freshly isolated monocytes provided the highest response, whereas untreated U937 cells generated the lowest amounts of factor Xa coagulant activity. Intermediate results were observed with THP-1 cells (Fig. 3*A*). In contrast, untreated HL-60, which have predominantly features of promyelocytes, did not express any factor Xa coagulant activity after ADP stimulation (data not shown).

We inquired whether the factor Xa coagulant activity produced by ADP-stimulated cells was related and/or supported by a structural change of factor X bound to its cellular receptor Mac-1. After ADP-induced binding of ^{125}I-factor X to Mac-1, SDS/PAGE of cell extracts revealed that monocytes and THP-1 and U937 cells actually promote a proteolytic modification of cell surface-bound ^{125}I-factor X. After a 20-min incubation, at a time when considerable amounts of factor Xa coagulant activity is associated with the cells in the clotting assay (Table 3), the single band of M_r 66,000, characteristic of the nonreduced ^{125}I-factor X offered to each ADP-stimulated cell suspension (Fig. 3*B*, lane 1), was consistently associated with a faster migrating band of M_r 51,000 in monocytes, THP-1 cells, and U937 cells (Fig. 3*B*, lanes 2–4). This derivative of ^{125}I-factor X was absent on ionomycin-stimulated monocytes and THP-1 cells (data not shown) and in supernatant medium of ADP-stimulated cells; however, when specifically eluted from the surface of cells exposed to ADP by treatment with 10 mM EDTA (20), this molecular species retained its direct coagulant properties and electrophoretic pattern.

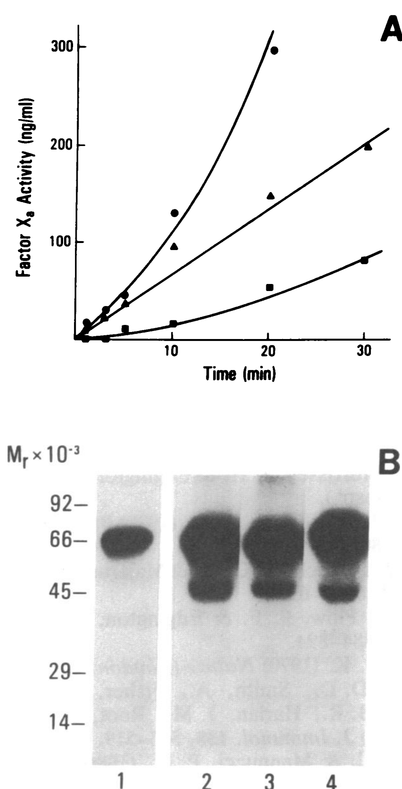

FIG. 3. (*A*) Generation of factor Xa coagulant activity by ADP-stimulated monocytes and monocytoid cells. Factor Xa coagulant activity was measured in ADP-stimulated suspensions of monocytes (●), THP-1 cells (▲), or U937 cells (■) and quantitated as described in Table 3. (*B*) Cell membrane-associated factor Xa. Monocytes (lane 2), THP-1 cells (lane 3), or U937 cells (lane 4) were separately incubated with 10 μM ADP, 2.5 mM $CaCl_2$, and ^{125}I-factor X at 2.5 μg/ml for 20 min at room temperature. Detergent-solubilized cell extracts were then analyzed by 10% SDS/PAGE and autoradiography. Lane 1, nonreduced ^{125}I-factor X consisting of a single band of M_r 66,000, which was offered to each cell suspension at the start of incubation. Lanes 2–4 show the proteolytic derivative composed of bands of M_r 66,000 and 51,000 characteristic of nonreduced factor Xa, associated with monocytes, THP-1 cells, and U937 cells.

DISCUSSION

We provide evidence for an additional pathway of activation of coagulation on the surface of cells of myeloid and macrophage lineage. Monocytes, whether as an effector cell of the immune response or after exposure to selected agonists, are remarkably effective in initiating coagulation by means of regulated expression of TF (11), the high-affinity receptor for factor VII (12, 13). These studies provide direct demonstration of the monocyte surface density of TF after a 6-hr stimulation with LPS and quantitate expression at about 17,000 molecules per cell, though with considerable heterogeneity.

In contrast to the relatively slow induction of PCA function in monocytes by most agonists and by means of cellular collaboration, we previously observed that exposure of monocytes to ADP induced, within minutes, the ability to initiate coagulation. This was effective in factor VII-deficient plasma (19), which suggests independence from the classical extrinsic pathway of activation by TF (12, 13). Now, the lack of inhibition of the ADP-mediated PCA by neutralizing anti-TF antibodies combined with the absence of TF on the surface of ADP-stimulated monocytes, as revealed by direct binding studies, conclusively exclude the participation of TF and TF:VII/VIIa complex in the initiation of this procoagulant response.

Rather, we demonstrated that after stimulation with ADP, monocytes and monocytoid cell lines bind factor X in a specific, Ca^{2+}-dependent, saturable manner that embodies the hallmarks of a valid receptor–ligand interaction (20). The high-affinity receptor for factor X on cells of the monocyte lineage was identified as the multifunctional leukocyte adhesive receptor Mac-1 (20, 23).

We now provide evidence that membrane-associated factor X is proteolytically converted to an active derivative characteristic structurally and functionally of factor Xa. The appearance of this derivative on the surface of stimulated cells is associated with appearance of factor Xa coagulant activity. The mechanism can be clearly separated into two steps (i.e., activation of Mac-1 to bind factor X and proteolytic activation of factor X by an enzyme of cellular origin). The enzyme responsible for activation of factor X is unknown; however, unlike Mac-1 activation for factor X binding, which can be elicited by ionomycin and thus is presumably induced by a Ca^{2+} flux, the proteolysis of factor X requires additional signals engendered by exposure to ADP.

We inquired whether all cells expressing Mac-1 (23) had the capacity to initiate coagulation by means of surface association and activation of factor X. Freshly isolated neutrophils stimulated with ADP specifically bound factor X in a saturable reaction identical to that observed for monocytes (20), which is consistent with the role of Mac-1 as a factor X receptor distributed on cells of monocytic/myeloid lineage (23). Similarly, Me_2SO-differentiated granulocyte-like HL-60 cells (27) bearing a functionally competent Mac-1 receptor (33) and free of other blood cell contamination bound factor X after ADP stimulation, with characteristics indistinguishable from those of circulating neutrophils, monocytes, or related cell lines (20). Moreover, although neutrophils do not express TF (8) and cannot initiate coagulation by the classic extrinsic pathway (12, 13), Me_2SO-differentiated HL-60 cells (27) produced factor Xa coagulant activity. Also, the monocytoid cell lines U937 and THP-1, which bind factor X, expressed factor Xa coagulant activity, thus initiating the procoagulant response. However, the efficiency to generate factor Xa coagulant activity correlates with the degree of cell differentiation since no activity was found in undifferentiated HL-60 and the least expression was found in U937 monoblast-like cells. This suggests that, although U937 can bind ^{125}I-factor X with affinity indistinguishable from that of more mature cells (20), the more efficient propagation and amplification of the procoagulant response, after the conversion of Mac-1 bound factor X to factor Xa-like derivative, requires additional events for which both the characteristics of delivered signals and the differentiation and maturation processes are crucial.

Remarkable features of this pathway of cellular initiation of coagulation are the rapid inducibility and the nature of the stimulus. We have observed that ADP induces rapid cellular responses in the monocyte, including specific receptor-site induction for fibrinogen (19) and factor X (20), activation of the arachidonic acid metabolism (34), and enzymatic proteolysis of cell surface-bound factor X. Adenine nucleotides such as ADP and ATP have been shown to mobilize cytosolic Ca^{2+} in endothelial cells (35) and neutrophils (36). In this context, ADP may elicit transients in the monocyte cytosolic Ca^{2+} relevant for intracellular signal transduction and rapid, highly specific cellular responses (19, 20, 34). This hypothesis is also suggested by our recent use of classical mobilizers of cytosolic Ca^{2+}, such as ionomycin and fMet-Leu-Phe (37) to recapitulate Mac-1 binding of factor X (ref. 20; D.C.A. and T.S.E., unpublished observations). Moreover, adenine nucleotides are ubiquitous *in vivo* and concentrations of ADP up to 20 μM can be physiologically obtained during hemostasis (38). The ADP-mediated activation of coagulation orchestrated by Mac-1 may then contribute to rapid fibrin deposition in the microenvironment of local injury and inflammation, in a collaborative interaction with activated platelets and vascular endothelium.

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1. Niemetz, J. & Fani, K. (1971) *Nature (London) New Biol.* **232**, 247–248.
2. Drake, T. A., Rodgers, G. M. & Sande, M. A. (1984) *J. Clin. Invest.* **73**, 1750–1753.
3. Colvin, R. B., Johnson, R. A., Mihm, M. C., Jr., & Dvorak, H. F. (1973) *J. Exp. Med.* **138**, 686–698.
4. Helin, H. & Edgington, T. S. (1983) *J. Exp. Med.* **158**, 962–975.
5. Gregory, S. A. & Edgington, T. S. (1985) *J. Clin. Invest.* **76**, 2440–2445.
6. Levy, G. A., Leibowitz, J. L. & Edgington, T. S. (1981) *J. Exp. Med.* **154**, 1150–1163.
7. Dindzans, V. J., Skamene, E. & Levy, G. A. (1986) *J. Immunol.* **137**, 2355–2360.
8. Edwards, R. L., Rickles, F. R. & Bobrove, A. M. (1979) *Blood* **54**, 359–370.
9. Levy, G. A. & Edgington, T. S. (1980) *J. Exp. Med.* **151**, 1232–1244.
10. Bevilacqua, M. P., Pober, J. S., Majeau, G. R., Cotran, R. S. & Gimbrone, M. A., Jr. (1984) *J. Exp. Med.* **160**, 618–623.
11. Morrissey, J. H., Fakhrai, H. & Edgington, T. S. (1987) *Cell* **50**, 129–135.
12. Broze, G. J., Jr. (1982) *J. Clin. Invest.* **70**, 526–535.
13. Ploplis, V. A., Edgington, T. S. & Fair, D. S. (1987) *J. Biol. Chem.* **262**, 9503–9508.
14. Nemerson, Y. & Gentry, R. (1986) *Biochemistry* **25**, 4020–4033.
15. Nesheim, M. E., Taswell, J. B. & Mann, K. G. (1979) *J. Biol. Chem.* **254**, 10952–10962.
16. Schwartz, B. S., Levy, G. A., Curtiss, L. K., Fair, D. S. & Edgington, T. S. (1981) *J. Clin. Invest.* **67**, 1650–1658.
17. Schwartz, B. S., Levy, G. A., Fair, D. S. & Edgington, T. S. (1982) *J. Exp. Med.* **155**, 1464–1479.
18. Shands, J. W., Jr. (1983) *Blood* **62**, 333–340.
19. Altieri, D. C., Mannucci, P. M. & Capitanio, A. M. (1986) *J. Clin. Invest.* **78**, 968–976.
20. Altieri, D. C. & Edgington, T. S. (1988) *J. Biol. Chem.* **263**, 7007–7015.
21. Hynes, R. O. (1987) *Cell* **48**, 549–554.
22. Ruoslahti, E. & Pierschbacher, M. D. (1987) *Science* **238**, 491–497.
23. Sanchez-Madrid, F., Nagy, J. A., Robbins, E., Simon, P. & Springer, T. A. (1983) *J. Exp. Med.* **158**, 1785–1803.
24. Wright, S. D., Rao, P. E., van Voorhis, W. C., Craigmyle, L. S., Iida, K., Talle, M. A., Westberg, E. F., Goldstein, G. & Silverstein, S. C. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 5699–5703.
25. Ralph, P., Harris, P. E., Punjabi, C. J., Welte, K., Litcofski, P. B., Ho, M. K., Rubin, B. Y., Moore, M. A. S. & Springer, T. A. (1983) *Blood* **62**, 1169–1175.
26. Tsuchiya, S., Yamabe, M., Yamaguchi, Y., Kobayashi, Y., Konno, T. & Tada, K. (1980) *Int. J. Cancer* **26**, 171–176.
27. Collins, S. J., Ruscetti, F. W., Gallagher, R. E. & Gallo, R. C. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 2458–2462.
28. Pepe, M., Morrissey, J. H. & Edgington, T. S. (1988) *FASEB J.* **2**, 391 (abstr.).
29. Fraker, P. J. & Speck, J. C., Jr. (1978) *Biochem. Biophys. Res. Commun.* **80**, 849–857.
30. Miletich, J. P., Jackson, C. M. & Majerus, P. W. (1978) *J. Biol. Chem.* **253**, 6908–6916.
31. Fair, D. S., Plow, E. F. & Edgington, T. S. (1979) *J. Clin. Invest.* **64**, 884–894.
32. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
33. Hickstein, D. D., Smith, A., Fisher, W., Beatty, P. G., Schwartz, B. R., Harlan, J. M., Root, R. K. & Locksley, R. M. (1987) *J. Immunol.* **138**, 513–519.
34. Altieri, D. C. & Mannucci, P. M. (1986) *J. Exp. Med.* **164**, 1815–1820.
35. Luckhoff, A. & Busse, R. (1986) *J. Cell. Physiol.* **126**, 414–420.
36. Ochs, D. L. & Reed, P. W. (1983) *J. Biol. Chem.* **258**, 10116–10122.
37. Lew, P. D., Wollheim, C. B., Waldvogel, F. A. & Pozzan, T. (1984) *J. Cell Biol.* **99**, 1212–1220.
38. Born, G. V. R. & Kratzer, M. A. A. (1984) *J. Physiol. (London)* **354**, 419–429.