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Fibrinogen Enhances Complement-Mediated Augmentation of Retention of Polymorphonuclear Leukocytes by Nylon Columns

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Retention of polymorphonuclear leukocytes (PMN) by nylon columns is significantly increased when PMN are suspended in zymosan-activated plasma. In zymosan-activated plasma, 25.8 ± 4.9% of PMN were retained as compared with 11.1 ± 2.7% suspended in zymosan-activated serum (ZAS) ($P = 0.001$) and 7.9 ± 3.2% in fresh serum ($P = 0.0005$). Addition of heated plasma (56°C for 30 min) to ZAS restored optimal retention. These results suggested a role for a heat-stable factor(s) in plasma, which augmented complement-mediated enhancement of PMN adhesiveness. This study was designed to determine whether fibrinogen enhanced retention by nylon columns of PMN in the presence of activated complement. Addition of defibrinated plasma to ZAS failed to enhance adhesiveness to nylon. The retention of PMN suspended in ZAS was 11.1 ± 2.7%, as compared with 10.5 ± 3.7% in ZAS plus defibrinated plasma. Retention of PMN suspended in ZAS plus fibrinogen was 27.92 ± 13.2% as opposed to 16.35 ± 7.3% ($P = 0.045$) in ZAS. In contrast, retention of cells in serum was 12.96 ± 7.5%. These results suggest that the process which enhances PMN adhesiveness involves the interaction of cells, activated complement, and fibrinogen.

The adherence of polymorphonuclear leukocytes (PMN) to endothelial cells is required for the initiation of the inflammatory response. Several investigations of the role of plasma proteins (4), cellular elements (13), and divalent cations (4) upon the adhesion of PMN and endothelial cells, in vivo and in vitro, have been reported. Adherence of PMN to nylon has been demonstrated by MacGregor et al. to represent an in vitro correlate of adherence to endothelial cells (9). PMN retention by nylon columns and adherence to monolayers is increased when PMN are suspended in plasma obtained from patients with inflammation (8). Augmented retention of PMN by nylon columns (11, 12) and PMN aggregation (2, 12) occur when cells are suspended in media containing activated complement. In addition, previous reports suggest that a heat-stable plasma factor is required to augment retention of PMN by nylon columns (7, 8). This investigation describes the requirement for fibrinogen, in addition to activated complement, to induce maximal PMN retention by nylon columns.

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MATERIALS AND METHODS

PMN preparation. Blood for PMN was obtained from fully informed volunteers. It was drawn into sterile syringes containing sufficient heparin to produce a final concentration of 7.5 U/ml of blood. Heparinized blood was mixed with dextran (type 200, lot no. 41C-154; Sigma Chemical Co., St. Louis, Mo.) and 0.15% M NaCl, placed in sterile plastic tubes, and kept at room temperature (25°C) for 45 min during sedimentation of erythrocytes. The leukocyte-rich supernatant was centrifuged at 180 × g for 5 min. The cells were washed twice in Hanks balanced salt solution (HBSS) (GIBCO Laboratories, Grand Island, N.Y.) and layered onto Ficoll-Hypaque (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) gradients. After separation, the PMN were suspended in HBSS at concentrations of between 10,000 to 20,000 cells per mm^3. The suspension contained approximately 90% PMN.

Plasma preparation. Plasma prepared with heparin, ethylenediaminetetraacetic acid (EDTA; 1.5 mg/ml), and sodium citrate (3.8 mg/ml) was used immediately or frozen at −70°C until required. "Heated" plasma (HP) was prepared in sterile plastic tubes which were placed in a 56°C water bath for 30 min. To activate the alternate pathway of complement, fresh plasma was incubated with 5 mg of Zymosan A (lot no. 105C 0351, Sigma) per ml for 30 min at 37°C (10). Plasma was defibrinated by addition of Ancord (Abbott Laboratories, North Chicago, Ill.), 4

U/ml of plasma, derived from the venom of the Malayan pit viper (3).

Serum preparation. Serum was obtained from whole blood clotted at 37°C for 1 h and stored at −70°C if not used immediately. Serum was incubated with Zymosan A (5 mg/ml) to obtain zymosan-activated serum (ZAS).

Fibrinogen. Fibrinogen was prepared by the glycine precipitation method of Kazal et al. and was 94% thrombin-clottable protein (6). When reconstituted in 1 ml of serum, the concentration of fibrinogen was 300 to 340 mg/dl.

PMN retention by nylon columns. Adherence of PMN was assayed by the method of MacGregor et al. (8) as previously reported from this laboratory (11, 12). Nylon fiber columns, 15 mm in height and weighing 40 mg, were constructed in glass pipettes. PMN suspensions (1 ml) were pipetted onto three columns. Adherence was calculated by averaging the percentage of PMN retained in the three columns.

Statistical analysis. All results were compared by using the Student *t*-test.

RESULTS

Addition of zymosan-activated plasma (ZAP) to PMN in HBSS resulted in augmented retention of the PMN by nylon columns (Table 1). Retention was 25.8 ± 4.9% with ZAP in contrast to 11.1 ± 2.7% with ZAS and 7.9 ± 3.2% with fresh serum ($P = 0.001$ and 0.0005). Addition of defibrinated plasma to ZAS did not increase PMN retention by the nylon columns (10.5 ± 3.7%). However, retention of PMN suspended in ZAS plus HP was greater than retention of PMN suspended in ZAS alone (Table 2). The augmenting activity was present in HP prepared with EDTA or sodium citrate as well as with heparin. Addition of heat-inactivated serum to ZAS did not augment retention induced by ZAS (data not shown).

Addition of fibrinogen to ZAS augmented retention of PMN by the columns (Table 3). Although the retention of PMN suspended in ZAS was increased as compared with PMN suspended in serum, this increment was not significant ($P = 0.22$). With the addition of fibrinogen to PMN-ZAS suspensions, the retention was increased from 16.35 ± 7.3 to 27.92 ± 13.2% ($P = 0.045$). In control experiments not shown, addition of fibrinogen to fresh serum did not augment PMN adherence to nylon (11.03 ± 4.7 versus 11.23 ± 3.7%).

TABLE 1. *Retention of PMN by nylon columns*[a]

Cell suspension	% Retention ± SD	P value
Serum (3)	7.9 ± 3.2	
ZAS (3)	11.1 ± 2.7	0.001, 0.0005
ZAP (3)	25.8 ± 4.9	0.39
ZAS + DP (3)	10.5 ± 3.7	

[a] PMN separated on Ficoll-Hypaque gradients suspended in HBSS plus fresh serum, ZAS, ZAP, or ZAS plus defibrinated plasma (DP). SD, Standard deviation. Number of experiments within parentheses.

DISCUSSION

MacGregor noted augmentation of PMN retention by nylon columns when cells were suspended in plasma, but not serum, obtained from rabbits receiving intravenous endotoxin. Furthermore, augmented retention of PMN was induced with addition of heated rabbit plasma (56°C for 30 min) to guinea pig complement (8). In other experiments, plasma from patients with inflammatory disease augmented retention but serum did not. Addition of fibrinogen did not restore the augmenting activity of serum (7).

In this experiment, ZAS induced less augmentation of retention than did ZAP. Addition of HP, prepared by using any of three anticoagulants, but not defibrinated plasma, restored optimal retention of PMN suspended in ZAS. These results suggested a role for fibrinogen in the complement-mediated increase in PMN adhesiveness. The failure of heated serum to augment retention induced by ZAS indicated that the enhancement associated with addition of HP to ZAS was not an artifact induced by heating serum or plasma. Addition of fibrinogen to ZAS

TABLE 2. *Augmentation of PMN retention: addition of HP to PMN in ZAS*[a]

Cell suspension	% Retention ± SD	P value
Serum (3)	13.9 ± 9.4	
Plasma (3)	9.9 ± 8.9	ns
ZAS (3)	19.7 ± 10.4	0.05
ZAP (3)	55.7 ± 11.3	0.05, 0.05, 0.05
ZAS + HP-H (3)	49.9 ± 13.4	
ZAS + HP-E (3)	51.6 ± 3.2	
ZAS + HP-NC (3)	42.8 ± 7.2	

[a] Ficoll-Hypaque-separated PMN suspended in HBSS plus fresh serum, plasma, ZAS, ZAP, and ZAS plus HP prepared with heparin (HP-H), EDTA (HP-E), and sodium citrate (HP-NC). Number of experiments within parentheses. SD, Standard deviation; ns, not significant.

TABLE 3. *Increase in retention of PMN in ZAS and ZAS plus fibrinogen*[a]

Cell suspension	% Retention ± SD	P value	
Serum (6)	12.9 ± 7.53	0.22	
ZAS (6)	16.4 ± 7.27	0.045	0.018
ZAS + Fib (6)	27.92 ± 13.24		

[a] Ficoll-Hypaque-separated PMN suspended in HBSS plus fresh serum, ZAS, and ZAS plus fibrinogen (Fib). Number of experiments within parentheses. SD, Standard deviation.

in physiological concentrations induced enhanced retention of PMN. The fibrinogen used in these experiments contains trace contaminants, plasminogen, and anti-hemophiliac globulin. These contaminants probably do not play a significant role in enhancement of PMN adherence, as demonstrated by the failure of fibrinogen to alter adherence of PMN suspended in normal serum; only in the presence of activated complement did fibrinogen augment PMN adhesiveness. These results raise the question of the role of fibrinogen and fibrin in the inflammatory response. As reviewed by Allison and Lancaster, fibrinogen has for many years been cited as contributing to the process which leads to the sticking of PMN to endothelium of capillaries adjacent to injured tissue. However, a clear definition of the role of fibrinogen in this early aspect of the inflammatory response has not been elucidated (1).

Pearson and co-workers, utilizing an in vitro system, noted enhanced adherence of leukocytes to endothelial cells when PMN were suspended in plasma as compared with PMN-saline suspensions. In addition, these authors noted that admixtures of erythrocytes and PMN enhanced, whereas platelets plus PMN reduced, adherence of PMN to endothelium in culture (13). Allison and Lancaster reported no inhibition of sticking of leukocytes to endothelial cells after reduction of the concentration of fibrinogen in vivo. Fibrinogen concentrations were lowered by induction of intravascular coagulation and fibrinolysis in rabbits. In these experiments, adherence of leukocytes was induced by heat injury and assessed in the rabbit ear chamber (1). However, intravenous administration of streptokinase, used to promote fibrinolysis, converts plasminogen to plasmin, which leads to the activation of complement (5). Thus, the sticking of PMN, produced by local injury, occurred during systemic activation of complement, which enhances PMN adhesiveness and may have obscured the effect of lowering plasma concentrations of fibrinogen.

In summary, PMN suspended in ZAP are retained by nylon columns to a greater extent than cells in ZAS or fresh serum. This augmented retention results from the effects of activated complement upon PMN adhesiveness in the presence of a heat-stable plasma factor, not present in defibrinated plasma. Addition of fibrinogen to ZAS resulted in significantly augmented retention of PMN suspended in ZAS. These results provide evidence that optimal PMN adhesiveness results from an interaction of cells, activated complement, and fibrinogen.

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