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Review Article

Natural Responses to Unnatural Materials: A Molecular Mechanism for Foreign Body Reactions

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Introduction

Modern medical practice involves the use of an increasingly broad array of implantable and blood contact devices. Many of the polymers that form the base materials of these devices were not designed to marry with the host environment but were commercially available materials pressed into service as needed. Unfortunately, almost all of these polymers, such as polyurethanes, silicone elastomers, and polyethylene terephthalate (e.g., Dacron®), trigger, to varying degrees, undesirable host responses including coagulation, inflammation, and fibrosis (1–8). These host responses can in turn lead to the degradation and/or failure of biomedical devices (see, e.g., ref. 9), sometimes with fatal consequences.

Inflammatory and fibrotic responses are also typical of so-called foreign-body reactions triggered by implants acquired by misadventure, such as bullets and splinters. It is fair to say that we still do not completely understand the pathophysiologic mechanisms by which foreign materials—purposefully or accidentally intruded into the body—initiate and propagate inflammation and fibrosis. These reactions are most puzzling in the case of biomaterials that generally lack leachable toxins, are nonimmunogenic, and are chemically inert. This leads to the rather obvious question of how the host first detects and then mounts an inflammatory response to these apparently innocuous biomedical materials.

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What Characteristics of Biomaterial Implants Trigger Inflammatory Responses?

The surfaces of most commonly used biomaterials spontaneously adsorb a layer of host protein within seconds to minutes after tissue or blood contact (10,11). Probably as a result of the hydrophobic surface properties of these materials, adsorbed proteins progressively change conformation, becoming denatured and impossible to remove with detergents such as sodium dodecyl sulfate (12–16). These primary protein–surface interactions typically precede the arrival of host inflammatory cells at implant surfaces. Therefore, inflammatory responses are probably influenced, if not dictated, by this spontaneously acquired chaotic layer of host protein and it is believed that the types of adsorbed proteins comprising this layer are critical determinants of biocompatibility (16,17). Working from this assumption, we set about to determine those host protein(s) of greatest importance in ensuing inflammatory responses to experimental implants. To do this, we employed an animal model in which test specimens (of polyethylene terephthalate film) were implanted intraperitoneally in mice, in some cases after being coated with various proteins (12,18). The extent of acute inflammatory response was monitored by measuring the numbers of neutrophils (PMN) and monocytes/macrophages (Mϕ) adherent to the test specimens 16–24 hr after implantation.

Albumin, fibrinogen, immunoglobulin G (IgG) and complement components are usually the most abundant spontaneously adsorbed proteins on the surfaces of polymeric biomaterials (17,19,20). Adsorbed albumin probably can be

ruled out as causal of subsequent inflammatory responses because biomaterials precoated with purified albumin are "passivated" and attract few phagocytic cells (11,21–24). Furthermore, although many earlier studies have shown that adsorbed IgG and complement activation caused by biomaterial surfaces will activate phagocytes (25–28), both IgG-deficient (severe combined immunodeficient) mice and complement-depleted (cobra venom factor–treated) mice exhibit "normal" inflammatory responses to implanted biomaterials (18). Therefore, neither spontaneous IgG adsorption nor surface-initiated complement activation is critical in the recruitment of inflammatory cells to biomaterial implants (18).

Instead, and rather unexpectedly, surface-bound fibrinogen appears to be essential for the genesis of inflammatory responses to implanted biomaterials (12). There are three lines of evidence for this. First, experimental implants precoated with serum, which is almost completely lacking in fibrinogen, fail to accumulate phagocytes whereas those coated with plasma do (to the same extent as seen following implantation of uncoated material). Importantly, implants precoated with fibrinogen-reconstituted serum engender "normal" inflammatory responses. Second, by the same token, implants precoated with afibrinogenemic plasma are passivated, but those coated with fibrinogen-reconstituted afibrinogenemic plasma cause inflammatory cell recruitment. Finally, mice pretreated with ancrod (and thereby depleted of almost all detectable fibrinogen) fail to mount an inflammatory response to uncoated implants but do so if the material is preincubated with either plasma or purified fibrinogen (12). It appears that the adsorbed fibrinogen does not provoke an inflammatory response via down-stream activation of further coagulation. Thus, animals pretreated with coumarin [and therefore incapable of further vitamin K–dependent clotting reactions such as thrombin-mediated conversion of fibrinogen to fibrin (29–31)] exhibit a "normal" inflammatory response to implanted materials (L. Tang, W.-W. Jiang, W.-J. Hu, unpublished results).

How Does Adsorbed Fibrinogen Trigger Phagocyte Accumulation on Implant Surfaces?

In order to determine the fibrinogen epitope(s) possibly responsible for phagocyte accumulation on implants, we prepared purified plasmin degradation fragments of fibrinogen and used these to coat experimental implants. The results indicate that implants precoated with plasmin degradation fragment D100 (MW = 105 kD), but not E50 (MW = 50 kD), accumulate large numbers of phagocytes (both PMN and Mϕ), equivalent to those found on fibrinogen-coated disks. The D100 fragment was further digested to smaller products, including D80 (MW = 80 kD) and D30 (MW = 35 kD). Both adsorbed D80 and D30, neither of which contains RGD sequences, are fully active in fostering the in vivo accumulation of phagocytes. This suggests that the necessary motif(s) for phagocyte–biomaterial interaction resides within the fibrinogen D30 fragment.

Altieri and colleagues (32) had earlier determined that one epitope within the D30 fragment of fibrinogen on the gamma chain (γ190–202) could mediate phagocyte adherence to tissue culture plastics via interactions with the phagocyte integrin Mac-1 (CD11b/CD18). We therefore suspected that γ190–202 (abbreviated as P1) might be critically important in phagocyte–surface fibrinogen interactions. To test this, 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 cellular responses to peptides (33–35)]. Indeed, implant surfaces precoated with the P1 peptide, but not the scrambled version, cause substantial phagocyte accumulation roughly equivalent to that on fibrinogen-coated surfaces (36). Recently, another Mac-1 receptor sequence, γ377–395 (abbreviated as P2), which also resides within the D30 fragment, has been identified (37). Our preliminary results indicate that disks coated with P2-albumin conjugates are as effective as fibrinogen-coated disks in causing phagocyte accumulation. We have recently found that when purified fibrinogen binds to, and denatures on, biomaterial surfaces, both P1 and P2 epitopes, which are normally occult in soluble fibrinogen, become exposed (i.e., reactive with monoclonal antibodies) (W.-H. Hu, T. P. Ugarova, E. F. Plow, J. W. Eaton, L. Tang, unpublished results). These exposed neo-epitopes may then serve as opsonins, prompting the subsequent phagocyte adherence/activation and adverse tissue reactions.

How Do Phagocytes Locate Foreign Materials?

The events involved in biomaterial-mediated inflammatory responses (or foreign-body reac-

tions) may be somewhat arbitrarily divided into three consecutive processes: (*1*) phagocyte transmigration through the endothelial barrier, (*2*) chemotaxis toward the implant, and (*3*) adherence to the biomaterials (38). Below, we briefly describe what we know about the mechanisms involved in each of these.

Phagocyte Transmigration through the Endothelial Barrier

We and others (39) have observed that the tissue adjacent experimental implants is often hyperemic and edematous, characteristics of a localized histaminic response. Furthermore, large numbers of degranulating mast cells are also present in the interface between host tissue and biomaterial implants. Because mast cells are known to play important roles in phagocyte transmigration during inflammatory responses (40–42), we thought that biomaterial-induced mast cell activation and associated histamine release might be required to enable phagocyte transmigration through the endothelial barrier. In support of the involvement of histaminic effects, the administration of a combination of H1 and H2 histamine receptor antagonists to mice given implants effectively blocks most phagocyte accumulation on the implant surfaces (43).

The likelihood that mast cells are the important source of histamine is supported by studies on mast cell–deficient (WBB6F1-Wv/W) and normal control (WBB6F1-+/+) mice. In mast cell–deficient animals, PMN and Mϕ recruitment to the peritoneal cavity and adherence to implant surfaces are almost completely blocked (43). Therefore, perhaps not too surprisingly, mast cell activation and associated histamine release are crucial in permitting the transmigration of phagocytes through the endothelial barrier to the site of the implant (43). This, however, leaves the question of how the incoming phagocytic cells might be directed to the surface of the implant itself, given that biomaterials do not elaborate chemotactic substances.

Phagocyte Chemotaxis toward Implant Surfaces

The preferential accumulation of phagocytes on biomaterial implants or foreign bodies is not a random event. It is likely that certain chemokines might engender the migration of phagocytes toward implant surfaces. We employed reverse transcript-polymerase chain reaction (RT-PCR) to assess the production of a variety of cytokine and chemokine mRNAs. Phagocytes actually adherent to implant surfaces show up-regulation of message for macrophage inflammatory protein 1α (MIP-1α) and monocyte chemoattractant protein-1 (MCP-1) whereas nonadherent phagocytes recovered by peritoneal lavage of the same animals do not show similar up-regulation. Using neutralizing antibodies against these chemokines, we have recently found that both MIP-1α and MCP-1, but not MIP-1β and MIP-2, are critical chemokinetic effectors of the accumulation of phagocytes on implanted biomaterials (44).

Phagocyte Adherence to Biomaterial Implants

As indicated above, certain fibrin(ogen) epitopes, particularly P1, are known to interact with the phagocyte Mac-1 integrin (32,37). Therefore, Mac-1 might be required for phagocyte recognition of fibrinogen-bearing foreign bodies and biomaterial implants. This was recently tested using CD11b knockout (KO) and CD18 KO mice. Compared with congenic normal controls, both CD11b KO and CD18 KO mice failed to accumulate phagocytes on implant surfaces, although the influx of phagocytes into the implant-bearing peritoneum was unaffected.

Adding a bit more complexity to the situation is the well-established finding that resting, unstimulated phagocytes express only low levels of Mac-1 (45) (and, in fact, do not preferentially adhere to albumin-coated vs fibrinogen-coated surfaces in in vitro adherence assays; W. W. Jiang, L. Tang, unpublished results). The explanation may be that Mac-1 is up-regulated by cytokines such as tumor necrosis factor α (TNF-α) (46–48) as the incoming phagocytes travel toward the implant surfaces. Indeed, not only is TNF-α released in relatively large amounts following the placement of intraperitoneal implants in mice, but administration of a neutralizing antibody to TNF-α greatly decreases the numbers of phagocytes that ultimately become adherent to the implant (38,44).

So, the overall sequence of events in biomaterial-mediated inflammatory responses (and probably in at least some types of other foreign-body reactions) appears to be as follows: (*1*) Implanted biomaterials somehow trigger the activation of mast cells, releasing histamine and other proinflammatory products such as preformed TNF-α. (*2*) The released histamine promotes the attraction of phagocytes to areas of the microvasculature adjacent to the implant, loosens inter-

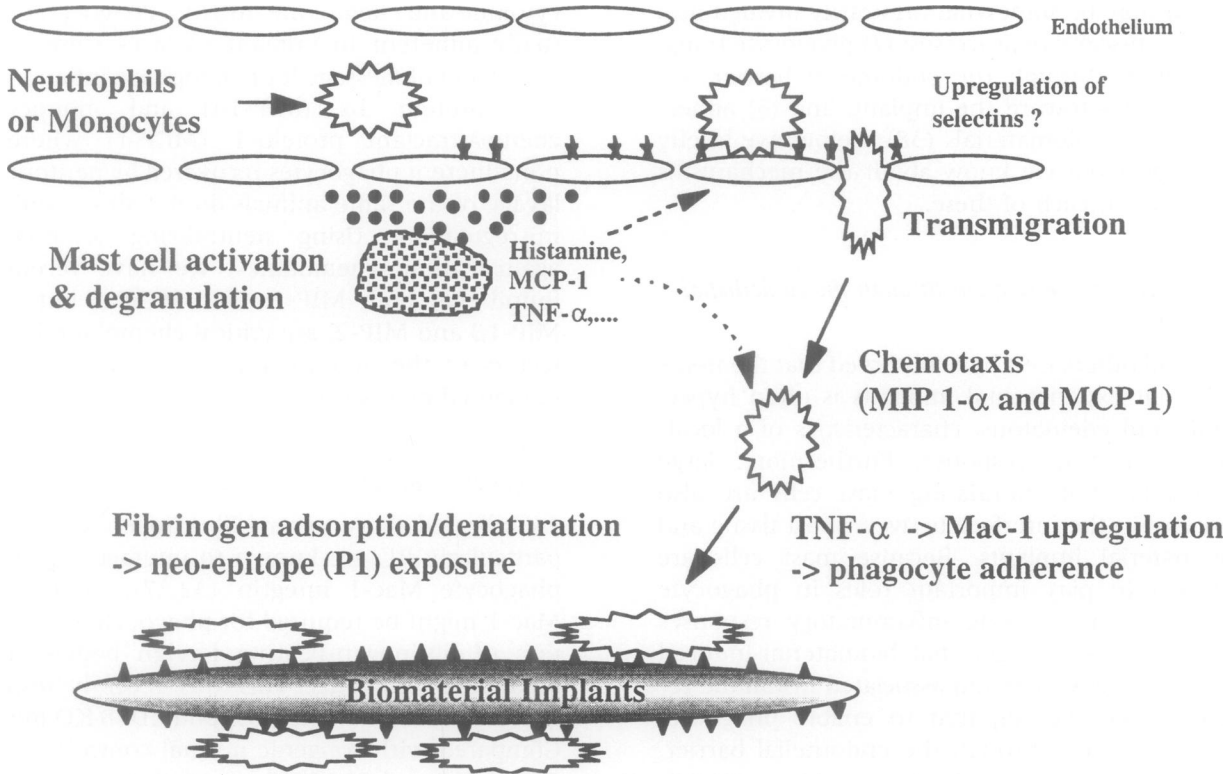

Fig. 1. Hypothetical sequence of events participating in acute inflammatory responses to implanted biomaterials.

endothelial bonds, and facilitates transmigration of phagocytes through the endothelial barrier. (*3*) The migration of incoming phagocytes toward biomaterial implants or foreign bodies involves at least two chemokines, MIP-1α and MCP-1, possibly first elaborated by pioneer cells that first reach the implant surface. (*4*) During the journey toward the implant, TNF-α (perhaps first released by mast cells and later by Mϕ) causes up-regulation of Mac-1 on phagocyte membranes. (*5*) Finally, the engagement of Mac-1 and the fibrin(ogen) epitope P1 (and possibly P2) enables the adherence and activation of phagocytes on implanted biomaterials and, quite likely, many types of implants acquired by misadventure (Fig. 1).

Why Should Surface-Bound Fibrinogen Promote Accumulation of Phagocytes on Implanted Materials?

Finally, on a more speculative note, we would like briefly to discuss why there is evidently a physiologic mechanism that enables inflammatory cells to recognize the partially denatured fibrinogen that accumulates on implant surfaces. Fibrin and fibrinogen degradation products, but not soluble fibrinogen, are known to participate in many situations that lead to inflammation and/or phagocyte recruitment. These include delayed-type hypersensitivity reactions, trauma, fibrosis, the reaction to many different types of solid tumors, and wound-healing reactions (49–58). It follows that surface-immobilized fibrinogen may resemble fibrin or fibrin degradation products. Fibrinolysis and fibrin degradation products are probably not involved in biomaterial-mediated inflammatory responses, because the powerful protease inhibitor, Trasylol, has no effect in vivo on the extent of acute inflammatory responses to biomaterial implants (59). Rather, the denatured fibrinogen bound to biomaterial implants may resemble normal insoluble fibrin as it occurs within coagulum. Especially on hydrophobic surfaces (which are typical of most implanted biomaterials), adsorbed proteins such as fibrinogen acquire an altered conformation and become tightly adherent (12–16). These

conformational changes of proteins adsorbed to hydrophobic biomaterial surfaces have been detected using many different techniques including sodium dodecyl sulfate (SDS) elution (60,61), scanning angle reflectometry (62), scanning force microscopy (63), and attenuated total reflectance Fourier transform infrared spectroscopy (14). In the case of fibrinogen it appears that interactions with hydrophobic surfaces cause the denaturation of the D domain (64) and the concomitant exposure of both P1 and P2 epitopes (which are ordinarily occult in soluble fibrinogen and exposed on fibrin) (37,65; W.-H. Hu, T. P. Ugarova, E. P. Plow, J. W. Eaton, L. Tang, unpublished results). This process is probably of importance in inflammatory responses because those types of biomaterials which prompt the greatest exposure of P1/P2 accumulate the highest numbers of phagocytic cells in vivo (W.-H. Hu, T. P. Ugarova, E. P. Plow, J. W. Eaton, L. Tang, unpublished results).

The reasons for fibrinogen behaving in this manner are not completely clear. On the molecular level, we do know that fibrinogen has a very high limiting viscosity number, $\eta = 0.24$ (66,67), indicating an unusual degree of hydration and elongation (68). The flexible, elongate shape of solution-phase fibrinogen also is supported by data from hydrodynamic measurements of model particles (69), low-angle X-ray scattering (70), neutron small-angle scattering (71), and fluorescence polarization (72). Within soluble fibrinogen, the fibrinogen D domain, which resides on both ends of the molecule, is highly folded and stacked with α-helices (73). Probably because of this highly folded configuration, the D domain is susceptible to conformational changes and denaturation caused by thrombin-catalyzed conversion to fibrin (74), minor environmental changes such as elevated temperature (74), or contact with hydrophobic surfaces (43). In the present instance, interactions between a hydrophobic foreign body and fibrinogen are likely to unfold the D domain, leading to the exposure of hidden proinflammatory epitopes, such as P1 and P2 (W.-H. Hu, T. P. Ugarova, E. P. Plow, J. W. Eaton, L. Tang, unpublished results).

Similar changes in state, with selective denaturation of the D domain, also occur upon the conversion of soluble fibrinogen to the insoluble fibrin clot (74). These state changes also are accompanied by the exposure of previously occult epitopes such as P1 and P2 (W.-H. Hu, T. P. Ugarova, E. P. Plow, J. W. Eaton, L. Tang, unpublished results). Therefore, the inflammatory reactions to biomaterial implants may actually recapitulate natural responses to hemorrhage and coagulation. In this latter circumstance, the homeostatic responses of greatest importance are increased phagocyte vigilance (enabling the search for, and destruction of, possible invading microorganisms) and the ultimate dissolution of the clot, promoted in part by the accumulated phagocytes (75). Ironically, in choosing hydrophobic polymers as base materials for medical devices, we may have inadvertently set off a cascade of responses meant for an entirely different purpose. It is not until we fully understand the genesis of these responses that we will be able to design more biocompatible materials and finally satisfy the dictum advanced by Robert Hooke:

> *The truth is, the science of Nature has been already too long made only a work of the brain and the fancy: It is now high time that it should return to the plainness and soundness of observations on material and obvious things (76).*

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