HUMAN MONOCYTES ARE ASSOCIATED WITH THE FORMATION OF FIBRIN*

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The complexity of the list of enzymes, factors, and receptors synthesized by mononuclear phagocytes suggests that they perform many functions in vivo. Recently monoclonal antibodies (mAb)[1] have been described which, when compared with other hemopoetically derived cells, recognize specific antigens on human or murine phagocytes (1–4). Although there is as yet little information about the cellular functions in which the recognized antigenic moieties might be engaged, their expression on monocytes and/or macrophages suggests that they participate in hitherto unrecognized activities of phagocytes.

One of these monocyte antigens recognized by the mAb, UC45 (2), is the topic of the present report. It will be shown that the antigen is well known, namely fibrin. There have been a number of reports linking immune responses and lymphoid cells with coagulation (5, 6). In particular, monocytes, when exposed to activated lymphocytes or their products in vitro, are able to express a procoagulant activity (7–10). Monocytes are known to bear receptors for fibrin and fibrinogen (11, 12); moreover, macrophages recovered from guinea pigs undergoing delayed-type hypersensitivity reactions were covered with fibrin (13). This present report establishes that a subpopulation of human monocytes, when exposed to plasma, are able to form on their surfaces needle-like structures of fibrin. These needles have unique antigenic properties detected by the mAb UC45, which reacts with the α chain of fibrin as it is found on monocytes, but very poorly with the amorphous network of the standard fibrin clot.

Materials and Methods

Human Monocytes and Plasma. Human monocytes from normal donors were prepared from peripheral blood mononuclear cells after Ficoll-Hypaque density ($P = 1.09$) centrifugation and subsequent adherence to petri dishes coated with microexudate from BHK cells (2, 14). After removal from the BHK microexudate with 3 mM EDTA and centrifugation, the cells were resuspended in Dulbecco's modified Eagle's medium containing 5% fetal calf serum and allowed to adhere to 13-mm round glass coverslips, usually for an overnight period unless otherwise specified. These cells were >99% monocytes as assessed by generalized cytoplasmic staining for α-naphthyl acetate esterase (NSE) enzyme (15).

The plasma used in the majority of the reported experiments consisted of the top layer of the Ficoll-Hypaque gradient used to fractionate heparinized or citrated blood. The plasma was

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[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; Fl GaRIg, fluorescein-conjugated goat anti-rabbit immunoglobulin; huF, human fibrinogen; kd, kilodalton; mAb, monoclonal antibody; NSE, nonspecific esterase; PBS-A, phosphate-buffered saline, pH 7.4, minus Ca^{+2}, Mg^{+2}; Rd GaMIg, rhodamine-conjugated goat anti-mouse immunoglobulin; SDS, sodium dodecyl sulfate.

thus diluted ~1:2 and was used within a few days after preparation. To induce fibers on the monocytes, coverslips were incubated for 10 min at 37°C with 25 or 50 μl of plasma. The coverslips were then washed thoroughly in phosphate-buffered saline, pH 7.4, minus Ca^{+2}, Mg^{+2} (PBS-A) before further treatment.

Tissue Macrophages. Lung macrophages from the uninvolved lungs of two Hodgkin's disease patients were isolated by adherence to microexudate coated plates (2, 14). These samples were a gift from Dr. John Habeshaw, St. Bartholemew's Hospital, London. In a similar manner, macrophages were prepared from three samples of breast milk (University College Hospital). Thymus tissue from two cardiac surgery patients (Institute of Child Health, London) and three pairs of tonsils (University College Hospital) were extensively teased, washed, and the macrophages isolated as above.

Antisera and Other Reagents. UC45 hybridoma tissue culture medium was used except where otherwise noted. As UC45 is an IgM antibody, two other IgM mAb were used as controls. IIC3, which has specificity for the leech nervous system, was obtained from Dr. Birgit Zipser (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY) and T13, which reacts with human T cells, was obtained from Dr. Peter Beverley (UCH Medical School, London). A fourth IgM mAb, J15, specifically reactive with platelets, was obtained from Dr. Andrew McMichael (John Radcliffe Hospital, Oxford). Unless otherwise specified, all mouse mAb were used undiluted in the form of tissue culture medium from the respective hybridoma cell lines. A rabbit antibody to human fibrinogen was obtained from Dr. Patrick Gaffney (Institute for Biological Standards, London). It was used at a dilution of 1:300. Normal rabbit serum (1:300) was used as a control. Human fibrinogen (huF) was obtained from Miles Laboratories, Elkhart, IN (code 82-311, lot A0.21). Human fibrin was obtained from the plasma of a single individual (MF) by freezing, then thawing partially clotted plasma. The resulting flocculant material was washed extensively in PBS-A and used as a source of fibrin after analysis by sodium dodecyl sulfate (SDS) gel electrophoresis. This procedure was adapted from a simple method for fibrinogen purification (17).

Indirect Immunofluorescence. In general, monocytes bearing fibers were tested as fixed preparations. Thus, coverslips were fixed for 5 min in 3.7% formaldehyde in PBS-A, rinsed in PBS-A and water, and extracted with acetone at −20°C for 5 min. After further rinsing, 20 μl of the required antibody was added to the coverslip for 20 min at 37°C. After washing three times in PBS-A, 20 μl of the second antibody, either rhodamine-conjugated goat anti-mouse immunoglobin (Rd GaMIg) or fluorescein-conjugated goat anti-rabbit immunoglobulin (Fl GaRIg) (N. L. Cappel Laboratories, Cochranville, PA) was added and a second incubation carried out for 20 min at 37°C. Both "second layer" antibodies were used at a dilution of 1:20. After three further washes, the coverslips were inverted over a drop of Gelvatol (Monsanto Co., St. Louis, MO) and visualized by epifluorescence microscopy.

In "double labeling" experiments, the mouse monoclonal antibodies were added either together with the rabbit anti-huF antibody or normal rabbit serum or mouse, and rabbit antibodies were added consecutively with a 20-min incubation for each antibody. Controls were done to make certain that neither Rd GaMIg or Fl GaRIg reacted with the inappropriate immunoglobulin.

Preparation of Monocyte Fibers. Large coverslips (25-mm) containing ~2 × 10^5 monocytes were incubated with 200 μl of fresh plasma (1:2 dilution) at 37°C. An equal number of coverslips containing monocytes but no plasma and coverslips containing plasma but no monocytes served as controls. After 20 min, the coverslips were carefully washed three times in PBS-A and then gently scraped with a rubber policeman. The cell and fiber mixture was then centrifuged for 5 min at 80 g, which separated the fiber "nets" from cells and any remaining plasma proteins. The resulting pellets were dissolved in SDS gel sample buffer before storing at −20°C. The supernatants were also stored at −20°C.

Protein Transfer to Nitrocellulose Paper (Western Blotting). Samples of huF, fibrin, monocyte fibers, and a mock fiber preparation from monocytes without fibers were subjected to SDS gel electrophoresis, followed by transfer to nitrocellulose paper (0.45-μm pore size; Millipore Corp., Bedford, MA) following the procedures described by Towbin et al. (18). One nitrocellulose strip was immediately stained with amido black and the others were incubated for 2 h in 3% bovine serum albumin (BSA)/PBS-A. These nitrocellulose strips were then incubated for 1 h at

room temperature with 2 ml of either diluted tissue culture medium containing mAb UC45 (1:5), mAb 11C3 (1:5), rabbit anti-F serum (1:20), or normal rabbit serum (1:20). The dilutions were done in 3% BSA. The strips were then washed four times over the course of one hour with PBS-A and were incubated for a second hour in either ^{125}I-goat anti-mouse immunoglobulin antibody or ^{125}I-goat anti-rabbit immunoglobulin antibody, kindly provided by Dr. Jim Lin and Dr. Fumio Matsumura (Cold Spring Harbor Laboratory). Both iodinated antibodies were used at ~2 × 10^6 cpm/ml of 3% BSA in PBS-A. The strips were finally washed in PBS-A five times over the course of several hours. The strips were blotted dry and analyzed by autoradiography.

Results

Plasma Induces Production of Fibers around Monocytes.   In a previous report (2), the IgM mAb UC45 was shown to react with needle-like projections found on a proportion of peripheral blood mononuclear cells which adhered to glass. These cells were identified as NSE-positive monocytes. In the present study it was found that when monocytes were purified by fractionation on microexudate-coated petri dishes (14), the fibers were removed from the cells. If these purified monocytes (>99% NSE) were allowed to readhere to glass and were then treated with human plasma, then needles or fibers were formed on a proportion of the cells. These are shown in Fig. 1 A, and the reaction of the fibers with UC45 is shown in Fig. 1 B. Reaction with a control IgM antibody, 11C3, was negative. The percentage of fiber-making monocytes ranged from 8 to 25% with an average of 18 ± 8% using cells obtained from 18 infrequently bled donors. These percentages were obtained from monocytes kept in culture overnight before treating with plasma. If the monocytes were treated within 15 min after isolation, usually 1-5% of the cells made fibers. 4 h of tissue culture, however, increased the percentage to a maximum. A similar percentage of monocytes kept in suspension in polycarbonate tubes also made fibers after 4 h at 37°C, indicating that adherence to a substrate was not necessary. Samples kept at 4°C did not make fibers.

In our previous study, 80% of monocytes were found to be UC45 positive (2). Cells were obtained from a group of frequently bled donors (50–100 ml/wk). It is of interest to know whether the increase in proportion of monocytes making fibers is related to the frequent phlebotomy.

Monocyte Fibers Labeled with Antibody to Fibrinogen.   Fiber-bearing monocytes were tested with a rabbit antibody to huF (dilution 1:300) and a positive reaction was obtained. Cultures of fixed monocyte fibers are shown simultaneously labeled with both UC45 (Fig. 2 A) and rabbit antibody to huF (Fig. 2 B). The two antibodies coincidentally label the monocyte fibers, strongly indicating that fibrinogen-related molecules form at least part of the monocyte fibrous needle structure.

To determine whether the huF and UC45 antigens might be present on the same molecule or at least spatially close on the fiber structure, an antibody competition experiment was done in which an attempt was made to block the binding of UC45 by rabbit anti-huF serum. Preincubation of fibers with the rabbit antiserum ranging in dilution from 1:25 to 1:300 did not alter the fluorescence intensity of UC45 staining. This suggested that the UC45 and huF antigens were either on different molecules, well separated on the same molecule, or that the rabbit anti-huF antibody did not contain specificities directed toward the UC45 antigen.

Fig. 3 presents a comparison of the binding to conventional plasma fibrin of rabbit anti-huF serum UC45 and control antibodies. The fibrin was formed by incubating
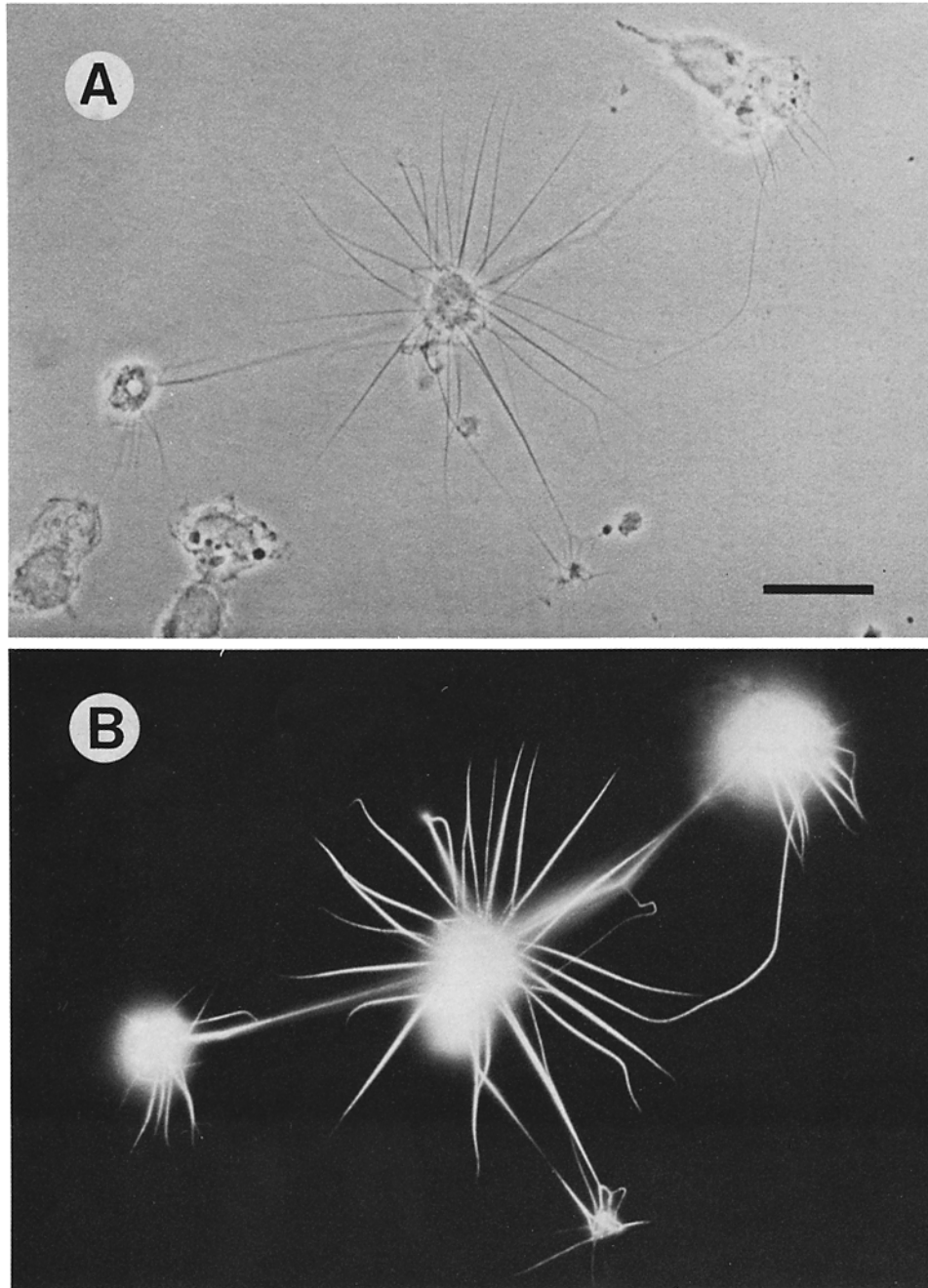
FIG. 1.   A human monocyte displaying needle-like projections formed after a 10-min incubation at 37°C with 25 μl fresh human plasma (1:2) shown by phase microscopy (A) and fluorescence microscopy (B) after reaction with monoclonal antibody UC45. Bar = 10 μm.
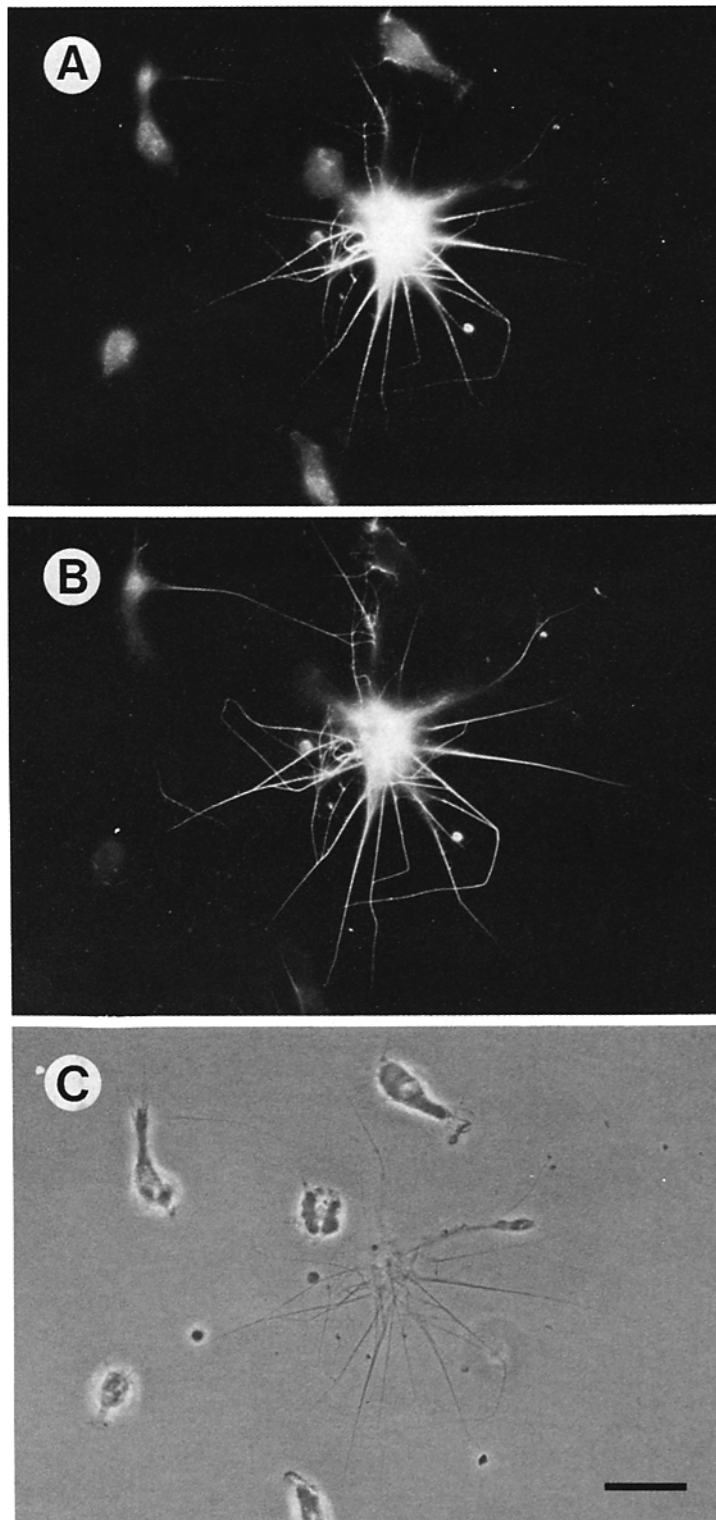
FIG. 2. A prefixed sample of human monocytes double labeled with rhodamine-positive UC45 monoclonal antibody (A) and fluorescein-positive rabbit anti-fibrinogen serum (B), phase microscopy (C). Note the complete overlap in rhodamine- and fluorescein-positive labeling of figures. Bar = 10 μm.
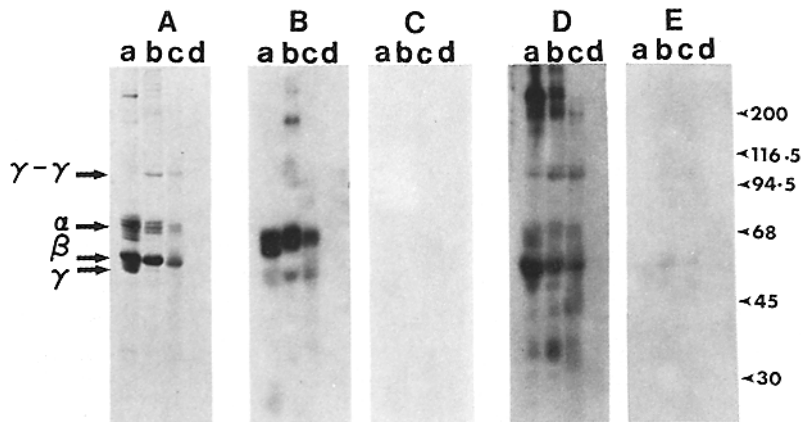
FIG. 3.   Reaction of conventional plasma fibrin with rabbit anti-fibrinogen serum and UC45. A phase micrograph of fibrin (A) labeled separately with rabbit anti-fibrinogen serum (1:300) (B), and normal rabbit serum control (1:300) (C) fibrin (D) was reacted with UC45 (E) or control monoclonal antibody, 11C3 (F) with both reactions detected by Rd GaMIg B and C were recorded with a 2-s exposure, and E and F with an 8-s exposure of Ektachrome, ASA 400 film (Eastman Kodak Co., Rochester, NY). Bar = 10 µm. detected by rhodamine-conjugated goat anti-rabbit immunoglobulin; second,

Fig. 4. Proteins reacting with UC45 and rabbit anti-fibrinogen serum after transfer from 10% SDS acrylamide gel to nitrocellulose paper (18). Samples of human fibrinogen (a), fibrin (b), monocyte fibers (c), and a mock fiber preparation from monocytes with no fibers (d) are shown, stained with amido black (A), reacted with UC45 (B), 11C3 (C), rabbit anti-fibrinogen serum (D), and normal rabbit serum (E). The molecular weight markers used were: myosin heavy chain, 200 kd; β-galactosidase, 116.5 kd; phosphorylase a, 94.5 kd; bovine serum albumin, 68 kd; ovalbumin, 45 kd; carbonic anhydrase, 30 kd. Bar = 10 μm.

50 μl of citrated plasma with 2.5 U bovine thrombin for 10 min at 37°C. Whereas rabbit anti-huF reacts strongly with fibrin (Fig. 3 B), the UC45 reaction (Fig. 3 E) is extremely weak. Fibrin formed by incubation of heparinized plasma with reptilase gave similar results. Thus, the antigen detected by mAb UC45 is either weakly represented or inaccessible on conventional fibrin.

UC45 Reacts with the α Chain of Fibrinogen and Fibrin.   In Fig. 4, samples of huF (a), fibrin (b), isolated monocyte fibers (c), and a mock fiber preparation from monocytes with no fibers (d) were reacted with UC45 (B), rabbit anti-huF serum (D), and control antibodies (C, E) by the protein or Western blotting technique as described by Towbin et al. (18). First, it is evident that the monocyte fiber pattern is almost identical to that of fibrin. Second, UC45 reacts very strongly with the α-chain banding pattern of huF, fibrin, and monocyte fibers. A second strongly staining band of 180 kilodaltons (kd) is seen in the fibrin sample (track b). This is in the correct position to be an α chain trimer (19). There is more faintly UC45-reactive material in the β and γ chain regions. The control IgM mAb, 11C3, gives no reaction. The pattern of reactivity with rabbit anti-F serum shows labeling of α, β, and γ chains and γ chain dimers (100 kd), which are characteristic of fibrin (19) and are most strongly labeled in the fibrin and monocyte fiber tracks.

In a previous report, it was noted that UC45 reacted with a 45-kd protein (2), which would seem to be in conflict with the reaction of UC45 with the 66 kd Aα-chain of fibrin(ogen). The explanation is that, previously, the restricted activity of UC45 for monocytes (and neurons) was determined by using immunofluorescent techniques on intact viable cells. When cells were subsequently permeabilized, it was found that UC45 reacted with mitochondria of all cells tested (unpublished). Work is in progress to determine whether the previously detected 45-kd entity is a mitochondrial protein or an Aα-chain cleavage product.

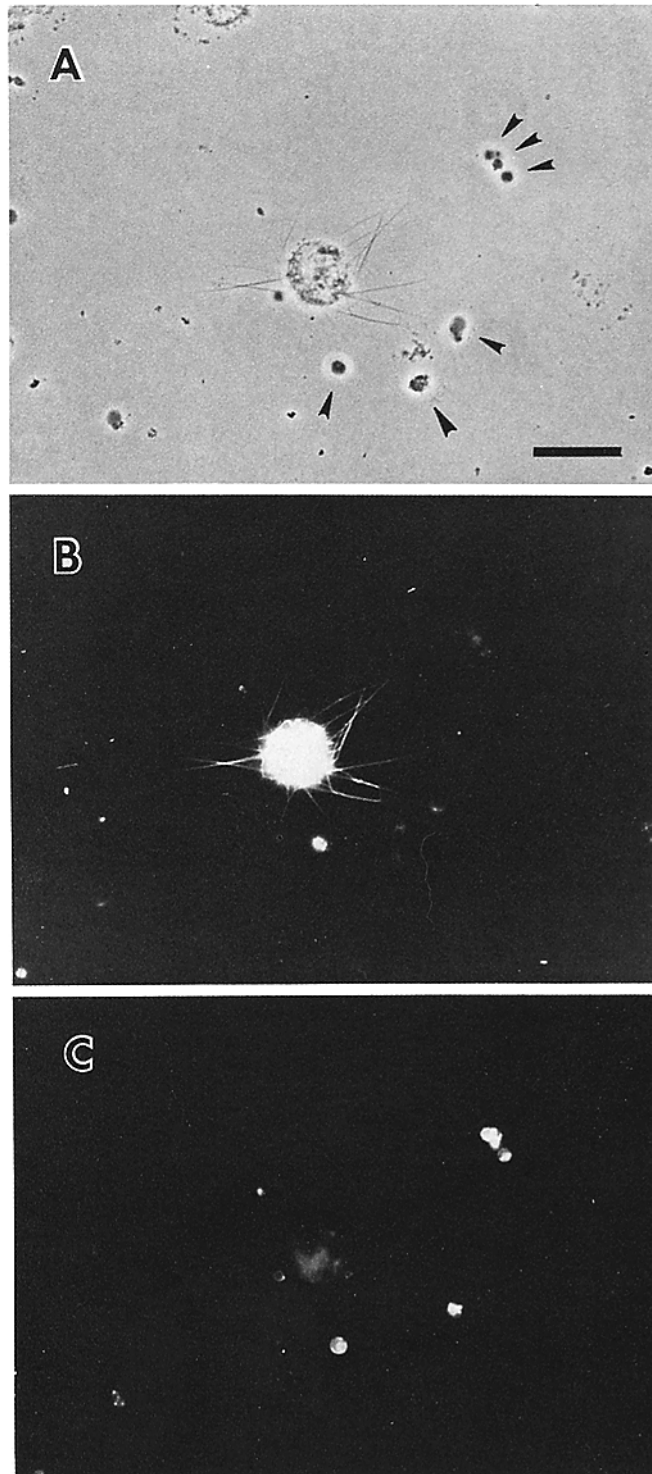*Blocking Fiber Production with UC45 Monoclonal Antibody.*   It was next asked whether

Fig. 5. A prefixed sample of monocytes and platelets (A) double labeled with fluorescein-positive rabbit anti-fibrinogen serum (B) and rhodamine-positive monoclonal antibody, J15, which specifically reacts with platelets (C). See arrows. Bar = 10 μm.

incubation of monocytes or plasma with UC45 would have an effect on the ability of monocytes to make fibers. Either T13 ascitic fluid or UC45 ascitic fluid was preincubated with plasma before addition to monocytes. Fiber production was assessed by labeling the cells with UC45 or rabbit anti-huF serum. Preincubation with T13 ascitic fluid gave the usual fiber pattern, whereas preincubation with UC45 ascitic fluid completely blocked fiber formation (data not shown). The reverse experiment in which monocytes were preincubated with UC45 or T13 ascitic fluid had no effect on fibrin formation. This result suggests that the UC45 antigen plays some role in the polymerization of fibrinogen by monocytes rather than acting at a site on the monocyte membrane.

Although the UC45 antigen was not easily detected on extracellular fibrin (Fig. 3 E), it was of interest to know whether the UC45 mAb could also affect the polymerization of this type of fibrin as assessed by the thrombin-time test. Accordingly, experiments were done parallel to the monocyte-blocking experiments using the same plasmas. The effect on the thrombin time was noted after addition of 0.1 ml saline containing 5 U of bovine thrombin (Diagnostic Reagents Ltd., Thame, England) to 0.2 ml of mAb-treated plasma. When the incubation contained 50 μl of T13, opacity began at 17 s, clot formation at 19 s, and retraction by 24 s. These times were comparable to a reaction to which no antibody had been added. When 50 μl UC45 was contained in the reaction, opacity began at 10 s, clot formation between 16 and 22 s, and retraction by 32 s. A similar time sequence of clotting was seen in seven similar experiments that included the treatment of heparinized plasma with reptilase. Therefore, although UC45 did perturb clot formation through initially hastening then protracting the process, it did not prevent it from occurring.

Platelets Play No Role in Fiber Formation. It was posible that platelets were associated with the formation of fibrin on the monocytes. That they were not involved in monocyte fiber formation was determined in the following way. First, plasma spun at 14,000 g to pellet platelets still readily caused fiber formation. Second, platelets adhering to monocytes are for the large part gone after an overnight incubation at a time when monocytes make fibers with facility. Third, the remaining platelets, detected with mAb J15 are scattered on the coverslips in a random fashion (Fig. 5 C) and are not associated in any significant way with the fiber-producing monocytes labeled with rabbit anti-huF serum (Fig. 5 B). Fig. 5 was selected for the unusually large number of platelets surrounding the monocyte (Fig. 5 A). More frequently, no platelets were seen in the vicinity of fiber-bearing monocytes.

Lack of Ability of Many Tissue Macrophages to Cause Fibrin Formation. Macrophages isolated from lung, thymus, and breast milk failed to cause fibrin needle formation when incubated with plasma for periods of time from 2 h to overnight in culture. In contrast, microexudate-adherent tonsil cells were active in fibrin formation. Extensive networks of UC45-positive fibrin enveloped the majority of tonsil cells that were 90% NSE positive. The morphology of the tonsil cells indicated that this population consisted of cells of monocyte size (31 ± 11%) as well as larger tissue macrophages (69 ± 11%). Because of the extensive nature of the UC45-positive network, it was difficult to be certain whether the fibrin formation initiated only from the smaller monocytic cells or whether the larger macrophages also participated. However, in a typical experiment, when plasma was added to the cells, 68 ± 1% of tonsil cells were also UC45 positive.

Discussion

The purpose of this study was to determine the antigenic and chemical nature of peculiar needle-like fibers that form around substrate-attached human monocytes in culture when they are exposed to plasma for as short a time as 15 s (Fig. 1). Several lines of evidence indicate that the major component of these needles is fibrin and that the UC45 mAb reacts with the α chain of this monocyte fibrin. However, there are a number of observations that suggest that the fibrin comprising the monocyte needles may differ from that found in the conventional fibrin clot. First, fibrin clots label very poorly with UC45 but very well with rabbit anti-huF serum (Fig. 3), suggesting that the UC45 antigen which is prominently displayed on monocyte fibrin is weakly expressed or inaccessible on fibrin clots. Furthermore, there was no competition by the polyclonal rabbit anti-huF serum for binding to the antigenic site recognized by the monoclonal antibody, UC45, as would be expected if both reacted with the same molecular species. This would suggest that the UC45 antigen on monocyte fibers, which was obviously immunogenic in the mouse, was either not immunogenic or not prominently expressed on human huF against which the rabbit antiserum was raised. These observations strongly indicate that the UC45 antigen present on α chain may be characteristic of the special needle-like fibrin formed by monocytes and only sparsely represented or inaccessible on extracellular fibrin and nonpolymerized huF. The fact that UC45 reacts with denatured Aα chain in the Western blotting procedure suggests that the antigen may be cryptic in native fibrinogen but exposed in an altered structure of fibrin in the monocyte needle.

There are several possible sources for the huF that is polymerized to form the fibrin needles. Macrophages bear receptors for huF and fibrin (11, 12), which indicates that the binding of huF from plasma to these receptors might initiate the needle-making process. However, one might speculate that macrophages themselves contain huF perhaps in granule form, as do platelets, which are present in plasma unless removed. Platelets, which have a crucial role in vascular clotting, bind huF when they are activated, contain huF in α granules, and adhere to freshly isolated monocytes. It was therefore possible that platelets bound to monocytes were responsible for the reported observations. This seems unlikely because, at a time when monocytes very avidly made their needle-like fibrin, a mAb specific for platelets, J15, detected very few platelets associated with monocytes, either on them or nearby (Fig. 5), and platelet-free plasma induced macrophage fiber formation. That the huF did not come from the monocytes themselves but from plasma is suggested by the ability of UC45 to block fiber formation when co-incubated with plasma, but not when preincubated with monocytes alone. Moreover, after the monocyte isolation procedure, neither viable nor permeabilized cells demonstrated a reaction with rabbit anti-huF serum by immunofluorescent techniques (data not shown). The blocking experiment also suggests that the UC45 antigen plays a role in the polymerization resulting in the monocyte needles. Although the antigen is poorly detected on conventional plasma fibrin, it was of interest to observe that, at the highest concentrations of antibody, the thrombin and reptilase times were prolonged, but clot formation was not prevented, again suggesting that the antigen plays a special role in monocyte fibrin.

There is heterogeneity amongst monocytes in their ability to make fibrin needles. In the present study, in which cells were obtained from 18 infrequently bled donors,

the percentage of fibrin-making monocytes ranged from 8 to 25%, with an average of 18 ± 8%. Although a proportion of the monocytes were able to make fibrin as soon as they were put into culture, the final percentage was reached between 4 and 6 h of culture and then did not alter. This did not depend on the adherence of monocytes to a substrate as cells maintained in suspension were also able to make fibrin. Thus it appears that a distinct subpopulation of monocytes has the ability to form fibrin on their membranes. Whether the same monocytes and macrophages also produce plasminogen activator, which leads to fibrinolysis, is unknown. It could be speculated that this subpopulation of fibrin-producing monocytes is detected because it lacks plasminogen activator and is thus able to retain fibrin, whereas the majority of monocytes may have it removed immediately.

Although two other studies have reported (13, 20), using immunofluorescent techniques, the presence of fibrin on phagocytes, the more widely observed phenomenon has been the induction of a procoagulant activity in phagocytes by exposure to activated lymphocytes and their products (7–10). Although this question was not addressed in this study, there was sufficient opportunity for interaction between monocytes and lymphocytes during the purification procedure, which included a 30-min incubation at 37°C. Procoagulant activity is also increased after 4–6 h of culture and has been shown to be dependent on new RNA and protein synthesis (21). This coincidence of timing in activation of procoagulant activity, thought to be tissue factor, the first event of the extrinsic pathway of coagulation, and the ability of monocytes to make fibrin, which is the final event of coagulation, suggest that the entire extrinsic pathway of coagulation is being activated in monocytes. None of the other factors involved in the extrinsic pathway, factors VII, X, V, or II, have yet been reported to be synthesized by human monocytes or macrophages, either before or after in vitro activation.

These observations raise the question of the possible role of coagulation in immune responses, particularly in tissue inflammation, where fibrin deposition is observed (5, 6). The evidence presented in this paper suggests that activated circulating monocytes are the responsible cells. More mature macrophages from lung, thymus, and breast milk did not have this capability, but a much higher proportion of phagocytes (70%) from inflamed tonsils were involved in fibrin formation. This may be the result of either a selective migration of cells with this capacity to the tonsil or in situ activation of circulating monocytes. A further observation linking fibrin formation with monocyte activation comes from the observation that acute monocytic leukemia cells, tested by immunofluorescence without any prior in vitro manipulation reacted with UC45 (22).

The in vivo effect of fibrin formation remains obscure. Hopper et al. (13) suggest that the induction of procoagulant activity is accompanied by a decrease in the ability of macrophages to respond to chemotactic agents. This would cause them to remain localized at the site of tissue damage, possibly phagocytosing debris. Furthermore, fibrin serves as a substrate for fibronectin, which precedes the appearance of collagen in the remodeling of loose connective tissue (23). Fibrin is covalently linked to fibronectin by factor XIII (24), and both fibronectin and factor XIII are synthesized by phagocytes (25, 26). This evidence suggests that the laying down of fibrin is the initiating event in tissue repair. Although the role of fibrin in in vivo activities other than blood clotting remain speculative, it is hoped that identification of the UC45

antigen will provide a means of assessing the importance of fibrin generated by monocytes in physiological events.

Summary

Purified populations of human monocytes (>99% monocytic) contain a subpopulation of cells (8–20%) that will surround themselves with a radial array of needles within 15 s after exposure to plasma. The needles are composed of fibrin as demonstrated by gel electrophoresis of isolated needles and by their reaction with a monoclonal antibody, UC45, that is specific for the α chain of fibrin. The addition of UC45 antibody to plasma inhibits the formation of the fibrin needles by monocytes, suggesting that the recognized antigen is important for polymerization. In contrast, UC45 binds weakly to conventional plasma fibrin and does not prevent clotting of plasma although the process is prolonged. Thus, the UC45 antigen is more prominently represented or more accessible on monocyte fibrin. At least 1% of the monocytes were able to form fibers at the earliest time that they could be tested. A greater proportion developed this ability between 2 and 6 h in culture. Macrophages from lung, thymus, and breast milk, when incubated for similar periods of time, do not form fibrin needles. However, phagocytes from tonsils were extremely active in this respect. It is speculated that monocytes may be induced to express fibrin as part of their activities in inflammatory lesions.

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