THE INDUCTION OF MACROPHAGE SPREADING: ROLE OF COAGULATION FACTORS AND THE COMPLEMENT SYSTEM*

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The mononuclear phagocytes of blood and tissues carry out many of their functions not as free swimming forms, but when they are attached to the surface of cells, basement membranes, or matrix proteins. Upon passage from blood into serous cavities these ameboid cells differentiate into macrophages and under various environmental stimuli can adhere to surfaces and show several manifestations of plasma membrane activity including pseudopod extension, ruffling, endocytosis, and translational motility.

In the absence of added exogenous factors, normal mouse peritoneal macrophages maintained in culture spread gradually reaching a maximum diameter within 12–24 h. In contrast, macrophages obtained from the peritoneal cavity of mice previously injected with endotoxin or thioglycollate medium spread immediately after attachment to the substrate (1). Similar spreading of macrophages occurs after the infection of mice with *Listeria monocytogenes* (2) or with *Trypanosoma cruzi* (3) and has been interpreted as an early expression of macrophage activation (4).

A variety of reagents has the property of inducing rapid spreading of unstimulated mouse peritoneal macrophages under in vitro conditions and when attached to a surface. This phenomenon has been observed with immune complexes, ATP, adenosine, proteases, dithiothreitol, manganese, and increased hydrogen ion concentration. This response occurs only in the absence of serum and is a subject that has been recently reviewed (5).

In this report we describe two systems in plasma and serum which when activated lead to the rapid spreading of unstimulated macrophages already attached to a glass or plastic substrate. The first is associated with the contact phase of blood coagulation and the second with the complement system.

Materials and Methods

Reagents. EDTA and EGTA, obtained from Sigma Chemical Co., St. Louis, Mo., were diluted to 0.1 M in H_2O and the pH adjusted to 7.4 with NaOH. Kaolin was purchased from Amend Drug Co., New York, washed three times with saline by centrifugation, resuspended at 20 mg/ml, and autoclaved. Celite 512 was a gift of Dr. Hymie Nossel from Columbia University and was treated in the same way as the kaolin. Inulin was purchased from Pfanstiehl Laboratories, Waukegan, Ill. Ellagic acid was bought from Aldrich Chemical Co., Milwaukee, Wis. and a solution at 10^{-5} in

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medium was prepared by sonication. Sodium heparin with no preservatives was bought from Connaught Medical Research Laboratories, Toronto, Canada. Zymosan was obtained from Sigma Chemical Co., St. Louis, Mo. Urokinase from Leo Pharmaceutical Products, Denmark was diluted in saline to a concentration of 1,000 Plough U/ml. Dog plasminogen prepared by affinity chromatography (6) was a gift of Dr. Saimon Gordon, The Rockefeller University. Thrombin, topical, from bovine origin was obtained from Parke, Davis & Co., Detroit, Mich. Bovine trypsin, soybean trypsin inhibitor, and lima bean trypsin inhibitor were obtained from Worthington Biochemical Co., Freehold, N. J. Purified cobra venom factor (CVF)[1] was obtained from Cordis Laboratory, Miami, Fla. Dulbecco's modified Eagle's medium, H-21, referred to as medium throughout this paper, and fetal calf serum were from Grand Island Biological Co., Grand Island, N. Y. Sheep erythrocytes, antibodies to sheep erythrocytes, and mouse complement were prepared as previously described (7).

Mice. Unless otherwise stated, macrophages, serum, and plasma were harvested from Swiss mice, female, 20–25 g, maintained at The Rockefeller University. The C5-deficient strains A/J and AKR/J (8) were obtained from The Jackson Laboratory, Bar Harbor, Maine.

Plasma and Serum Collection. Mice were bled under ether anesthesia, by cutting the axillary vessels. Blood from each mouse was collected with siliconized Pasteur pipettes (Siliclad, Clay Adams, Inc., Div. of Becton, Dickinson & Co., Parsippany, N. J.) and placed into plastic vials containing either 10 U of heparin, 0.1 ml of 0.1 M EGTA, or no anticoagulant. After 15 min at room temperature they were spun for 3 min in a microfuge B (Beckman Instruments, Palo Alto, Calif.). The cell-free sera and plasma were pooled and stored in aliquots at −70°C until use. Celite treatment was performed by incubating a 50% dilution of serum or plasma in medium with 10 mg/ml celite for 20 min at 37°C with constant shaking. A similar procedure was used with kaolin.

Solubilization of Immune Complexes. Solubilization of immune complexes was kindly performed by Doctors Joyce Czop and Victor Nussenzweig as described (9).

Macrophage Spreading Assay. Macrophages were obtained as described by Cohn and Benson (10). Total peritoneal cells were suspended in medium with 10% fetal calf serum and adjusted to 2×10^6/ml. 0.1 ml of the cell suspension was added to each 13-mm round glass cover slip (Clay Adams Inc.) or 0.3 ml to each well of a 24-well tissue culture tray (Linbro Chemical Co., New Haven, Conn.). They were incubated for 30 min at 37°C under a 5% CO_2, 100% humidity atmosphere and then washed three times with medium to remove nonadherent cells. Cover slips were transferred to a dry Petri dish and covered with 0.1 ml of the solution to be tested, whereas culture wells received 0.3 ml. The usual incubation time was 1 h at 37°C. At the end of this period the cells were fixed by the addition of 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, prewarmed to 37°C.

Macrophages were considered spread when they showed a membrane apron occupying an area double that of unspread cells, or when they were elongated. A calibrated micrometer eye piece (Bausch & Lomb Inc., Rochester, N. Y.) was used for comparison.

Area Occupied by Macrophages. Area occupied by macrophages was determined in 8 × 10 prints of microphotographs using a grid with parallel lines at 1-cm intervals. A picture of the grid of a Neubauer counting chamber was used for size reference. A detailed discussion of the method has been published (11). No attempt was made to determine the actual membrane surface area of the cells as reported previously (11).

Dialysis. Dialysis was performed either in dialysis bags (Arthur H. Thomas Co., Philadelphia, Pa.) previously washed and autoclaved in 1 mM EDTA pH 7.0, then boiled and rinsed, or in collodion bags (Schleicher & Schuell, Keene, N. H.). Plasma was placed inside the bags, and an equal volume of phosphate-buffered saline containing 1% heat-inactivated mouse serum and 10 U/ml heparin placed on the outside. Dialysis was carried out overnight on a shaker.

Gel Filtration. Gel filtration was carried out in the cold in Sephadex G-50 medium (Pharmacia Fine Chemicals, Piscataway, N. J.), packed in a siliconized column 2.5 × 40 cm. The buffer was 0.03 M NH_4HCO_3 pH 7.9. The excluded fraction and the included fraction were separately pooled, lyophilized, and reconstituted to the initial volume with phosphate-buffered saline. 0.1% bovine serum albumin was added to the included fraction. Plasma and serum were used for these experiments. In the case of plasma, heparin (10 U/ml) was added to the reconstituted material. The complement activation by Sephadex was prevented by the addition of 10 mM EDTA to the plasma before filtration. This plasma was reconstituted with Ca^{++} and Mg^{++} before use.

[1] *Abbreviation used in this paper:* CVF, cobra venom factor from *Naja naja*.

TABLE I
Plasma-Induced Spreading of Mouse Peritoneal Macrophages

Preparation added to macrophage monolayers	Percent of macrophages spread on	
	Glass surface	Plastic surface
Fresh plasma* containing		
Heparin, 10 U/ml	95	8
EGTA, 10 mM	97	6
EDTA, 10 mM	10	9
Incubated 56°C 1h	12	10

* Plasma added at a concentration of 50% in culture medium and incubated with the cells for 1 h at 37°C. Similar results were obtained with serum.

Results

Glass-Dependent Spreading. Mouse peritoneal macrophages cultivated on a glass surface show an intense spreading reaction when exposed to fresh mouse plasma diluted to 50% with culture medium (Table I). Similar results are obtained with serum. This phenomenon is observed with unspread freshly explanted macrophages, as well as with macrophages maintained in culture for 24 h in medium containing 10% fetal calf serum, which already show a certain degree of spreading. The spreading reaction depends on the nature of the substrate to which the cells are attached, and as shown in Table I, it does not occur during the period if the cells are maintained on a plastic surface. Plasma prepared with EGTA, heparin, or serum collected in siliconized vessels was equally efficient in inducing spreading. On the other hand, addition of 10 mM EDTA or heat inactivation at 56°C for 1 h abolished the spreading activity.

The glass-dependent spreading activity could be quantitatively removed by treating the plasma or serum with kaolin or celite, agents that are known to bind and activate some factors involved in the contact phase of blood coagulation (Fig. 1).

Materials adsorbed to celite could be subsequently eluted with 1.71 M NaCl (12). The eluate, after overnight dialysis against medium, was itself unable to induce spreading. However, it could reconstitute the spreading activity of a celite-treated plasma by 50%, when compared to a control of untreated plasma.

The addition of ellagic acid to macrophage monolayers prepared on plastic dishes and overlayered with plasma induced intense spreading. 98% of the macrophages spread with concentrations of 5×10^{-5} and 10^{-6} M. No spreading was observed with 10^{-7} M ellagic acid. Controls using kaolin-treated plasma showed no spreading. Ellagic acid is known to be a soluble activator of the Hageman factor (13).

Plasma or serum maintained at 4°C in plastic containers for more than 24 h lost its ability to induce glass-dependent spreading. Storage at −70°C preserved full activity for at least 1 mo.

Complement-Dependent Spreading. The activation of the alternative pathway of complement fixation in the celite- or kaolin-treated plasma generates spreading activity, as described in Table II. The complement activator could be added directly to the plasma overlaying the monolayer, as was the case with CVF, or it could be preincubated with the plasma, removed, and this activated

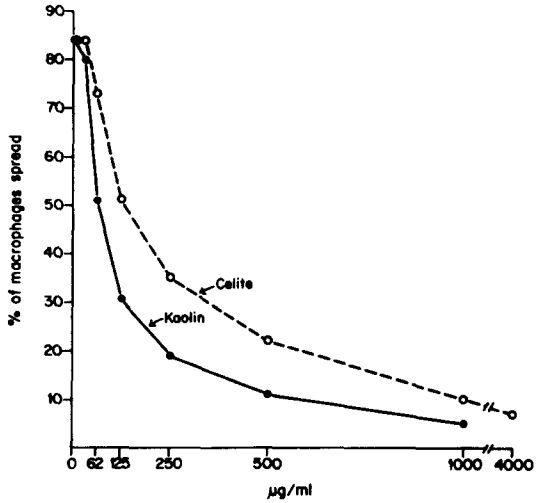

FIG. 1. Removal of glass-dependent spreading activity from plasma by treatment with celite and kaolin. The plasma was preincubated with these materials, centrifuged, diluted to 30% in medium, and added to macrophage monolayers. The percent of macrophages spread was determined after 1 h incubation at 37°C.

TABLE II

Complement-Induced Spreading of Mouse Peritoneal Macrophages Attached to a Glass Surface

Plasma pretreatment*	Complement activator		
	None‡	Zymosan§	CVF‖
	% spreading		
Celite	15	74	85
Kaolin	11	78	90
56°C, 1 h	8	9	11
None	79	61	91

* Fresh plasma containing 10 U heparin/ml, diluted to 50% in medium.

‡ Percentage of macrophages spread determined after 1 h of incubation at 37°C.

§ Zymosan (10 mg/ml) preincubated with plasma for 30 min at 37°C, then removed by centrifugation.

‖ CVF added to cultures in final concentration of 5 U/ml.

plasma then added to the macrophages, as was done with the zymosan particles. CVF by itself or CVF that had been heated at 72°C for 30 min added to kaolin-treated plasma was unable to generate spreading activity. The addition of inulin to kaolin-treated plasma, followed by its removal by centrifugation, induced spreading in a manner comparable to that of zymosan.

Spreading activity of complement-generated products, under conditions in which glass-dependent spreading factors were not involved, could also be accomplished by the addition of CVF and untreated plasma to macrophages attached to a plastic surface.

The induction of spreading activity by the activation of complement required low molecular weight plasma components. These low molecular weight sub-

TABLE III
Requirement for Low Molecular Weight Components in the Induction of Complement-Dependent Spreading by Zymosan

Plasma preparation	Percent of macrophages spread* with a plasma preparation pretreated with		
	None	Celite	Celite + Zymosan
Plasma	85	6	58
Excluded fraction‡ (Sephadex G-50)	69	8	7

* Assay was carried out with monolayers prepared on glass cover slips; the plasma concentration was 50% or equivalent for the fraction.

‡ Fresh plasma containing 10 mM EDTA was passed through a Sephadex G-50 column. All fractions comprising the void volume were pooled, lyophilized, reconstituted to original volume of plasma, and dialyzed against medium containing 10 U/ml heparin.

stances were not required for the induction of the glass-dependent spreading activity. This was demonstrated by using plasma (containing 10 mM EDTA) which had been passed through a Sephadex G-50 column. The excluded peak was pooled, lyophilized, the Ca^{++} and Mg^{++} reconstituted, and the fraction tested for spreading activity. The results are described in Table III. The addition of zymosan to this fraction did not generate spreading. The excluded fraction may lack factor D, which in humans has a mol wt of 24,000 and is a component necessary for the activation of the alternative pathway of complement fixation (reviewed in reference 14).

Plasma obtained from mice genetically deficient in C5 (AKR/J and A/J) were able to induce both glass-dependent and complement-dependent spreading. The number of cells spread was reduced by approximately 20% when compared to the activity of NCS mouse plasma. No attempts were made to further characterize this difference.

The spreading activity generated by complement activation remained unaltered after incubation of the activated plasma for 3 h at 37°C or several days at 0°C.

Experiments were performed to exclude the influence of particulate complement activators per se, such as zymosan and inulin, on the spreading process. For this purpose cover slips containing attached cells were inverted over 2 × 10-mm lucite chambers. These already contained kaolin-treated plasma and the complement activators which settled to the bottom and were not in direct contact with the macrophages. The results were identical to experiments in which the complement activators were removed from preincubated plasma and in both instances rapid spreading ensued.

The lucite chamber also allowed the use of sheep erythrocytes coated with IgM antibodies to activate the serum and induce spreading, demonstrating that the phenomenon could also be elicited by the classical pathway of complement fixation. 46% of the macrophages were spread after 1 h when 0.2 ml of AKR serum pretreated with kaolin and 10^8 E(IgM) were added to the chambers.

Kinetics and Morphology. In both the glass-dependent and the complement-dependent spreading activities, the number of macrophages spread were directly related to the concentration of plasma or serum and the time of incubation. The curves obtained with plasma and macrophages cultivated on glass surface are shown in Figs. 2 and 3. After a 1-h incubation a plateau is reached with plasma concentrations above 30%. With 50% plasma, almost all the macrophages in the preparation are spread by 3 h.

The morphological appearance of the spread cells is similar in both the glass-dependent and the complement-dependent systems. Fig. 4 shows phase contrast microphotographs of macrophages fixed with glutaraldehyde at several time points during the spreading reaction. Initially the cells are round and show very little activity until they project a unipolar wedge-shaped segment of membrane. This apron extends smoothly, and many ruffles are observed at the edges. While the cells occupy the maximal surface area, the projected veil contains no visible organelles. After 2–3 h, the macrophages acquire a more elongated shape. Intracellular structures such as mitochondria are then redistributed throughout the entire cytoplasm. As previously mentioned, this sequence is similar in both the glass-dependent and in the complement-dependent spreading. On several occasions, when CVF was used to activate complement, spike-like projections could be seen at the margins of the membrane.

The spreading phenomenon could also be observed with live macrophages plated on glass cover slips exposed to fresh plasma and maintained in an enclosed chamber at 37°C. Undulating ruffles arose from the central cell body moving toward the cell periphery, whereas pinocytic vesicles moved centripetally towards the nuclear region. No significant translational movement of the macrophages was seen during the 3-h experiments.

The substrate surface area occupied by individual macrophages changed substantially as the spreading reaction proceeded (Fig. 5). It increased rapidly during the 1st h, reached a plateau between 60 and 120 min, and was then reduced as the macrophages assumed an elongated shape. At maximal spreading, the area occupied by the cells was approximately eight times larger than that occupied by the unspread macrophages.

Attachment and Spreading. Preattachment of the macrophages to a solid substrate was required for the spreading reaction to occur.

Plasma, plasma containing ellagic acid (5×10^{-5} M), or kaolin-treated plasma containing CVF (20 U/ml) were added to normal mouse peritoneal cells in suspension for 1 h at 37°C. The cells were fixed by the addition of 2.5% glutaraldehyde and examined by phase contrast microscopy. No morphological alteration could be detected. In other experiments, the peritoneal exudate cell suspensions exposed to the various plasma preparations were washed and placed on glass cover slips for 60 min at 37°C in the presence or in the absence of 10% fetal calf serum. No spreading was observed.

Celite, Kaolin, and Complement Fixation. The ability of kaolin and celite to remove complement components and influence activation was next evaluated. For this purpose, the solubilization of immune complexes by serum was employed as an assay (9). Fig. 6 demonstrates that kaolin- or celite-treated sera exhibit only a slight delay in the initial rate of complex solubilization. Addi-

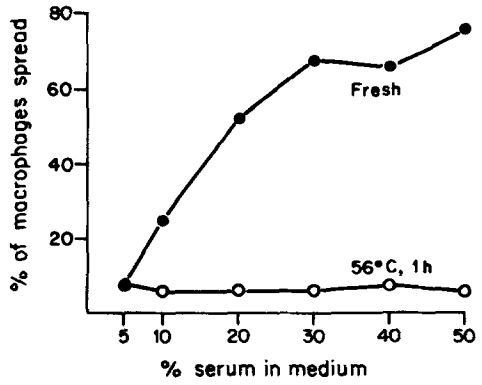

FIG. 2. Concentration dependence of the serum-spreading activity. Serum was added to the macrophage monolayers on glass cover slips for 1 h. Fresh (●—●) and heat-inactivated sera (○—○) compared.

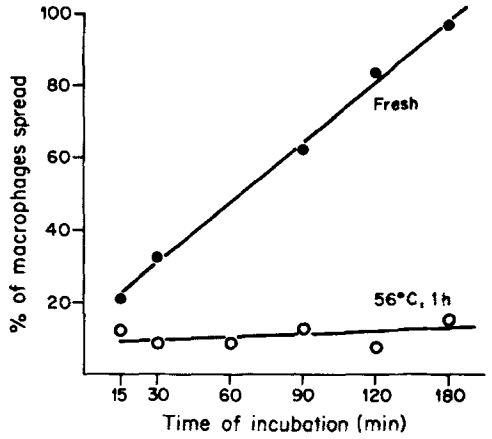

FIG. 3. Time-course of the glass-dependent spreading. 30% plasma in medium was added to macrophage monolayers on glass cover slips. Both fresh plasma (●—●) and plasma that had been heat inactivated (○—○) were used.

tional experiments carried out for a prolonged time in the presence of excess immune complexes showed only a 10–15% reduction in complexes solubilized when compared to untreated sera. Therefore, celite and kaolin treatment of sera has only a slight effect on the generation of complement-dependent spreading and immune complex solubilization.

Proteases and Protease Inhibitors. Rabinovitch (15) had previously shown that proteases such as trypsin were able to produce rapid spreading of freshly explanted macrophages. This finding, as well as the possibility that proteases were being generated in activated plasma, prompted us to examine the role of protease inhibitors and naturally occurring enzymes. As shown in Table IV, soybean trypsin inhibitor and lima bean trypsin inhibitor blocked the glass-induced spreading activity without affecting the CVF-induced activity. These agents clearly dissociated the two spreading pathways and suggested the importance of a protease in the former system.

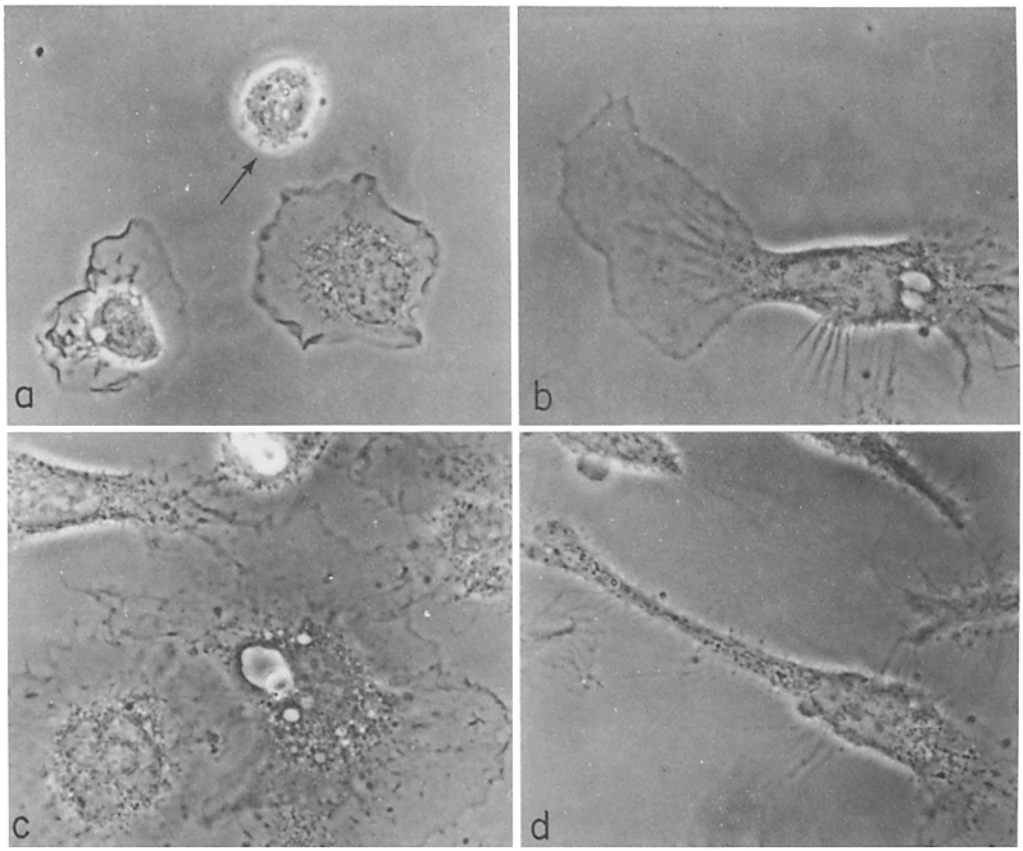

FIG. 4. Macrophage monolayers spread after exposure to fresh plasma and CVF at 37°C for (a) 15 min, (b) 30 min, (c) 60 min, and (d) 180 min. The predominant morphological appearance of the unspread cells at time 0 is that of the cell indicated by an arrow in (a). The preparations were fixed with glutaraldehyde and examined under phase contrast. Original magnification 1,000 ×.

The influence of proteases on macrophage spreading in the presence and absence of plasma is seen in Table V. The addition of urokinase to plasma fails to induce spreading, probably because the plasmin generated is controlled by natural inhibitors. However, if additional plasminogen is added to the system spreading takes place. Similarly, large amounts of trypsin induce spreading, whereas thrombin does not have an appreciable effect. In each instance in which activity occurred, its action could be blocked by soybean trypsin inhibitor.

Molecular Size of the Inducers of Complement-Dependent Spreading. The spreading activity generated by CVF added to plasma was stable in the cold for several days allowing its partial characterization. Fresh plasma was incubated with CVF and then studied by dialysis and gel filtration.

After extensive dialysis, the CVF-induced activity was lost, while the celite-depletable activity was retained inside the dialysis bag. When dialysis was carried out against a small volume of fluid, substantial activity could be recovered in the dialysate from the CVF-treated plasma.

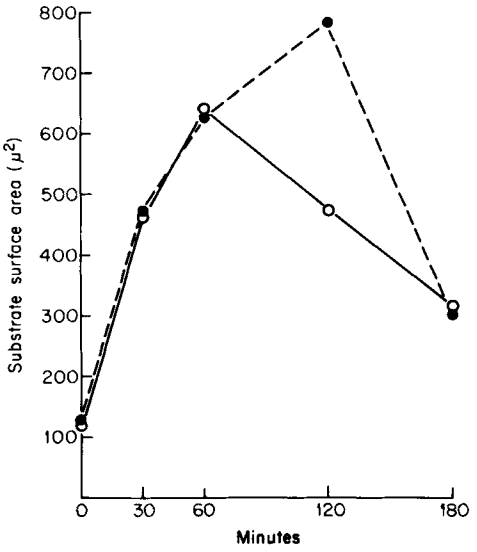

FIG. 5. The influence of plasma on the surface area occupied by macrophages. (●---●) 50% fresh plasma, (O---O) plasma preincubated with celite and then zymosan. Each point is the average area of at least 20 cells determined as described in the Materials and Methods section.

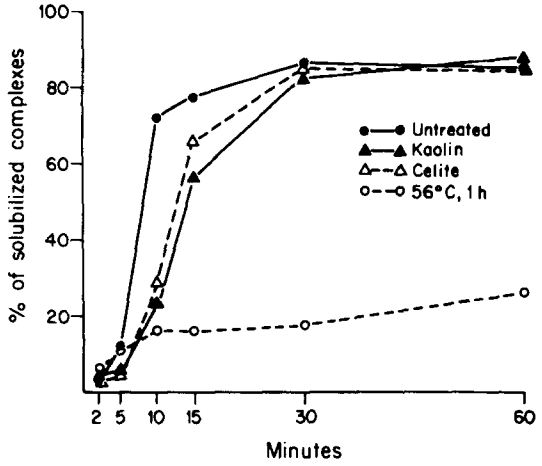

FIG. 6. Effect of kaolin and celite treatment on mouse complement function. Kinetics of the complement-dependent solubilization of ^{125}I-BSA-anti-BSA (bovine serum albumin) immune precipitates. The ordinate represents the percent of the counts per minute in the precipitate released in soluble form after incubation with the mouse serum preparation. The method is described in reference 8.

The size of the active molecules generated by complement activation was also studied by gel filtration of CVF-treated serum on Sephadex G-50. The included fraction induced the spreading of 72% of the macrophages plated on plastic, while the excluded fraction induced only 12% spreading. The activity in the included fraction was eluted after bovine serum albumin and ovalbumin which were used as markers.

TABLE IV
Effect of Protease Inhibitors on Spreading of Macrophages Cultivated on Glass

Plasma* pretreatment	CVF	Percent spreading‡ in presence of		
		No inhibitor	Soybean trypsin inhibitor (1 mg/ml)	Lima bean trypsin inhibitor (500 μg/ml)
None	Not added	85	3	24
None	Added	92	97	89
Celite	Not added	6	10	9
Celite	Added	97	94	96
56°C, 1 h	Not added	7	5	10
56°C, 1 h	Added	8	9	11

* Plasma collected with heparin, 10 U/ml, and diluted to 50% with culture medium.
‡ Percentage of macrophages spread determined after 1 h of incubation at 37°C.

TABLE V
Macrophage Spreading Induced By Proteases

Enzyme added*	Percent of macrophages spread‡ in the presence of			
	Medium		50% plasma	
	None	STI§	None	STI
Urokinase, 0.5 U/ml	2	4	0	0
Plasminogen, 0.5 U/ml	2	3	7	1
Urokinase (0.5 U/ml) + plasminogen (0.5 U/ml)	57	11	39	16
Trypsin, 200 μg/ml	79	19	47	22
Thrombin, 2 U/ml	6	3	6	0

* Enzymes were added to monolayers immediately after the addition of medium or plasma.
‡ Macrophages were cultivated on a plastic surface. The percentage of cells spread was determined after 1 h at 37°C. The background spreading in the absence of enzyme was subtracted from the data for each group. It varied from 12 to 21%.
§ Soybean trypsin inhibitor (STI) (1 mg/ml) was added before the addition of the enzyme.

Discussion

The experiments above show that rapid macrophage spreading can be induced by the activation of two distinct pathways in plasma. One of the pathways is initiated by contact of the plasma with a glass surface and the other by activation of the complement system. The glass-dependent spreading activity has characteristics that can be attributed to the activation of the contact phase of the intrinsic system of blood coagulation (reviewed in reference 16). It can be efficiently initiated by glass surfaces and by ellagic acid, but not by plastic

surfaces. Furthermore, the spreading activity can be aborted by pretreatment of the plasma with kaolin or celite. Celite and kaolin are known to bind and activate Hageman factor (Factor XII) of the coagulation system (17) as well as Factor XI, Fletcher factor or prekallikrein (18), and Fitzgerald factor or high molecular weight kininogen (19, 20). The activation of Hageman factor initiates a complex set of enzymatic reactions and feedback mechanisms which culminate with the generation of several serine proteases from their zymogens, among them plasmin (reviewed in references 21 and 22). The half-life of these enzymes is short due to protease inhibitors present in large amounts in normal plasma, among them α_2-macroglobulin and α_1-antitrypsin (reviewed in 23). The fact that soybean trypsin inhibitor inhibited the glass-induced spreading activity suggests that these proteases play a role in the spreading phenomenon. Actually, macrophages show a peculiar response when exposed to proteases. While most cells cultivated as monolayers round off and detach upon treatment with trypsin, macrophages quickly spread and remain adherent to the surface (5). The addition of urokinase and plasminogen to kaolin-treated plasma induced spreading supporting the possibility that the proteases may act directly on the plasma membrane of the macrophage. The high efficiency of plasma in the glass-dependent spreading despite the presence of large amounts of protease inhibitors is probably consequent to the proximity between the activating substrate and the cell surface.

The later components of the coagulation system such as thrombin and fibrinogen do not seem to participate in spreading. Thrombin does not induce spreading by itself. On the other hand serum, which has lost most of its fibrinogen, is almost as efficient as plasma.

The independence between the glass-initiated and the complement-induced spreading was demonstrated by (*a*) the fact that kaolin and soybean trypsin inhibitor completely blocked the glass-dependent spreading without affecting the complement-dependent spreading, and (*b*) by the depletion of small molecular weight plasma components by chromatography in Sephadex G-50 which prevented only the complement-mediated spreading. The small molecular weight product which mediates the complement-dependent spreading awaits further characterization. It should be stressed that even if the initiation of the two spreading pathways is certainly independent, the possibility still remains that the actual effector molecule which induces spreading is the same in both systems. The activation pathways of the complement and coagulation systems may superimpose. Plasmin, for instance, has been reported to cleave C3 and generate C3a (24).

At present there are no indications that "de novo" synthesis of membrane components is required for the spreading to occur. The unspread cells seem to have a large reserve of membrane in folds and ridges which could account for the surface of the spread cells. Furthermore, in some situations macrophages can be made to round up and spread in cycles of only a few minutes, indicating an ability to rapidly reorganize their membranes (25). Microfilaments, microtubules, and contractile proteins may all be involved in the spreading process and the subsequent movement of organelles into cytoplasmic veils. In this regard, macrophages attached to glass and plastic substrates show a rich array of fibrils

beneath the plasma membrane of the dish surface (26), and both cytochalasin B and colchicine inhibit the spreading reaction (unpublished observation). It should also be mentioned that proteases acting on plasma membrane components may modify the contact points of the fibrillar system with the inner surface of the plasma membrane (27).

The substrate on which the cells are plated plays at least two well-defined roles. First, it provides the matrix upon which the cells can adhere and spread, and second it can activate either the complement or coagulation systems. This matrix may include agents such as immune complexes for which the macrophage has plasma membrane receptors. A sequential interaction of membrane receptors with substrate ligands would lead to rapid spreading as described by Griffin et al. for phagocytosis (28). In fact, macrophages plated on a surface coated with immune complexes spread rapidly (29).

Many of the substances known to induce macrophage activation are able to activate Hageman factor and/or the alternative pathway of complement fixation under in vivo conditions. Consequently, the characteristics of macrophages obtained from animals that received these irritants may reflect the prolonged interaction of resident and/or emigrating cells with polypeptides generated during activation of these systems. Such plasma-derived enzymes and polypeptides may influence mononuclear phagocytes not only in terms of spreading but in other sequelae of activation such as enhanced endocytosis and secretion.

Summary

Unstimulated mouse peritoneal macrophages, attached to either glass or plastic substrates, responded to factors generated in serum and plasma by spreading and increasing their apparent surface area up to eightfold. Two distinct and dissociable systems were involved. The first appears related to the contact phase of blood coagulation. It is activated by glass and not plastic surfaces, depleted by kaolin adsorption, and inhibited by soybean trypsin inhibitor. In contrast, a separate complement-dependent system can be generated in kaolin-adsorbed plasma. Activation of the complement system can occur either by the alternate or classical pathways and generates a relatively small effector molecule which is dialyzable. These factors presumably influencing the surface membrane and underlying structures may explain the rapid spreading of activated macrophages observed after both infections and chemical peritoneal inflammatory agents.

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