Molecular Determinants of Acute Inflammatory Responses to Biomaterials

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Abstract

The frequent inflammatory responses to implanted medical devices are puzzling in view of the inert and nontoxic nature of most biomaterials. Because implant surfaces spontaneously adsorb host proteins, this proteinaceous film is probably important in the subsequent attraction of phagocytes. In fact, earlier we found that acute inflammatory responses to experimental polyethylene terephthalate implants in mice require the precedent adsorption of one particular host protein, fibrinogen. The present investigations were aimed at defining the molecular determinants of fibrinogen-mediated acute inflammatory responses to implanted biomaterials. We find: (*a*) plasmin degradation of purified fibrinogen into defined domains reveals that the proinflammatory activity resides within the D fragment, which contains neither the fibrin cross-linking sites nor RGD sequences; (*b*) the major (and, perhaps, exclusive) proinflammatory sequence appears to be fibrinogen γ190–202, previously shown to interact with CD11b/CD18 (Mac-1). The chemically synthesized peptide, cross-linked to albumin (which itself does not promote inflammatory responses), mimics the proinflammatory effect of adsorbed native fibrinogen; and (*c*) this sequence probably promotes inflammatory responses through interactions with Mac-1 because phagocyte accumulation on experimental implants is almost completely abrogated by administration of recombinant neutrophil inhibitory factor (which blocks CD11b–fibrin(ogen) interaction). We conclude that improved knowledge of such surface–protein–phagocyte interactions may permit the future development of more biocompatible implantable materials. (*J. Clin. Invest.* 1996. 97:1329–1334.) Key words: fibrinogen • Mac-1 • neutrophils • macrophages • inflammation

Introduction

Implanted biomaterials frequently trigger inflammatory responses which may, in turn, presage serious iatrogenic consequences such as stress cracking of pacemaker leads (1, 2), osteolysis adjacent artificial joints (3–5), and fibrosis and capsule contracture affecting mammary prostheses (6–10). The causes of these adverse responses are still obscure, particularly in view of the nontoxic, nonimmunogenic, and chemically inert nature of most implantable biomaterials. However, after implantation, biomaterials spontaneously acquire a layer of host protein. Therefore, this surface protein layer actually may be responsible for subsequent phagocyte attraction and activation on the surfaces of implants (11–15).

In the case of blood-contact biomaterials (e.g., polytetrafluoroethylene, polydimethylsiloxane, polyurethane, polyethylene, and Dacron) three host proteins predominate: albumin, IgG, and fibrinogen (16, 17). Of these, albumin is known to "passivate" biomaterial surfaces, blunting both proinflammatory and thrombogenic responses (14, 15, 18–20). We also have tentatively ruled out the necessity for either surface-bound IgG or complement activation in triggering acute inflammatory responses to biomaterial implants. Polyethylene terephthalate (PET)[1] disks implanted intraperitoneally in mice with either severe combined immunodeficiency (with almost undetectable plasma IgG) or complement deficiency (induced by injection of cobra venom factor) have near-normal recruitment of phagocytic cells to the implant surfaces (14).

Subsequent results from the same experimental implantation model suggest that spontaneously adsorbed fibrin(ogen) is most important in mediating the short-term accumulation of inflammatory cells on implanted biomaterials. Whereas implants precoated with either serum or hypofibrinogenemic human plasma fail to accumulate many inflammatory cells, addition of fibrinogen to these coatings fully restores the normal phagocyte accumulation. Perhaps most convincingly, severely hypofibrinogenemic mice do not mount an inflammatory response to implanted PET unless the material is coated with fibrinogen or the animals are injected with fibrinogen before implantation (15).

However, the mechanisms underlying the recognition, attraction, and adhesion of inflammatory cells to surface fibrinogen remain unknown. In this study, we have proteolytically dissected fibrinogen to identify the domain(s) of this large and complex protein which might be responsible for proinflammatory activity. Surprisingly, a single and small peptide (residues 190–202 of the fibrinogen γ chain), representing $< 1\%$ of the total molecule, appears responsible for the proinflammatory activity of adsorbed fibrinogen. This small epitope is recog-

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Received for publication 6 November 1995 and accepted in revised

J. Clin. Invest.

0021-9738/96/03/1329/06 $2.00
Volume 97, Number 5, March 1996, 1329–1334

1. *Abbreviations used in this paper:* Mϕ, macrophages/monocytes; MPO, myeloperoxidase; NIF, neutrophil inhibitory factor; NSE, nonspecific esterase; PET, polyethylene terephthalate.

nized by the phagocyte integrin, Mac-1 (CD11b/CD18) (21), and we find that blockade of Mac-1 almost totally prevents in vivo phagocyte accumulation on implant surfaces. The results of this study provide a fundamental piece of information about the interactions between phagocytes and biomaterials. A more comprehensive knowledge of these interactions may permit the rational design of biomaterial surfaces which discourage adverse inflammatory and/or fibrotic reactions.

Methods

Materials. Human albumin (AlbumarC™ 25%) was purchased from Baxter Healthcare Corp. (Glendale, CA). Human plasmin, horseradish peroxidase, guaiacol (*o*-methoxyphenol), hydrogen peroxide (30% solution), eserine (physostigmine), *o*-nitrophenyl butyrate, β-NADH (from yeast), sodium pyruvate, Triton X-100 (toctyl phenoxy polyethoxyethanol), NaN_3, iodoacetamide, EDTA, Mes, and *N*-hydroxysulfosuccinimide (NHS) were obtained from Sigma Chemical Co. (St. Louis, MO). Trasylol® VLE was purchased from Miles Inc. (Diagnostics Division, Kankakee, IL). CM Sephadex C-50 was obtained from Pharmacia Biotech Inc. (Piscataway, NJ). 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) was purchased from Pierce Chemical Co. (Rockford, IL). PET (Mylar®) film (type A, 0.005 mm thick) was obtained from Cadillac Plastic and Chemical Co. (Birmingham, MI). Neutrophil inhibitory factor (NIF) was generously provided by Dr. M. Moyle (Corvas International, Inc., San Diego, CA).

Purification of fibrinogen and generation of proteolytic fragments. In the experiments reported here, for reasons of economy, human fibrinogen was used in animal studies. Our earlier results (15) indicate that no heterologous foreign protein reaction occurs in our short-term in vivo implantation model. Fibrinogen was purified from fresh human blood by differential ethanol precipitation (22). Plasmin degradation fragments D100 (105,000 mol wt) and E50 (50,000 mol wt) were prepared by digestion of fibrinogen in the presence of 5 mM $CaCl_2$ and plasmin (0.013 U/mg of fibrinogen). The D and E fragments were separated by ion exchange chromatography (CM Sephadex C-50) (23, 24). These fragments were not cross-contaminated as judged by SDS-PAGE. In the presence of 5 mM EDTA and 2 M urea, fragment D100 was further degraded to fragments D80 (80,000 mol wt) and D30 (30,000 mol wt) by plasmin (25). All proteins were stored at −70°C. To quantitate the amount of surface-adsorbed protein, samples of purified proteins and synthesized peptides were iodinated with $Na^{125}I$ by the lactoperoxidase labeling method (26, 27).

Peptide synthesis and characterization. Peptides were synthesized by the solid-phase method using Fmoc chemistry in a peptide synthesizer (model 431A; Applied Biosystems, Foster City, CA). After synthesis, the peptide was deprotected and cleaved from the resin with 95% trifluoroacetic acid. The structure of synthesized peptides was verified by amino acid analysis and by molecular mass analysis by electrospray mass spectrometry. Lyophilized crude peptides were purified by preparative reverse-phase HPLC on a C-18 column. A variant peptide (P1) duplicating the fibrinogen γ chain sequence was synthesized with the addition of the residues Lys-Tyr at the NH_2 terminus to provide an additional site for cross-linking to albumin. The total amino acid sequence of P1 is G^{190}WTVFQKRLDGSV^{202} (21). A control scrambled peptide having the sequence FRLGWVQ TSVDKG was also used.

Conjugation of the peptides to albumin. Synthetic peptides, both P1 and the scrambled peptide, were chemically conjugated to human albumin using the carbodiimide, EDC. This was done because the small size of this peptide precludes its direct adherence to biomaterial surfaces and because the coupling of synthetic peptides to larger carrier proteins has been shown to improve cell adhesion activities (28–30). The conjugation of the peptides to albumin was carried out with a modified two-step coupling procedure (31, 32). Briefly, 5 mg of the peptides and 5 mg of albumin were solubilized in 1 ml of activation buffer (0.1 M Mes, 0.5 M NaCl, pH 6.0). The reaction was started by addition of EDC (final concentration 2 mM) and NHS (final concentration 5 mM). After 4 h of incubation at room temperature, the albumin-coupled peptides were dialyzed extensively against PBS (10 mM, pH 7.3) to remove excess linker and uncoupled peptides.

Preparation of PET disks. Disks of 1.2 cm in diameter were cut from PET film. The disks were stirred for > 96 h in 70% ethanol with multiple changes to remove dust and sterilize the surface, and stored in 100% ethanol. Before use, the disks were hydrated by immersion in sterile, pyrogen-free saline for at least 1 h. Protein-coated plastic disks were produced by incubating hydrated PET disks with solutions of human albumin (10 mg/ml), human fibrinogen (60 μg/ml), human fibrinogen degradation products (60 μg/ml), peptide-albumin (60 μg/ml), or saline (as control) at room temperature in a rotary shaker (25 rpm) for 16 h under sterile conditions. The coated disks were then blocked with human albumin (10 mg/ml) for 3 h to ensure complete surface coverage. After brief rinsing with sterile PBS, these disks were implanted. Control experiments with ^{125}I-labeled proteins (fibrinogen and degradation products) indicated that the concentration of surface proteins ($\sim$ 400 ng/cm^2) before implantation was roughly equivalent to a continuous monolayer.

Implantation of PET disks. As an in vivo model for assessing inflammatory responses to various surfaces, sterile protein-coated PET disks were implanted intraperitoneally in male Swiss Webster (SW) mice (20-gram body wt) (Taconic Farms Inc., Germantown, NY) as described earlier (14, 15). The extent of inflammatory responses to implanted biomaterials may vary in mice of different ages, batches, or even with the season of the year. Therefore, only mice from the same shipment were used in individual experiments and control groups were included in every experiment for comparison. Experimental values shown in all graphs represent the results of single implantation experiments using five animals per treatment. All such experiments were repeated at least three times and the results shown are representative of all trials. In some experiments, NIF was repeatedly injected intraperitoneally at 0, 4, 8, and 12 h after implantation at a dose of 40 mg/kg body wt.

Explantation was performed at 16 h after implantation (earlier found to be the approximate time of maximal phagocyte accumulation). After careful removal from the peritoneal cavity, explanted disks were gently washed with PBS. The adherent cells were then lysed by incubating the disks with 0.5 ml of 1% (vol/vol) Triton X-100 for 1 h (to release cytosolic and granular contents of adherent cells). The numbers of adherent phagocytes were then estimated by enzyme activities (myeloperoxidase [MPO] for neutrophils [PMN] and nonspecific esterase [NSE] for macrophages/monocytes [Mϕ]). Previous control experiments indicate that measured enzyme activity is a reliable indicator of the numbers of surface-adherent phagocytes (14, 15).

Measurement of enzyme activities. Our earlier results showed that > 95% of material-associated peroxidase activity was from MPO and < 5% represented eosinophil peroxidase (15). MPO (predominantly from PMN although monocytes may have small amounts) was measured by a guaiacol reaction (33). Control studies on purified PMN from mice indicated that the MPO activity of mouse peripheral blood PMN is $\sim$ 23 nU/cell (15).

NSE is relatively restricted to Mϕ (34). The activity of NSE was determined by following the rate of hydrolysis of *o*-nitrophenyl butyrate (35) in the presence of eserine (10 mM final concentration), which will eliminate possible interference by plasma cholinesterase (36). Enzyme assays on nonelicited mouse Mϕ (obtained by peritoneal lavage) indicated that the NSE activity of these Mϕ is 11 nU/cell (15). Our earlier control experiments showed that measured enzyme activity was a reliable indicator of microscopically enumerated surface-adherent phagocytes (r = 0.90 and 0.96 for NSE and MPO, respectively; n = 8).

Determinations of endotoxin contamination. Subsamples of PET disks, both untreated and preincubated with various protein preparations, were assayed for endotoxin contamination by the chromogenic limulus amebocyte lysate test (Whittaker Bioproducts, Walkersville,

MA). In no case was significant surface-associated endotoxin found (i.e., no sample contained > 0.01 ng endotoxin/cm^2 of material surface).

Statistical analysis. Significance of differences was assessed using two-tailed Student's *t* test.

Results

Inflammatory cells accumulate on fibrinogen fragment D–, but not fragment E–, coated surfaces. To directly determine the fibrinogen epitopes responsible for phagocyte accumulation on implants, we used material coated with purified fibrinogen fragments. As shown in Table I, substantial amounts of fibrinogen and its degradation products adsorb to PET surfaces after 18 h of incubation. After intraperitoneal implantation in mice, D100-coated disks accumulate large numbers of phagocytes (both PMN and Mϕ), as many as do fibrinogen-coated disks. On the other hand, E50-coated material has no evident proinflammatory effect (Fig. 1). With degradation of D100 to D80 (molecular mass of 80 kD), similar numbers of adherent phagocytes are recruited to D80- versus fibrinogen-coated disks in vivo (data not shown). In an attempt to determine the specific portion of fibrinogen responsible for phagocyte accumulation, D80 was digested still further to D30 (molecular mass of 30 kD). D30 is at least as potent as the parent molecule in causing the attraction of both PMN and Mϕ (Fig. 2). Therefore, D30 retains the motif(s) necessary for phagocyte attraction.

Glycine 190–Valine 202 of the fibrinogen γ chain is sufficient to mediate phagocyte accumulation. Altieri and colleagues (21) have determined recently that one segment of D30, γ190–202 (abbreviated as P1), is critically important in other phagocyte–fibrinogen interactions. Therefore, we hypothesized that this peptide might be the crucial determinant of phagocyte interactions with implanted fibrinogen-bearing surfaces. To test this hypothesis, P1 and a P1-based scrambled peptide were synthesized and covalently linked to human albumin, which has been used widely as a carrier to enhance the cellular responses to peptides (28–30). Peptide–albumin complexes were precoated on implant surfaces and the cellular responses to these surfaces were then tested in vivo. In agreement with our hypothesis, implant surfaces precoated with P1 peptide, but not scrambled peptide, induce substantial phagocyte accumulation, equivalent to that caused by fibrinogen-coated surfaces (Fig. 3). These results clearly indicate that the fibrinogen γ190–202 sequence may be of signal importance in mediating the attraction of phagocytes to fibrinogen-bearing implant surfaces.

Table I. Surface-adsorbed Fibrinogen and Fibrinogen Fragments on PET Disks

Proteins	Sample number	Protein concentration
		ng/cm^2
Fibrinogen	4	340.0±31.8
Fragment D100	4	508.1±99.5
Fragment E	4	438.3±7.5

PET disks were incubated with various ^{125}I-labeled protein solutions (50 μg/ml) for 18 h at room temperature. After blocking with human albumin (10 mg/ml) for 4 h at room temperature, the surface protein was estimated by gamma counting. Values shown represent the means ±1 SD.

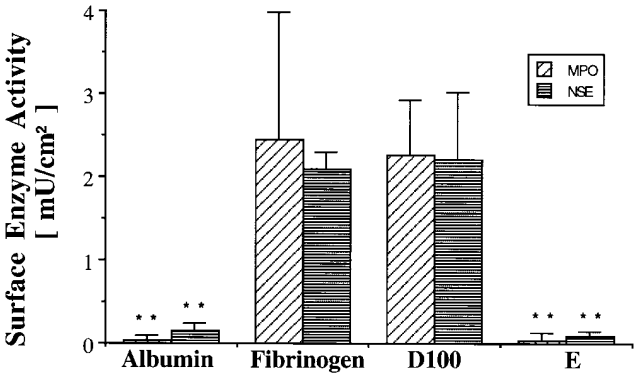

Figure 1. Phagocyte accumulation on the surfaces of PET disks preincubated with human fibrinogen (60 μg/ml), fragment D100 (60 μg/ml), fragment E (60 μg/ml), and albumin (10 mg/ml) after implantation in SW mice for 16 h. Vertical lines denote ±1 SD (n = 5 in all cases). **P < 0.01 vs. fibrinogen-coated implants. Estimated numbers of PMN: 106,100±67,000/cm^2 on fibrinogen-coated disks, 98,300±28,700/cm^2 on fragment D100–coated disks, 1,900±3,800/cm^2 on fragment E–coated disks, and 1,900±1,900/cm^2 on albumin-coated disks. Estimated numbers of Mϕ: 190,900±18,200/cm^2 on fibrinogen-coated disks, 200,000±74,500/cm^2 on fragment D100–coated disks, 9,100±3,600/cm^2 on fragment E–coated disks, and 12,700±9,100/cm^2 on albumin-coated disks.

Blockade of P1–Mac-1 interaction inhibits phagocyte accumulation. In aggregate, the preceding results indicate that one short sequence on fibrinogen (P1) is probably of predominant importance in phagocyte attraction and binding. Altieri et al. (21) have found that P1 is recognized by the Mac-1 integrin on leukocytes. To evaluate the possible importance of P1–Mac-1 interaction in phagocyte accumulation on implants, we used recombinant NIF for two reasons. First, NIF appears to impair

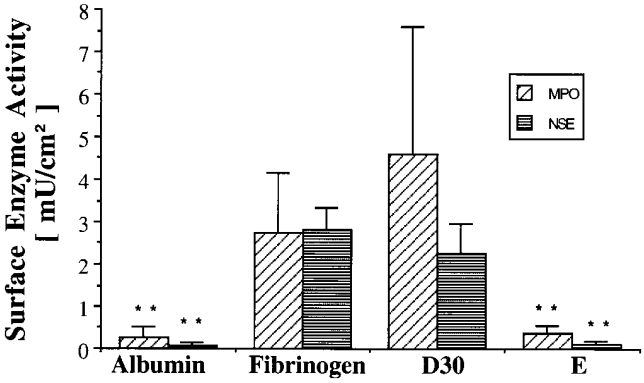

Figure 2. Phagocyte accumulation on the surfaces of PET disks preincubated with human fibrinogen (60 μg/ml), fragment D30 (60 μg/ml), fragment E (60 μg/ml), and albumin (10 mg/ml) after implantation in SW mice for 16 h. Vertical lines denote ±1 SD (n = 5 in all cases). **P < 0.01 vs. fibrinogen-coated implants. Estimated numbers of PMN: 119,700±60,300/cm^2 on fibrinogen-coated disks, 200,300±129,900/cm^2 on fragment D30–coated disks, 15,700±8,600/cm^2 on fragment E–coated disks, and 11,700±10,200/cm^2 on albumin-coated disks. Estimated numbers of Mϕ: 254,500±48,200/cm^2 on fibrinogen-coated disks, 204,500±63,600/cm^2 on fragment D30–coated disks, 10,000±10,900/cm^2 on fragment E–coated disks, and 8,200±6,400/cm^2 on albumin-coated disks.

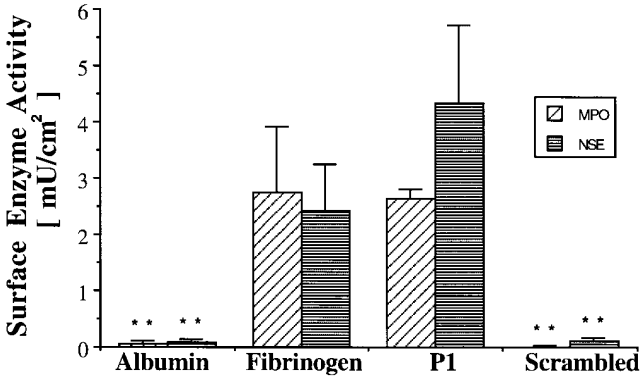

Figure 3. Phagocyte accumulation on the surfaces of PET disks preincubated with human fibrinogen (200 μg/ml), P1-albumin (200 μg/ml), scrambled peptide-albumin (200 μg/ml), and albumin (200 μg/ml) after 16 h of implantation in SW mice. Vertical lines denote ±1 SD ($n = 5$ in all cases). $^{**}P < 0.01$ vs. fibrinogen-coated implants. Estimated numbers of PMN: 120,000±50,900/cm^2 on fibrinogen-coated disks, 115,200±64,300/cm^2 on P1-albumin–coated disks, 400±900/cm^2 on scrambled peptide-albumin–coated disks, and 2,100±2,500/cm^2 on albumin-coated disks. Estimated numbers of Mϕ: 220,900±75,500/cm^2 on fibrinogen-coated disks, 392,700±128,200/cm^2 on P1-albumin–coated disks, 9,100±7,300/cm^2 on scrambled peptide-albumin–coated disks, and 7,300±5,500/cm^2 on albumin-coated disks.

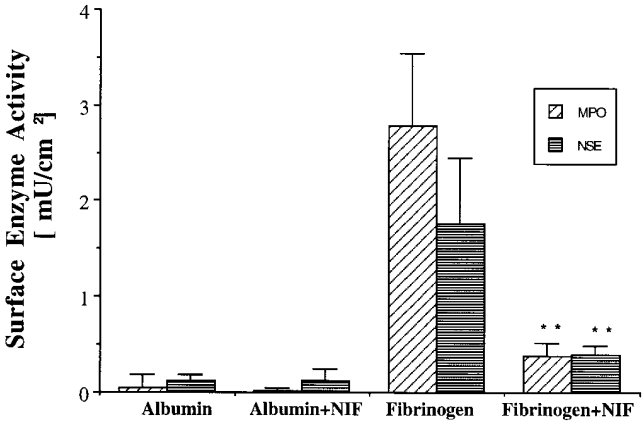

Figure 4. rNIF prevents most phagocyte accumulation on the surfaces of PET disks preincubated with human fibrinogen (60 μg/ml) and albumin (10 mg/ml) after 16 h of implantation in SW mice. Vertical lines denote ±1 SD ($n = 5$ in all cases). Significance of differences versus the same protein-coated implants in control (non–NIF-treated) mice: $^{**}P < 0.01$. Estimated numbers of PMN: on fibrinogen-coated disks, 121,400±33,000/cm^2 in control mice, 16,500±5,800/cm^2 in rNIF-treated mice; on albumin-coated disks, 1,900±3,700/cm^2 in control mice, 900±1,300/cm^2 in rNIF-treated mice. Estimated numbers of Mϕ: on fibrinogen-coated disks: 160,000±62,700/cm^2 in control mice, 36,400±7,300/cm^2 in rNIF-treated mice; on albumin-coated disks, 10,900±5,500/cm^2 in control mice, 10,900±10,900/cm^2 in rNIF-treated mice.

CD11b–fibrin(ogen) interaction without affecting other CD11b functions such as binding to coagulation Factor X (37, 38). Second, and more importantly, our preliminary results indicate that NIF does not block the recruitment of phagocytes to inflammatory sites, as does a monoclonal antibody to murine CD11b (5C6) (39). Accordingly, mice receiving fibrinogen- and albumin-coated implants were treated repetitively with NIF. The results (Fig. 4) show that Mac-1 blockade by NIF inhibits by > 75% phagocyte accumulation on fibrinogen-coated surfaces. Thus, interactions between one motif on fibrinogen (γ190–202) and Mac-1 may be particularly important in the accumulation of phagocytes on implant surfaces and, therefore, in early biomaterial-mediated inflammatory responses.

Discussion

Despite the increasing usage of biomaterials as implants and/or medical devices, little is known of the determinants of "biocompatibility" of such materials (40). Indeed, adverse reactions such as device-associated inflammation, fibrosis, coagulation, and infection are frequent and sometimes life-threatening. Within a few hours after implantation, most materials trigger acute inflammatory responses, reflected by an accumulation of phagocytic cells on implant surfaces. The ensuing chronic inflammation may prompt a number of serious iatrogenic consequences such as stress cracking on pacemaker leads (1, 2), osteolysis adjacent artificial joints (3–5), and fibrosis and capsule contracture surrounding mammary prostheses (6–10). In the search for the proximate trigger of these responses, a chaotic layer of spontaneously adsorbed host proteins on implant surfaces has been indicted as a possible culprit (11–15). This is because the adsorption of plasma proteins to implant surfaces is almost instantaneous; therefore, inflammatory cells likely interact with this protein layer rather than the material itself.

Earlier, we found that adsorbed fibrinogen plays a pivotal role in initiating acute inflammatory responses to biomaterial implants (15). In an effort to further elucidate the mechanisms underlying the recognition and adhesion of inflammatory cells to surface fibrinogen, we have attempted to identify the portion(s) of fibrinogen responsible for inflammatory cell accumulation. We first tested two proteolytic products, D100 (molecular mass of 105 kD) and E (molecular mass of 50 kD), which together comprise 76% of the intact fibrinogen molecule (molecular mass of 340 kD). After implantation, D100-coated surfaces are as proinflammatory as fibrinogen-coated surfaces. However, fragment E–coated surfaces are as inert as albumin-coated surfaces. Fragment D100 possesses two important functional sites in its γ chain remnant: a fibrin cross-linking site and the site recognized by integrin $\alpha_{IIb}\beta 3$ on platelets. These sites are lost when D100 is digested to D80. Nonetheless, D80-coated surfaces attracted an equally large number of phagocytes, equivalent to fibrinogen-coated and D100-coated surfaces. Even with further digestion to generate D30, D30-coated surfaces are as effective as fibrinogen-coated surfaces in prompting inflammatory responses. Several earlier studies have suggested that some phagocyte integrins can recognize and bind to the Arg-Gly-Asp sequences of many adhesive proteins including fibrinogen (41) which has two separate RGD sites: RGDF at Aα 95–98 and RGDS at Aα 572–575 (42). RGD sequences in extracellular matrix and clotting cascade proteins also are thought to be important in adhesion, chemotaxis, and phagocytosis (43–46). However, D30 contains no RGD sequence and still induces significant inflammatory responses. Therefore, in the special case of fibrinogen adsorbed to PET implants, RGD sequences may not be crucial in the process of phagocyte accumulation.

Importantly, however, D30 retains a motif recently found

to mediate the binding of phagocytes via the Mac-1 (CD11b/CD18) integrin (25). The specific cell binding region for Mac-1 has been identified as fibrinogen γ190–202 (21). Therefore, we hypothesized that this peptide might be involved in phagocyte accumulation on biomaterial implants. In support of this, P1 peptide (immobilized to albumin), but not a control (scrambled) peptide, induces phagocyte accumulation at least as marked as that on fibrinogen-coated surfaces.

Finally, it appears that the P1 sequence within adsorbed fibrinogen indeed may interact with the phagocyte integrin, Mac-1. This integrin has been found to be important in mediating the adhesion of both activated Mϕ and PMN to fibrinogen and/or fibrin in a variety of inflammatory responses (21, 25, 47–50). In support of this tentative conclusion, implants in mice also treated with NIF, which blocks CD11b–fibrin(ogen) interaction without affecting other CD11b functions (37), show minimal phagocyte accumulation.

We earlier concluded that the precedent surface adsorption of fibrin(ogen) was an absolute requirement for acute inflammatory responses to PET implants (15). However, in a number of other pathologic circumstances, there is also evidence of an intimate relationship between fibrin(ogen) accumulation and inflammatory responses. Thus, fibrinogen depletion has been shown to markedly limit the extent of septic abscess formation (51). Similarly, hypofibrinogenemic rats have substantially decreased proteinuria and glomerular PMN accumulation in a model of immune-mediated glomerulonephritis (52). This latter observation was tentatively attributed to a generalized decrease in both inflammatory cell recruitment and activation within the glomerulus. Furthermore, the proinflammatory influences of fibrin(ogen) may not be limited to direct effects on phagocytic cells. Very recent in vitro observations indicate that interactions between fibrin and peripheral blood mononuclear cells prompt greatly enhanced expression of the proinflammatory cytokine, IL-1β (53). In further support of this, we find that the in vitro interaction of rodent peritoneal lavage mononuclear cells with fibrinogen-coated (but not albumin-coated) PET surfaces also upregulates messages for IL-1α and TNF-α (Tang, L., unpublished observations).

Thus, this is by no means the first indication of a possible causal connection between the deposition of fibrin(ogen) and promotion of inflammation. However, to the best of our knowledge the present results constitute the first clear in vivo evidence of the possible molecular determinants of this interrelationship. At least in the case of biomaterial implants, it appears that phagocyte accumulation is mediated by interactions between fibrinogen γ190–202 and the phagocyte integrin Mac-1 (CD11b/CD18). Further knowledge of the interplay between biomaterials, adsorbed proteins, and host cells may permit the rational design of surfaces which discourage adverse inflammatory and/or fibrotic reactions. In a broader context, understanding of such interactions may lead to the development of new therapeutic strategies for the moderation of other types of pathogenic inflammatory responses.

Acknowledgments

We wish to thank Dr. Matthew Moyle for generously providing NIF and Diane Konzen for expert manuscript preparation.

This work was funded by grants from the American Heart Association (L. Tang) and the National Institutes of Health (J.W. Eaton and E.F. Plow).

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