GENERATION OF COAGULATION FACTOR V ACTIVITY BY CULTURED RABBIT ALVEOLAR MACROPHAGES

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Coagulation occurs not only intravascularly in a milieu containing the complete plasma clotting system, but also extravascularly during hemostasis and inflammation (1–3). To more fully understand extravascular coagulation reactions, especially those occurring in lung disorders characterized by alveolar edema and capillary leak syndromes (3, 4), we recently investigated alveolar macrophage populations isolated from rabbits. In these studies, the macrophages were found to contain tissue factor activity in amounts that increase in vivo and in vitro with cell maturation. Immunologic lung reactions induced by bacillus Calmette-Guérin had the effect of stimulating pulmonary accumulation of the cells, mostly as mature forms expressing large amounts of tissue factor procoagulant (5). The role of tissue factor in coagulation is to initiate the extrinsic clotting pathway by complexing with factor VII, and this complex activates factor X to Xa, which in turn interacts with factor V. The latter protein is required for rapid proteolytic prothrombin to thrombin conversion by factor Xa, and is therefore a central cofactor in the cascade of reactions leading to fibrin formation (6, 7). To date, only the hepatocyte is widely accepted as a cellular source of factor V (8, 9).

In experiments reported here, we investigated production by alveolar macrophages of additional procoagulants comprising the extrinsic and common final clotting pathways. We found that these macrophages generate factor V activity, particularly at intermediate stages of cellular maturation. Factor V procoagulant activity derived from this macrophage source may contribute to clotting reactions and fibrin deposition in the lung.

Materials and Methods

Animals and Cell Procurement. Alveolar macrophages were isolated from New Zealand White female rabbits by bronchoalveolar lavage and separated into subpopulations using discontinuous Percoll gradients as previously described (5). Briefly, animals were killed by intravenous injection of 3 ml of pentobarbitol (1 g/ml). The lungs were excised and lavaged with 80 ml of 0.15 M sterile saline. Cells in lavage fluids were pelleted by centrifugation, suspended in saline, and further purified by centrifugation through a discontinuous Percoll gradient of densities 1.055, 1.060, 1.064, 1.068, and 1.070 g/ml. Cells banding at density interfaces were carefully collected using a peristaltic pump, washed, and suspended in phosphate-buffered saline at a concentration of 4×10^6 cells/ml. Maturation was evaluated by examining Wright-stained smears of the individual, freshly isolated macrophage subpopulations, as previously described (5). Briefly, by mor-

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phologic examination using a light microscope, it was observed that cells of low density (more mature) had relatively large cytoplasmic diameters (C), low ratios of nuclear to cytoplasmic diameters (N/C), and extensive cytoplasmic granulation and vacuolation as compared with denser (less mature) cells. For quantitative morphometric studies, a light-pen digitizer system was used to measure diameters of projected cell images, and an on-line computer calculated mean values of C and N/C. These morphometric studies showed that C increased progressively from a mean of 13 μm in the 1.055 g/ml fraction to 19 μm in the 1.070 g/ml fraction. By contrast, N/C ratios progressively decreased from 5.6×10^{-1} to 6.7×10^{-1} from the highest- to lowest-density subpopulations. These quantitative findings of stepwise increases of C and decreases of N/C, correlating with density changes and morphologic appearance, provide evidence that maturity in the macrophage subpopulations progressively increased from the 1.055-g/ml to the 1.070-g/ml fractions (5). The unfractionated cell preparations and the five purified subpopulations contained 98% macrophages as determined by nonspecific esterase and Wright staining, and the cells were 95% viable, as tested by trypan blue dye exclusion.

Cell Culture. Unfractionated macrophages and the subpopulations were suspended in phosphate-buffered saline and incubated at a concentration of 4×10^6 cells/ml in either Labtek flaskettes (Miles Laboratories, Inc., Naperville, IL) or in 24-well Costar plates (Costar, Data Packaging, Cambridge, MA) in 1-ml or 0.25-ml cultures, respectively. After incubation for 2 h at 37°C in humidified air with 5% CO_2, the phosphate-buffered saline overlying adherent macrophages was removed and replaced by culture medium consisting of minimum essential medium (Flow Laboratories, Inc., McLean, VA). Added to the minimum essential medium was 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.5% heated (100°C, 30 min) lactoalbumin hydrolysate (Becton, Dickinson & Co., Cockeysville, MD). The serum-free cultures were continued for periods ranging from 2 to 48 h as reported in the Results. In some experiments, endotoxin type BII (kindly provided by Dr. David Morrison of Emory University, Atlanta, GA) was added to cultured cells at 0.1–10 μg/ml. After cell culture, supernatants to be assayed for coagulation factors were recovered with a pipette and centrifuged to remove any detached cells. The detached cells, pelleted by centrifugation of supernatants, were then recovered and added to attached cells harvested separately by scraping the flasks or microtiter plate wells with a rubber policeman. Samples not assayed immediately were stored at −20°C in 20% glycerin. Under these conditions, factor V activity was stable during storage, but in the absence of glycerin, factor V was labile.

Coagulation Assays. Factor V activity was measured in culture supernatants using a two-stage coagulation test with purified coagulation factors (6). Preliminary studies showed that similar results were obtained if assays were performed using cultured cells suspended in the supernatants rather than supernatants alone. In the first stage of the assay, thrombin was generated from prothrombin by factor Xa during a 5-min incubation at 37°C in the presence of lipid vesicles, factor V in the sample, and calcium chloride. This reaction was stopped by diluting the reaction mixture 10-fold with chilled saline. The amounts of thrombin produced were measured in the second stage of assay as clotting times (seconds) obtained after adding the diluted first-stage mixture to fibrinogen, using a Fibrometer (Becton, Dickinson & Co.). These clotting times were converted to thrombin equivalents (National Institutes of Health thrombin units per milliliter of supernatant) by referral to calibration curves made daily. The curves showed log-log plots of clotting times measured after adding known amounts of serially diluted thrombin standard to fibrinogen (6). The standard curves were linear from 5 to 19 thrombin units/ml at lower limits to 395 units/ml at upper limits. Duplicate measurements varied by <10%. Incubation mixtures for the first stage contained 30 μl of test material (e.g., supernatants) plus 30 μl of components at the following concentrations: purified bovine Xa at 0.04 unit/ml, purified human prothrombin at 0.2 unit/ml, rabbit brain cephalin at 6.6 nmol lipid phosphorus/ml, and 0.025 M $CaCl_2$. For the second stage of assay, 90 μl of purified bovine fibrinogen at 5 mg/ml was added along with 90 μl of the incubated first-stage mixture diluted 10-fold. Controls showed that reaction mixture components used for assaying cultured cells and supernatants were present in excess in the two-stage test, and using 10-fold-higher

concentrations of $CaCl_2$, lipid, factor Xa, prothrombin, and fibrinogen had no effect on the specificity of the assay. A linear relationship between the logarithms of the (initial) rates of thrombin generation and the concentrations of factor V from macrophage cultures was found. Brain thromboplastin standards were without effect on clotting times obtained in the two-stage assay for factor V. In selected experiments, test samples were preincubated with catalytic amounts of thrombin (0.5–2 units/ml) to determine if the factor V activity in the samples was present as V or Va (10). The thrombin-activated samples were diluted two- to five-fold before testing for factor V to avoid interference in the assay by the added thrombin. Purified reagents for the two-stage assay of factor V activity were from Sigma Chemical Co., St. Louis, MO. Some experiments used lysates, which were prepared by freezing and thawing cells in medium with and without added glycerin at 20% for stabilization of factor V.

Tissue factor activity was quantitated using a one-stage clotting test. Culture supernatants or cell lysates that were suspended in fresh medium or supernatant at 4×10^6 cells/ml (60 μl) were added to 60 μl of 0.025 M $CaCl_2$ and platelet-poor rabbit plasma (60 μl). Clot formation time was measured using a Fibrometer, and times were converted into tissue factor units using log-log plots of the clotting times obtained with serial dilutions of a thromboplastin standard (5). By definition, 1,000 units of thromboplastin is the amount giving a coagulation time of 50 s in this test. Thromboplastin standards were fresh rabbit brain homogenates prepared in our laboratory and rabbit alveolar macrophage lysates. Duplicate measurements differed by <10%.

To determine if coagulation factors other than factor V and tissue factor were present in the cultures, plasmas from patients with severe congenital monodeficiencies in factors V, VII, and X (from the Clinical Coagulation Laboratory, University of North Carolina, Chapel Hill, NC) and Dade factor II–deficient substrates (American Dade, Miami, FL) were used in one-stage clotting tests. For these tests, 60 μl of the culture supernatant or cell lysates was incubated with 60 μl of an excess of rabbit tissue factor standard at 37°C. After 30 s, 60 μl of 0.25 M $CaCl_2$ plus 60 μl of substrate plasma was added and clotting times were measured in a Fibrometer.

Chromogenic Assay for Factor X or Xa. The presence of factor X/Xa in the cultures was also investigated in a two-stage test using the chromogenic substrate S-2222 (Kabi Diagnostica, Stockholm, Sweden). 100 μl of culture supernatant or standard solution of purified factor X (0.1–0.001 units/ml), purified factor Xa (0.04–0.02 units/ml), or rabbit plasma dilutions of 1/50–1/5,000 were incubated with 15 μl of 0.1 M $CaCl_2$ and 25 μl of Russell's viper venom (Sigma Chemical Co.) at 1 unit/ml. After 30 min at 37°C, 100 μl of the mixture was transferred to wells of microtiter plates (Dynatech Immulon, Scientific Products, Charlotte, NC) containing 50 μl of 0.3 M Tris buffer, pH 8.3, and 100 μl of S-2222. The incubation was continued for periods ranging from 5 min to 24 h. The reaction was stopped by adding 10 μl of 50% acetic acid to each microwell and the optical density was read with a plate reader at 405 nm.

Results

Factor V Procoagulant Activity Is Generated by Alveolar Macrophages in Culture. Alveolar macrophages isolated from rabbits and cultured for 22 h were examined for their ability to generate clotting factor V using cells separated into five subpopulations by Percoll density gradient centrifugation. With this technique, the cells are purified at several stages of maturation, and advancing maturity, characterized morphometrically, correlates inversely with cell density (5). Factor V activity was found to be generated predominantly by intermediate density subpopulations of these macrophages. Thus, in six representative experiments, factor V activity was not observed in assays before culture using either intact or lysed cells. By contrast, factor V activity was consistently found in supernatants harvested from 22-h cultures containing subpopulations of inter-

mediate density (1.060, 1.064, and 1.068 g/ml; Table I). However, low density cells (1.055 g/ml) generated little or no measurable factor V activity (Table I). The highest-density subpopulation (1.070 g/ml) contained too few cells for study in half of the experiments shown (Table I). Additional studies were carried out using fewer cells (1×10^6 rather than 4×10^6 per culture). In these smaller cultures, factor V activity was occasionally generated by the 1.070-g/ml subpop ulation, and when this occurred the amounts were comparable to those found with intermediate-density cells cultured under the same conditions. (In the smaller cultures, 1.055-g/ml subpopulations again contrasted with remaining subpopulations by failing to generate factor V activity in detectable amounts and intermediate-density subpopulations consistently generated factor V activity.) Since only intermediate-density cells consistently generated factor V activity, data presented in the following experiments are for these cells only.

Time-dependent Factor V Activity Generation by Alveolar Macrophages, Inhibition by Puromycin, and Lack of Stimulatory Effects of Endotoxin. Experiments were carried out to examine the time course of factor V activity generation by alveolar macrophages. Activity was first detected in culture supernatants at 8 h, and by 22 h, peak activity was reached (Fig. 1). Thereafter, activity declined. Including puromycin in the cultures at 0.1–10 μg/ml inhibited the generation of factor V activity, which indicates requirements for protein synthesis (Fig. 2). Dose-response studies showed that relatively low concentrations of puromycin inhibited generation of factor V activity as compared with tissue factor activity production

TABLE I

Amounts of Factor V Activity Generated by Cultured Alveolar Macrophage Supopulations

Fraction number (percent total cells)*	Cell density	Factor V activity‡		Ng–Nr§
		Clotting time	Thrombin equivalents	
			U/ml	
1 (16%)	1.055	178 ± 93	<19	2–6
2 (39%)	1.060	51 ± 6	75	6–6
3 (34%)	1.064	54 ± 5	69	5–5
4 (9%)	1.068	60 ± 5	55	5–6
5 (<1%)	1.070	>500	<19	0–3
Unfractionated cells		91 ± 17	35	3–6
Medium blank		>500	<19	6–6

Amounts of factor V activity measured in 30 μl of supernatants from alveolar macrophage cultures containing 4×10^6 cells/ml in 1 ml of serum-free medium are shown as clotting times in two-stage assays and alternatively as units of thrombin equivalents per milliliter.

* Proportions of cells purified in each fraction are shown as a percent of the total number of cells recovered from the density gradients.

‡ Factor V activity measured in seconds in a two-stage test is expressed as thrombin equivalents (or units) per milliliter as explained in Materials and Methods. Results are means for those experiments in which factor V was generated.

§ Ng–Nr represents the number of experiments in which measurable amounts of factor V activity were generated, followed by the number of times subpopulations were recovered from Percoll gradients in numbers sufficient for culture and subsequent assay (e.g., fraction 1 contained sufficient cells for study in all six experiments, but factor V activity was generated only twice, and the amounts were below limits of the assay, <19 units).

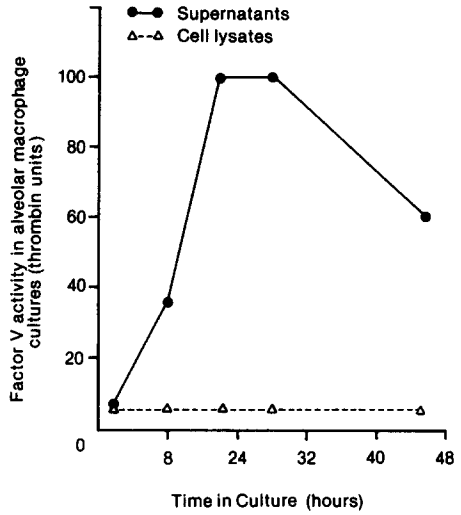

FIGURE 1. Alveolar macrophages of density 1.060 were cultured at 4×10^6 cells/ml for the time intervals indicated. Supernatants and cells were harvested separately and amounts of factor V activity were measured by a two-stage coagulation assay. The data show factor V activity as thrombin units (or equivalents) per milliliter in one of four similar experiments. Factor V activity was found only in the supernatants, which indicates a lack of intracellular accumulation of this procoagulant during culture.

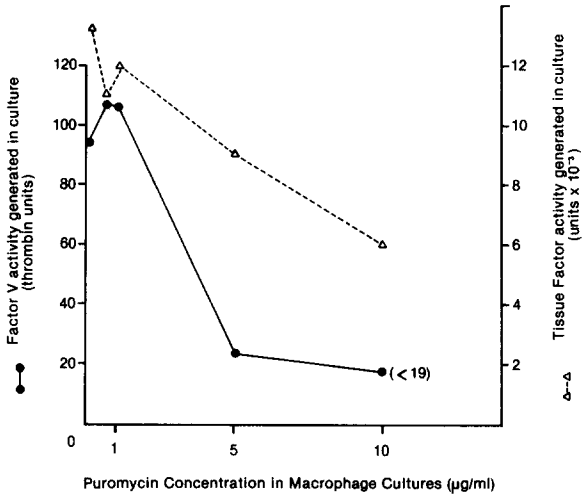

FIGURE 2. Alveolar macrophage cultures containing 4×10^6 cells/ml were assayed for both factor V and tissue factor activities. Amounts of procoagulant activity generation are shown as net increases per milliliter as compared with results before culture, and sensitivities to the protein synthesis inhibitor, puromycin, are compared. Data are for one of four similar experiments.

(Fig. 2). Addition of endotoxin to the alveolar macrophage cultures failed to stimulate further increases of factor V generation, but added endotoxin markedly stimulated tissue factor production by the same cells (Table II).

Characterization of Alveolar Macrophage Factor V/Va Activity by Specific Requirements in One- and Two-Stage Tests. Factor V activity in the supernatants was

TABLE II
Lack of Stimulatory Effects on Factor V Activity Generation of Endotoxin Added to Cultures

Endotoxin	Factor V activity*		Tissue factor activity‡	
μg/ml	*s*	*U/ml*	*s*	*U/ml*
0	68	50.0	65	7,470 (4,731)
0.1	67	51.1	60	9,130 (6,391)
1.0	70	43.5	43	28,220 (25,481)
10.0	69	46.6	40	31,540 (28,801)
Blank	>500	<19	>500	<1

Alveolar macrophages of density 1.060 were cultured with the indicated concentrations of added endotoxin for 24 h at 37°C. Data are from one experiment measuring Factor V and tissue factor in the same cultures. In three additional experiments using endotoxin as a stimulus (1 μg/ml), average tissue factor activity increased 14-fold compared with levels in control cultures without added endotoxin, whereas generation of factor V activity was not stimulated in the same cultures (0.1-fold).

* Amounts of factor V were measured in a two-stage assay of culture supernatants as described in Table I. Before culture, factor V was undetectable in the cell preparations (clotting times were >500 s).

‡ Tissue factor was measured in a one-stage test using cells recovered from the culture flaskette and lysed before assay. Results in parentheses indicate net changes of activity in culture compared with results before culture. The remaining data show actual clotting times and units of activity measured after culture.

TABLE III
Characterization of Factor V Activity Generated in Culture Using One-Stage Assays in Deficient Substrate Plasmas

	II^-	V^-	VII^-	X^-
Supernatants	107 ± 3 (4)	55 ± 2 (5)	115 ± 3 (5)	>250 (5)
Lysates	124 ± 5 (4)	83 ± 2 (4)	114 ± 4 (4)	>250 (4)
Saline control	119 ± 5 (4)	82 ± 2 (4)	112 ± 5 (4)	>250 (4)

The results shown are mean times (s ± SEM) for assays of culture supernatants, lysates of cultured cells, and saline blanks in one-stage clotting factor assays using plasmas monodeficient in the factors shown. The assays utilized 60 μl of supernatant fluid or cell lysate resuspended in fresh medium from 22-h alveolar macrophage cultures, and the samples were tested in the presence of an excess of added tissue factor. The number of experiments performed is indicated, in parentheses.

initially characterized in a one-stage coagulation assay. Supernatants from cultured macrophages, but not lysates of these cells, corrected the coagulation defect in factor V–deficient substrate plasmas. Neither supernatants nor the lysates corrected defects in substrates deficient in factors, II, VII, or X. Thus, the procoagulant activity identified in supernatants is specifically factor V, and factor II, VII, and X activities were not detected (Table III).

Specific functional characterization of factor V activity was also carried out by demonstrating strict requirements for factor Xa, prothrombin, lipid, and Ca^{++} for the formation of thrombin in a two-stage test. During the first stage of this assay, supernatants were incubated for 5 min with factor Xa, substrate (prothrom-

bin), lipid vesicles, and Ca^{++}. For the second stage, the first reaction mixture was added to fibrinogen, and the time required for fibrin clot formation was the measured endpoint. Adding supernatants directly to fibrinogen did not induce fibrin formation, and all components of the first-stage mixture, including supernatants plus the other three reactants, were found to be needed for fibrin formation (Table IV). Thus, the procoagulant activity detected in culture supernatants was not a direct prothrombinase, but it amplified the effects of the classical prothrombinase, factor Xa, in a reaction dependent on lipids and Ca^{++}. This observed specificity characterizes the supernatant procoagulant activity as factor V. Pretreatment of supernatants with catalytic amounts of thrombin did not significantly increase the amounts of factor V measured, which indicates that factor V generated by cultured alveolar macrophages is in an activated (factor Va) form (9). (In three experiments, supernatants were preincubated with either thrombin or saline and diluted to avoid effects of added thrombin in the assay. In the thrombin-and saline-treated supernatants, amounts of factor V activity, expressed as units of thrombin equivalents per milliliter, were 55.6 ± 8.2 and 51.0 ± 7, respectively. Buffer blanks containing either appropriately diluted thrombin or saline had activities of <7.5 units/ml.)

Control Experiments Showing a Lack in Alveolar Macrophages of Factor V Activatible by Thrombin or Inhibitors of Factor V Activity. In additional experiments, alveolar macrophages were studied either directly after isolation or after culture for 22 h. Lysates of the uncultured and cultured cells (separated from supernatants) were pretreated with catalytic amounts of thrombin before assay in a two-stage test. Factor V activity was not detected in these thrombin-treated cells. (Clotting times in two-stage tests were >500 s in assays using 4×10^6 cells/ml, as routinely present in culture. Assays using excess numbers of uncultured and cultured cells, at 5×10^7/ml, gave similar results, i.e., clotting times were >500 s.) In other experiments, uncultured and cultured cells at a concentration of 4×10^6/ml were mixed with supernatants containing factor V activity generated in culture. The mixtures were incubated at 37°C for 30 min before assay in a two-stage test. In these mixing experiments, there was no evidence found to indicate inhibitory effects of the cells on supernatant factor V activity (Table V).

TABLE IV

Effects of Deleting Components of the Factor Xa-dependent Prothrombinase Complex on Assay of Culture Supernatants for Factor V

Component deleted and resultant clotting time (s)						
None	Lipids	Prothrombin	Fibrinogen	Xa	Ca^{++}	Supernatant
50	>500	>500	>500	>500	>500	>500

Results are for a series of two-stage assays carried out as described in Table I after individually replacing components of the first-stage reaction mixture with saline. The resultant clotting time prolongations (as compared with the 50-s time obtained when none of the components were omitted) demonstrate that the macrophage procoagulant has specific requirements for each of the omitted reactants and therefore is factor V. The result shown after replacing Ca^{++} with saline uses a supernatant dialysed to remove Ca^{++}, which was a constituent of the culture medium. Using the undialysed supernatant containing Ca^{++}, the clotting time was 121 s.

TABLE V
Lack of Inhibition of Factor V Activity by Alveolar Macrophages

	Clotting time	Factor V activity (thrombin equivalents)
	s	*U/ml*
Supernatant + control medium	63.0	60.1
Supernatant + cultured cells	55.0	72.3
Supernatant + fresh cells	56.5	69.0
Cultured cells	>500	<5.0
Fresh cells	>500	<5.0

Alveolar macrophages at 4×10^6 cells/ml were tested either directly after isolation or after culture for 22 h. Cell lysates were mixed 1:1 with supernatants containing factor V activity generated in culture, and the mixtures were assayed for factor V activity in a two-stage test as described in Table I. Data show the means of duplicate assays from one of three similar experiments.

Discussion

The present experiments demonstrate that coagulation factor V activity is generated by alveolar macrophages in culture. Thus, factor V activity was not found in or on fresly isolated alveolar macrophages, even when excess numbers of cells were assayed following preincubation with thrombin, which was added in catalytic amounts to activate any inactive factor V in the cells. After initiating alveolar macrophage culture, amounts of factor V activity increased in a time-dependent manner. Within ~8 h, the procoagulant attained readily measureable levels, and peak amounts were reached by 22 h. For generation of factor V activity, live cells synthesizing protein were required, as shown by results with controls containing lysed rather than viable cells and by inhibitory effects of puromycin included in the culture medium. Rather than being intracellular or on cell surfaces, the factor V activity was found in culture supernatants, which indicates that this procoagulant generated in its functional form is released from alveolar macrophages into the extracellular environment. Using Percoll density gradients to obtain a series of cells at different stages of maturation characterized morphometrically (5), we found that alveolar macrophages are heterogeneous with respect to their ability to generate factor V activity. Macrophages of intermediate maturity (and density) consistently generated this activity, while fully mature (low density) cells generated little or none of the procoagulant. The least mature (most dense) subpopulation obtained, which accounted for <1% of the total cells, showed variable ability to generate factor V activity.

In additional experiments, we found that addition of endotoxin to cultures did not stimulate generation of factor V activity. This contrasted with effects on tissue factor generation, which was stimulated by added endotoxin. Moreover, tissue factor generation was found at all stages of macrophage maturation in our earlier studies (5), rather than being restricted to cells at particular stages of maturity as seen here with generation of factor V activity. Another contrast

observed was that inhibition of factor V generation occurred with relatively low concentrations of puromycin compared with amounts needed to inhibit generation of tissue factor. Finally, alveolar macrophage tissue factor was found to be cell associated (5), while factor V activity was released into culture supernatants but was not detectable on macrophage surfaces (intact cell measurements) or intracellularly (lysed cell measurements).

Alveolar macrophages have not been previously shown to generate factor V activity or coagulation factors other than tissue factor. However, freshly isolated human monocytes and lymphocytes have been found to possess surface receptors for factor V, and addition of purified factors Va and Xa and Ca^{++} to these intact cells results in formation of a functional prothrombinase complex (11). Earlier reports (12, 13) suggested that factor V activity is produced by murine peritoneal macrophages simultaneously with prothrombin, factors VII, and Xa, tissue factor activities. These reports are apparently controversial (14). A subsequent study (15) showed that human monocytes have surface receptors for factor VII/VIIa, and the receptor in all likelihood is tissue factor. It has also been shown (14, 16, 17) that rabbit hepatic macrophages, murine peritoneal exudate macrophages, and human monocytes generate factor VII–like or factor VII activity.

In the present study, the assay for factor V relied on demonstrating that a prothrombinase is formed by adding culture supernatants to mixtures containing a prothrombin substrate together with purified factor Xa, lipid vesicles, and Ca^{++}. Omitting supernatants or any of these reaction mixture components required for prothrombinase complex assembly resulted in a loss of detectable prothrombinase formation. This specificity characterizes the procoagulant in supernatants as factor V. Furthermore, the observed specific requirements for reaction mixture components indicates a lack of significant generation by the macrophages of thrombin or prothrombinases independent of factor X (18, 19). Evidence that the macrophages generated factor V activitiy was additionally confirmed by using a one-stage assay in substrate plasma severely monodeficient in factor V. Factors X/Xa, II, and VII were not detected in testing supernatants (or cells) in an amidolytic assay for factor X/Xa and in one-stage assays in substrates monodeficient in these factors. Further studies will be needed to elucidate the molecular structure of rabbit factor V, which, in contrast to human and bovine factor V, has not been isolated to date.

The precise physiologic role of factor V activity produced by alveolar macrophages is unknown. However, it is clear that the coagulation system provides a vital defense against lung hemorrhage. Moreover, in certain types of pulmonary inflammation, the process of coagulation within alveolar exudates favorably affects hemodynamics and improves oxygenation (20). Tissue factor is available within the alveolus (5; McGee, M. P., and H. Rothberger, manuscript submitted for publication), and for thrombin formation to occur through the tissue factor (extrinsic) pathway in this extravascular space, sources of factors V, VII, II, and X would be required. As determined in studies of human and bovine plasma, factor V is by far the largest of the latter clotting proteins (330,000 vs. 50,000–70,000 daltons) (7, 21, 22). Based on these differentials of molecular size and known characteristics of alveolar wall permeability (4), it seems likely that in pulmonary inflammatory reactions accompanied by subtle capillary damage and

leakage of plasma, diffusion of factor V from interstitial areas into the alveolar space would be relatively limited. Therefore, in these reactions, factor V activity generated by alveolar macrophages may serve to critically increase the rate of local thrombin formation. In purified systems, factor V amplifies the rate of factor Xa catalysis of prothrombin to thrombin by more than 10^4-fold (7).

Summary

Alveolar macrophages are cells important in immune defense and inflammation in the lung, and the coagulation system participates in these reactions. In earlier experiments, it was found that alveolar macrophages contain and produce tissue factor, the extrinsic clotting pathway activator. The present experiments explore possible production by alveolar macrophages of the sequence of the clotting proteins that interact to form thrombin following initiation of coagulation by tissue factor. In studies using alveolar macrophages purified from rabbits, factor V activity was not detected in cell preparations assayed directly after isolation. However, after short-term culture, we found generation and release of factor V activity by these cells, which was predominantly from subpopulations with densities of 1.060–1.068 g/ml, corresponding to intermediate stages of alveolar macrophage maturation. Cell viability and protein synthesis were required for generation of the activity as shown by inhibitory effects of cell lysis before culture and by effects observed after including puromycin in cultures with viable cells. The activity generated was characterized as factor V by demonstrating specific functional requirements in one- and two-stage coagulation tests. There was no detectable generation in these cultures of factors II, VII, X, or the more recently described factor X–independent monocyte/macrophage prothrombinases. Factor V activity generated by alveolar macrophages may contribute to prothrominase assembly, activation of clotting, and fibrin formation within the alveolus.

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