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Fibronectin Levels During Intraperitoneal Inflammation

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Fibronectin is a high-molecular-weight opsonic protein known to influence macrophage uptake of nonbacterial particulate matter. The concentration of fibronectin in serum and the quantity of fibronectin in the inflamed peritoneal space were examined in rats after intraperitoneal casein injection. Fibronectin levels were studied in relation to the opsonic activity of the serum, as assayed by the uptake of gelatin-coated, ^{51}Cr-labeled, fixed sheep erythrocytes by adherent peritoneal macrophage monolayers. Intraperitoneal inflammation resulted in a marked increase in peritoneal fluid fibronectin that lasted throughout a 4-day observation period. The opsonic activity of serum also increased after casein challenge at 24, 48, and 72 h. The elevation in the level of fibronectin in the peritoneal space appeared to coincide with or closely precede the maximal increase in concentration of inflammatory peritoneal macrophages. After casein injection, when serum immunoreactive fibronectin increased, an enhancement in phagocytic clearance of blood-borne gelatin-coated test particles was also observed. It is suggested that the elevation of fibronectin in blood during intraperitoneal inflammation may mediate enhanced liver phagocytic function. The increased amount of fibronectin in the inflamed peritoneal space may also influence the phagocytic activity of peritoneal macrophages.

Part of the response to the intraperitoneal injection of an inflammatory stimulant consists of a well-characterized increase in the number of peritoneal macrophages (5). Such macrophages were shown by Van Furth et al. (24) to be derived from the pool of circulating blood monocytes and have been characterized as actively phagocytic cells (8). Inflammatory peritoneal macrophages can be harvested and used in a sensitive bioassay for the opsonic activity of fibronectin (4). Fibronectin has been shown to be a major factor influencing the phagocytic behavior of reticuloendothelial (RE) cells with respect to particle clearance from the blood and uptake by isolated mononuclear macrophages (3, 4, 8, 18, 22). Under in vitro conditions, resident peritoneal macrophages obtained from mice appear to synthesize fibronectin (11). If fibronectin is produced by these cells in vivo, it may influence the ingestion of foreign or denatured microparticulates in the peritoneal space (4, 8, 11).

The present study was designed to assess the influence of intraperitoneal inflammation, a condition associated with the influx of macrophages into the peritoneal space, on peritoneal fibronectin. In addition, the systemic effects of intraperitoneal inflammation with respect to RE function and circulating fibronectin concentration were also evaluated. It was observed that serum fibronectin was significantly increased by intraperitoneal inflammation. This elevation in serum fibronectin was associated with increased RE system (RES) phagocytic function. Also, the appearance of large quantities of fibronectin in the inflamed peritoneal space coincided temporally with the rise in serum fibronectin but preceded the peak of the mononuclear infiltrate.

MATERIALS AND METHODS

Experimental animals. Male Sprague-Dawley rats weighing 200 to 250 g were used in these studies and were allowed access to food and water ad libitum. The animals were lightly anesthetized with ether before casein injection, RES evaluation by colloid clearance, or surgical intervention.

Casein-induced intraperitoneal inflammation. A solution of 1% sodium caseinate (Eastman Chemical Products Inc.) in normal saline was injected intraperitoneally at a dose of 15 ml per 100 g of body weight to induce the inflammatory response. Control animals received an equivalent dose of normal saline.

Recovery of peritoneal cells. Cells were harvested from the peritoneal space at 24-h intervals after saline or casein challenge. Eight rats were sacrificed at each interval (four saline and four casein injected). Cell collection was enhanced by injecting the rats intraperitoneally with 10 ml of heparinized normal saline, followed by gentle massage of the abdominal region immediately before fluid collection. From 6 to 8 ml of fluid was usually recovered unless endogenous inflammatory exudative fluid was present in the peritoneum; this condition was associated with recovery of greater amounts of fluid. Differential cell counts were made on

Dif-Quik-stained smears of peritoneal lavage fluid. A hemacytometer was used to assess the number of cells recovered by the lavage procedure.

Purification of plasma fibronectin and antiserum development. For isolating rat plasma fibronectin, an affinity column as originally described by Engvall and Ruoslahti (6) was prepared, consisting of gelatin coupled to cyanogen bromide-activated Sepharose 4B (Pharmacia Fine Chemicals, Inc.). Plasma fibronectin biospecifically binds to gelatin and can subsequently be recovered in biologically active form as described previously in detail (3). The rat fibronectin was eluted from the column with 4 M urea in phosphate-buffered saline (PBS). The eluted fibronectin was collected and dialyzed at 4°C overnight against PBS containing 0.02% mercaptoethanol. Antiserum to rat fibronectin antiserum was developed in rats as previously described (4).

Gradient polyacrylamide gel electrophoresis. A drop of glycerol was mixed with 100-μl samples of whole citrated rat plasma and affinity-purified rat fibronectin in PBS. The samples were then applied to commercially prepared polyacrylamide gel (2.7 to 27% concave gradient; Isolabs, Inc.) and electrophoresed at 80 V for 24 h in 0.088 M Tris-EDTA-borate buffer (pH 8.2). Coomassie blue was used to stain the gels after electrophoresis.

Determination of antiserum specificity by immunoelectrophoresis. The rabbit anti-rat fibronectin antiserum was allowed to diffuse against samples of affinity-purified rat plasma fibronectin and normal rat plasma that had previously been electrophoresed in 1% agarose at 200 V for 24 h. Antiserum specificity was assessed from the appearance of the precipitant arcs that were stained with Coomassie blue.

Electroimmunoassay of peritoneal fluid and serum fibronectin. Electroimmunoassay (13) with monospecific antiserum to rat fibronectin made in rabbits was used to determine the immunoreactive fibronectin concentration as previously described (2). Serum or peritoneal lavage fluid fibronectin was assayed by incorporating the antiserum into a warm solution of agarose in Tris-tricine buffer (pH 8.6). The solution was then poured onto a glass plate and allowed to cool and harden. Serum or peritoneal fluid samples were diluted 1:10, and 10-μl portions were applied to wells cut into the agarose gel. Electrophoresis was carried out for 24 h at 7 V/cm in Tris-tricine buffer. The gels were dried, washed free of nonprecipitated protein, and stained with Coomassie blue dye. Rocket heights were compared with a standard curve developed by assaying dilutions of standardized rat serum. Fibronectin in serum was expressed as micrograms per milliliter. Peritoneal fibronectin (in micrograms) represents the total amount of fibronectin in the recovered (cell-free) lavage fluid, i.e., the lavage volume times the fibronectin concentration.

Preparation of phagocytizable test particles. Evaluations of in vitro plasma opsonic activity and systemic RE clearance capacity were carried out with the gelatinized, ^{131}I-labeled RES test lipid emulsion, the preparation of which has been previously described (20, 21). For the in vitro bioassays, a 1% emulsion with a gelatin concentration of 0.1% was used. For the in vivo studies, a 10% emulsion with a gelatin concentration of 0.3% was used. Gelatinized, fixed, ^{51}Cr-labeled sheep erythrocytes (SRBC) were used as test particles in the macrophage monolayer assay for serum opsonic activity (4).

Labeled test particles were prepared for the macrophage monolayer assay by isolating SRBC from citrated whole blood, washing them five times with saline, and then suspending them in 40 ml of PBS (pH 7.4) containing 500 μCi of $Na^{51}CrO_4$, as previously described (4). The suspension was gently agitated for 1 h at room temperature. After incubation, the SRBC were recovered by centrifugation at 5,000 × *g* for 10 min, washed, and collected in PBS. The ^{51}Cr-labeled SRBC were fixed with formaldehyde and then gelatin coated as described by Blumenstock et al. (4). After gelatinization, the SRBC preparation was washed five times by centrifugation at 4°C with PBS and then suspended in Dulbecco modified Eagle medium to a final hematocrit of 4%.

Determination of plasma opsonic activity. The isotopic in vitro liver slice bioassay was used to assess the opsonic activity (2, 4, 20, 21) of the plasma. For the in vitro assay, the flasks contained 2.0 ml of Krebs-Ringer phosphate buffer (pH 7.4), 100 USP units of heparin, 1.0 ml of test plasma, and 0.2 ml (2 mg) of gelatinized, ^{131}I-labeled RES test lipid emulsion. Uptake of the labeled test particles by the liver slices was determined by isotopic procedures after 30 min of incubation with oscillation at 37°C under 95% O_2 and 5% CO_2. The opsonic activity of the plasma was expressed in reference to its influence on particulate uptake, calculated as the percentage of the added 2-mg dose removed per 100 mg of liver slice (wet weight). This assay was described previously for animal and human studies (18, 22) to evaluate plasma opsonic activity as well as the activity of purified fibronectin (2–4).

Determination of serum opsonic activity. The macrophage monolayer assay of Blumenstock et al. (4) was used to assay the opsonic activity of the serum. Rats were used as donors of peritoneal macrophages at 96 h after the intraperitoneal injection of casein. The macrophages were recovered by peritoneal lavage and washed three times by refrigerated (4°C) centrifugation at 150 × *g* with Hanks balanced salt solution. A suspension containing 2 × 10^6 total cells per ml was then prepared in medium supplemented with 30% fetal calf serum.

Samples (1 ml) of the cell suspension were added to the wells of a multiwell tissue culture dish (Falcon Plastics) and then incubated for 2 h in a humidified 5% CO_2 atmosphere at 37°C. Monolayers of adherent macrophages were present on the bottoms of the culture wells after incubation. For assaying opsonic activity in serum, the culture medium was removed and the cell monolayers were incubated for 2 h (37°C, 5% CO_2) with 1 ml of medium containing 10 U of heparin, 20 μl of the serum to be assayed, and 100 μl of the gelatinized, ^{51}Cr-labeled, fixed SRBC suspension. After incubation, the medium was removed and the monolayers were washed three times with PBS. The cell layers were then digested with 1 M NaOH for 20 min and placed in counting tubes for radioactivity determination. Uptake was expressed as the percentage of added labeled test particles taken up per 2 × 10^6 adherent cells. Blanks without serum were tested, and the background was subtracted. Triplicate assays were performed on each serum sample. Although the peritoneal macrophage monolayer assay reflects the intense

ingestion shown by electron microscopic studies with gelatin-coated target particles (8), the existence of particles firmly bound to the outer surface of the macrophages, not removed by the washing procedure, can obviously be incorporated into the assay isotopic counts.

Light-microscopic evaluation of SRBC uptake by peritoneal macrophage monolayers. A qualitative assessment of macrophage-particle interaction was performed by light microscopy after incubation of the macrophages alone or in the presence of SRBC, heparin, and normal rat serum (fibronectin concentration ≅ 460 μg/ml). These incubations were carried out in Lab-Tek tissue culture chambers mounted on glass microscope slides. The monolayers with or without SRBC were washed three times with PBS before Dif-Quik staining and microscopic observation.

Assessment of in vivo RE phagocytic activity. RE function was evaluated by the clearance of the gelatinized ^{131}I-labeled RES test lipid emulsion particles from the circulation (17, 21). The emulsion was injected intravenously at a dose of 50 mg/100 g of body weight. Serial blood samples were obtained at 2-min intervals from the cut tail vein and analyzed for radioactivity. The rate of clearance of the particles from the blood was expressed as the half-time ($t/2$) or the phagocytic index (K) as previously described (1, 17). Duplicate portions of the liver, lung, spleen, and bone marrow were taken at 15 min postinjection for the determination of test colloid distribution. Localization of test colloid was expressed as the percent injected dose removed per total organ (*%ID/TO*).

Statistical analysis and isotopic determinations. Student's t test was used to perform statistical analyses, with the confidence level of significance set at 95%. Radioactivity (^{131}I and ^{51}Cr) was determined with an autogamma counter equipped with a two-inch, thallium-activated (NaI) crystal. All samples were corrected for background activity.

RESULTS

Gradient polyacrylamide gel electrophoresis of the affinity purified rat plasma fibronectin showed one large band in the high-molecular-weight range (Fig. 1). The fibronectin preparation was sufficiently homogenous to generate highly monospecific antiserum in rabbits, as shown by immunoelectrophoresis (Fig. 2). This antiserum was subsequently used in the measurement of serum and peritoneal fibronectin by rocket electroimmunoassay.

Immunoreactive serum fibronectin levels after casein-induced intraperitoneal inflammation became significantly ($P < 0.05$) elevated at 24, 48, and 72 h after casein injection, with an apparent decline toward control levels by 96 h (Table 1). Serum fibronectin at 72 h after injection appeared at a maximum and was approximately 100% above control levels. Comparable changes were not seen after saline injection, although a slight upward trend was seen by 72 h in this initial series.

The amount of fibronectin recoverable from the peritoneal space by lavage rose dramatically from trace levels (barely detectable by electroimmunoassay) before casein injection to approximately 3,138 μg by 24 h and 5,316 μg by 48 h after injection (Table 2). These values, which reflect the calculated total amount of fluid-phase fibronectin in the peritoneal space, were determined by multiplying the volume of total lavage fluid by its fibronectin concentration.

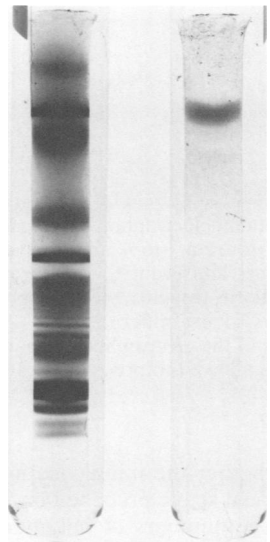

FIG. 1. Gradient polyacrylamide gel electrophoresis of normal rat plasma (left) and affinity-purified rat plasma fibronectin (right). Electrophoresis was performed at 80 V for 24 h in 0.08 M Tris-EDTA-borate buffer.

Approximately 10 ml of endogenous inflammatory fluid was characteristically present at 48 h after casein injection. The fibronectin concentration in this fluid was as high as that typically measured in the serum of a normal rat. Although distinct variability existed in the degree of elevation of fibronectin in the peritoneal space of each rat individually, a rapid and distinct elevation ($P < 0.001$) existed in all of the animals.

To assist in interpretation of the fibronectin response, we evaluated the nature of the cellular infiltrate developed during casein-induced intraperitoneal inflammation (Table 3). Mononuclear cells (peritoneal macrophages and lymphocytes) were the predominant cell type before casein injection (zero time), although their number was not as great as would be expected, i.e., approximately 1.30×10^6 cells per rat (used as the base line for both groups). The number of peritoneal polymorphonuclear leukocytes became markedly elevated within 24 h after casein injection and declined as a percentage of the total cells thereafter. From the current data, it is difficult to

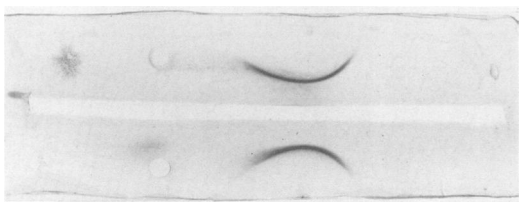

FIG. 2. Immunoelectrophoresis of affinity-purified rat plasma fibronectin (top) and normal rat plasma (bottom) against antiserum to rat fibronectin developed in rabbits (in trough). Electrophoresis was carried out in Tris-tricine buffer (pH 8.6) at 200 V for 90 min. Diffusion of the electrophoresed samples and the antiserum was allowed to proceed for 48 h at 25°C.

determine whether the initial rise in fibronectin in the peritoneal space preceded or followed the arrival of large numbers of polymorphonuclear leukocytes into the inflamed region. However, the temporal response suggests that fibronectin is markedly elevated in inflammatory peritoneal fluid before the peak elevation in the number of mononuclear cells is reached over the 48- to 72-h period after casein injection. At 48 h, the total fibronectin content in the peritoneal space was highly elevated ($P < 0.001$) (Table 2). Microscopic examination of stained smears of lavage fluid revealed that the majority of mononuclear cells recovered at 48, 72, and 96 h after casein challenge were large, highly vacuolated inflammatory macrophages (Fig. 3c, d, and e). They were different from the peritoneal cells normally residing in the peritoneal space (Fig. 3a) or present early after inflammation (Fig. 3b).

To determine whether the elevation of immunoreactive fibronectin in the blood was associated with an increase in opsonic activity, we harvested the plasma 72 h after casein injection and evaluated it by the liver slice bioassay. This assay was only done at the 72-h point since it required sacrifice of the animals. The plasma of casein-injected rats showed significantly ($P < 0.05$) greater opsonic activity than did plasma obtained from saline-injected control animals; the values (mean ± standard error of the mean for seven determinations) were 18.26 ± 1.66% and 12.91 ± 1.69% of the injected dose per 100 mg (wet weight) of liver, respectively. This elevated bioassayable opsonic activity of the plasma at 72 h was due to a significant increase in the immunoreactive fibronectin concentration of the plasma, which was >100% higher (485.4 ± 13.2 and 1,028.3 ± 111.5 μg/ml for the control and casein-injected rats, respectively).

To further develop a functional association between elevated fibronectin and increased expression of opsonic activity, we studied another set of animals (six saline- and six casein-injected rats) over a period of 3 days for opsonic activity by the peritoneal macrophage bioassay. This bioassay was used instead of the liver slice bioassay because its requirement for small portions of serum allowed us to observe the same animal over time. Microscopic observations (Fig. 4) indicated that particle uptake by the macrophages in this assay system reflected both target particle ingestion by peritoneal macrophages and some adherence to the outer surface; both aspects reflect opsonic activity. The opsonic activity in the casein-injected rats as studied by the monolayer assay was found to be significantly ($P < 0.05$) greater than the preinjection level at 24, 48, and 72 h after injection (Table 4). Immunoreactive fibronectin levels were also markedly elevated in response to casein (Table 4). There was a mild drop in fibronectin at 72 h after casein injection, but its level in this second group was still well above the zero-time (preinjection) level. An upward trend for fibronectin was also seen this time in the control saline-injected rats, but was not as pronounced as that after casein challenge. This upward trend might be due to the loss of blood volume by these rats over the sampling period. Thus, 0.7 ml of blood per rat was collected daily to obtain sufficient serum for both opsonic and immunoreactive assays versus 0.1 ml when only immunoreactive fibronectin was evaluated (Table 1). However, the elevated immunoreactive fibronectin levels were at all times related to increased opsonic activity.

To verify that enhanced opsonic activity was reflected in vivo by increased RES phagocytic function, we determined the clearance of the gelatinized, ^{131}I-labeled RES test emulsion in control and casein-challenged rats at 72 h after casein injection. RE function was significantly ($P < 0.01$) enhanced in vivo in the casein-injected rats (Table 5). The $t/2$ for clearance of

TABLE 1. Immunoreactive serum fibronectin after induction of intraperitoneal inflammation with casein[a]

Time after injection (h)	Immunoreactive fibronectin (μg/ml) (mean ± SEM)	
	Controls ($n = 4$)	Casein injected ($n = 4$)
0	403.5 ± 30.7	503.5 ± 37.4
24	432.4 ± 27.0	927.1 ± 59.4[b]
48	408.6 ± 23.4	787.8 ± 85.7[b]
72	485.4 ± 13.2[b]	1028.3 ± 111.5[b]
96	475.0 ± 26.6[b]	643.2 ± 38.5

[a] Casein solution was injected intraperitoneally at a dose of 15 ml/100 g of body weight. Controls received the same dose of normal saline.

[b] Significantly ($P < 0.05$) greater than preinjection levels.

TABLE 2. Effect of intraperitoneal injection of casein on immunoreactive peritoneal fluid fibronectin[a]

Time after injection (h)	Fibronectin concn in lavage fluid (μg/ml)	Fibronectin content (μg)[b]
0	6.3 ± 0.8	42.5 ± 7.1
24	118.5 ± 4.9	3138.7 ± 107.6
48	231.7 ± 20.4	5316.6 ± 1007.6
72	225.5 ± 55.2	2947.6 ± 1060.8
96	43.3 ± 11.6	379.3 ± 219.0

[a] Values represent the mean ± standard error of the mean for four animals tested at 0, 24, 48, and 72 h and three animals tested at 96 h.

[b] Values are expressed as the total amount of fibronectin (immunoreactive fibronectin content) per peritoneal space. This was calculated as the peritoneal lavage volume (milliliters) times the lavage fluid fibronectin concentration (micrograms per milliliter). Lavage volume equals the endogenous inflammatory fluid volume plus the recoverable exogenously injected saline lavage volume.

the test colloid was 3.47 ± 0.38 min in the casein-injected rats versus 10.81 ± 1.25 min in the saline controls. Liver uptake of the colloid in the casein-injected rats was greater ($P < 0.01$) on both a per gram and total organ basis. Spleen and bone marrow contained less test colloid at the 15-min point. This was anticipated, since an inverse relationship between liver uptake and extrahepatic particle uptake exists, especially with intense hepatic Kupffer cell removal of the particles from the blood (17). It should be noted that although the circulating level of immunoreactive fibronectin at 72 h had essentially doubled (Table 1), the $t/2$ for clearance and liver uptake was not as great on a percentage basis (Table 5). This reflects both the time interval selected for analysis of particle distribution in the tissues (i.e., 15 min) and the fact that the clearance rate began to approach its maximum (i.e., >90% in liver and spleen). If tissue uptake were determined earlier, i.e., at 5 or 10 min, the difference between control and treated rats could be exag-

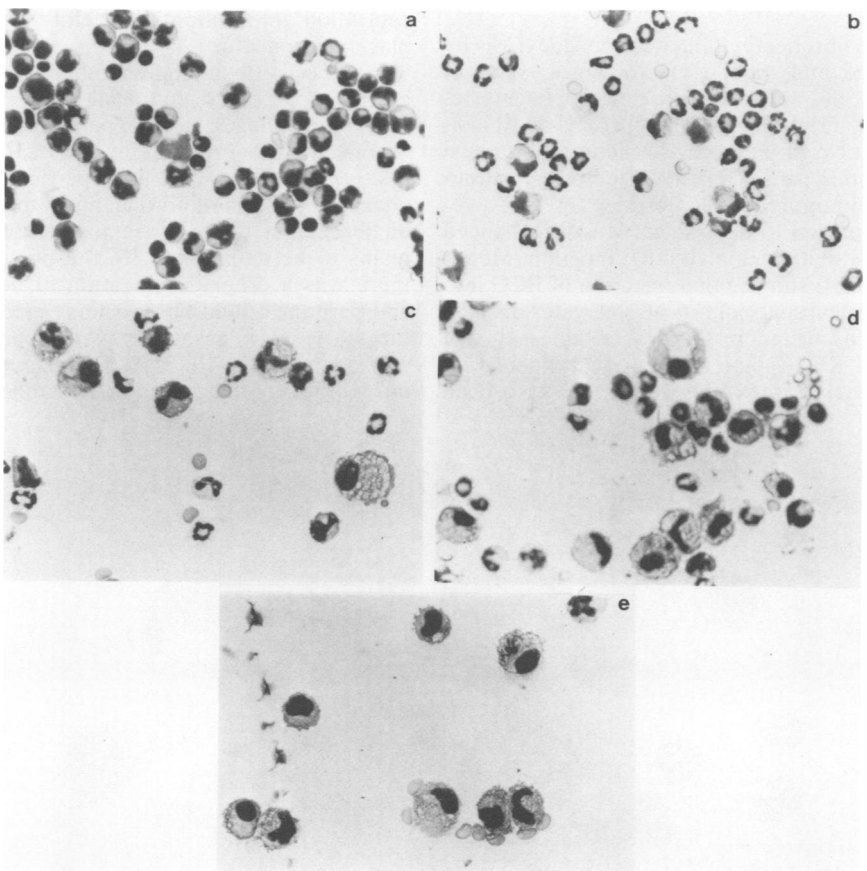

FIG. 3. Dif-Quik-stained lavage fluid cell smears prepared before (a) and at 24 (b), 48 (c), 72 (d), and 96 h (e) after intraperitoneal casein injection. A small number of red cells are present in the fluid as a result of the lavage procedure (×120).

TABLE 3. Effect of intraperitoneal casein injection on the number of peritoneal mononuclear and polymorphonuclear cells[a]

Time after injection (h)	Cell types (no. and %) in:			
	Control (saline-injected) rats		Casein-injected rats	
	Mononuclear	Polymorphonuclear	Mononuclear	Polymorphonuclear
0	1.30 ± 0.41 (91)	0.13 ± 0.03 (9)	1.30 ± 0.41 (91)	0.13 ± 0.03 (9)
24	3.59 ± 1.90 (61)	2.34 ± 1.39 (39)	7.29 ± 1.60[b] (20)	29.24 ± 7.24[b] (80)
48	2.00 ± 0.68 (75)	0.68 ± 0.46 (25)	42.34 ± 4.62[b] (45)	52.26 ± 8.34[b] (55)
72	2.01 ± 0.87 (82)	0.45 ± 0.08[b] (18)	34.80 ± 3.20[b] (53)	30.71 ± 4.75[b] (47)
96	2.17 ± 0.49 (89)	0.26 ± 0.10 (11)	11.64 ± 6.06 (75)	3.71 ± 1.19[b] (25)

[a] Values are given as the mean ± standard error of the mean for three animals tested at zero time (preinjection) and four animals in each of the other time groups. A 1% solution of casein in normal saline was injected intraperitoneally at a dose of 15 ml/100 g of body weight. Control animals received an equivalent dose of normal saline. Values represent the number of cells per rat ($\times 10^6$); values in parentheses are the percentage of total cells.
[b] Significantly ($P < 0.05$) different from zero time (preinjection).

gerated, since uptake is a function of the rate of particle removal (1, 17). However, the response was the same: increased RES clearance with high fibronectin levels.

DISCUSSION

Plasma fibronectin influences phagocytosis by both fixed and mobile macrophages, such as Kupffer cells and peritoneal macrophages, respectively (3, 4, 8, 18). The current study indicates that casein-induced, sterile intraperitoneal inflammation causes a marked increase in circulating immunoreactive fibronectin. This response appears to be associated with enhanced opsonic activity and in vivo RE function. Megirian et al. (14) showed that injection of BCG into the peritoneal space of rats also elevates opsonic activity, as tested by liver slice bioassay and increased RES function. Peritoneal inoculation with Ehrlich ascites tumor cells has also been reported to induce a rise in plasma fibronectin in mice as measured by immunoassay (26). In the latter instance, fibronectin was also found to be produced by Ehrlich ascites tumor cells under in vivo conditions and to be present in high concentration in ascitic fluid before the rise of plasma fibronectin.

Many cells, including endothelial cells, fibroblasts, hepatocytes, epithelial cells, and peritoneal macrophages, can apparently synthesize fibronectin in vitro (10, 11, 16, 23, 25). The relative contribution of local peritoneal macrophage production of fibronectin to the elevation of fibronectin observed in the peritoneum remains to be determined. In the present study, there was a dramatic elevation of both serum and peritoneal fluid fibronectin. Since fibronectin deficiency is associated with depressed RE phagocytosis (18, 19, 21, 22), its elevation after intraperitoneal inflammation may reflect the ac-

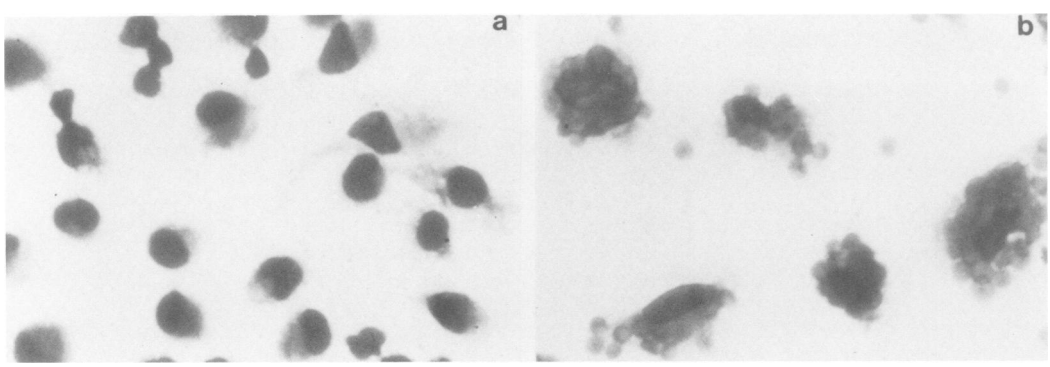

FIG. 4. Adherent inflammatory peritoneal macrophages after a 2-h incubation (a) alone or (b) in the presence of 2% normal rat serum, 10 U of heparin, and gelatinized, fixed, ^{51}Cr-labeled SRBC (100 μl of a 4% hematocrit was added, to a final incubation volume of 1 ml). The macrophage monolayers, with or without added SRBC, were washed three times with PBS before being fixed and stained (Dif-Quik stain) (×450).

TABLE 4. Serum opsonic activity and immunoreactive fibronectin concentration after intraperitoneal saline or casein injection[a]

Time after injection (h)	Saline-injected rats		Casein-injected rats	
	Serum opsonic activity (%)	Fibronectin concn (μg/ml)	Serum opsonic activity (%)	Fibronectin concn (μg/ml)
0	23.6 ± 2.0	476.7 ± 41.1	24.9 ± 1.7	446.9 ± 40.4
24	25.3 ± 2.4	687.5 ± 39.7	31.4 ± 1.2[b]	1019.6 ± 50.9
48	23.3 ± 2.8	633.6 ± 56.8	32.5 ± 1.8[c]	1136.3 ± 43.6
72	23.6 ± 2.2	613.5 ± 41.4	32.5 ± 1.6[c]	781.7 ± 62.0

[a] Two groups of animals (six saline- and six casein-injected rats in each) were studied over a 3-day period. Values in each case represent the mean ± standard error of the mean for six rats; for serum opsonic activity, values represent the percent uptake of added labeled test particles per 2×10^6 adherent cells.

[b] In terms of the peritoneal macrophage monolayer assay, the opsonic activity at 24 h was significantly ($P < 0.05$) greater than preinjection levels.

[c] In terms of the peritoneal macrophage monolayer assay, the opsonic activity at 48 and 72 h was significantly ($P < 0.01$) greater than preinjection levels.

TABLE 5. RE phagocytic function 72 h after intraperitoneal casein injection[a]

Group	Phagocytic index (*K*)	*t*/2 (min)	Relative localization of test colloid (*%ID/TO*)[b]			
			Liver	Lung	Spleen	Bone marrow
Control (saline injected)	0.030 ± 0.003	10.81 ± 1.25	70.75 ± 4.08	0.61 ± 0.07	5.56 ± 0.43	4.20 ± 0.65
Casein injected	0.094 ± 0.010[c]	3.47 ± 0.38[d]	88.84 ± 3.52[c]	0.44 ± 0.08	2.98 ± 0.39[d]	1.23 ± 0.32[d]

[a] The gelatinized, ^{131}I-labeled RE test lipid emulsion was injected intraperitoneally into rats at a dose of 50 mg/100 g of body weight. There were eight rats in each group. Values are mean ± standard error of the mean.

[b] Tissue uptake of the test colloid was determined at 15 min postinjection and is expressed on a total organ basis (*%ID/TO*).

[c] Significantly increased ($P < 0.01$) from control.

[d] Significantly decreased ($P < 0.01$) from control.

tivation of a defense against the products of tissue injury in both the peritoneal space and circulation. Accordingly, recent studies by Lanser and Saba (12) revealed an acute depletion in the blood, followed by a pronounced elevation in serum, of fibronectin caused by *Staphylococcus aureus* peritonitis. Thus, the response appears to be a general event triggered by irritation or inflammation of the peritoneal space with either bacterial or nonbacterial substances.

In the present study, the increase in serum fibronectin appeared to occur before or in parallel with the increase in peritoneal fibronectin. The sustained high level of fibronectin suggests the potential existence of a gradient for fibronectin flux from blood into the peritoneal space during inflammation. Indeed, although the total amount of fibronectin recoverable from the peritoneal space was very high (about 3,000 to 5,000 μg), the absolute concentration per milliliter of peritoneal fluid was still lower than the blood concentration at 48 to 72 h. The soluble form of fibronectin in blood apparently has the potential to become incorporated in vivo into the insoluble tissue fibronectin pools (15). Such incorporation has been speculated to be an important aspect of the improved microvascular and organ function response observed in septic, injured patients after the reversal of fibronectin deficiency which follows the infusion of fibronectin-rich plasma cryoprecipitate (18, 22). Accordingly, fibronectin elevation in the peritoneal space during inflammation may serve an adhesive role in the organization of new tissue (7); an opsonic role for macrophage phagocytosis of collagenous tissue debris (4, 22); or perhaps as a chemotactic stimulus for cells (9). At a minimum, the current findings suggest that elevated fibronectin levels, as they may influence macrophage defense against intraperitoneal inflammation and the products of tissue injury, warrant investigation.

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