Analysis of Inflammation

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Abstract
In the past, inflammation has been associated with infections and with the immune system. But more recent evidence suggests that a much broader range of diseases have telltale markers for inflammation. Inflammation is the basic mechanism available for repair of tissue after an injury and consists of a cascade of cellular and microvascular reactions that serve to remove damaged and generate new tissue. The cascade includes elevated permeability in microvessels, attachment of circulating cells to the vessels in the vicinity of the injury site, migration of several cell types, cell apoptosis, and growth of new tissue and blood vessels. This review provides a summary of the major microvascular, cellular, and molecular mechanisms that regulate elements of the inflammatory cascade. The analysis is largely focused on the identification of the major participants, notably signaling and adhesion molecules, and their mode of action in the inflammatory cascade. We present a new hypothesis for the generation of inflammatory mediators in plasma that are derived from the digestive pancreatic enzymes responsible for digestion. The inflammatory cascade offers a large number of opportunities for development of quantitative models that describe various aspects of human diseases.
INTRODUCTION

Inflammation or the inflammatory process is a medical term, not an engineering one. Since the early nineteenth century, the inflammatory process has been one of the most intensively investigated areas of experimental medicine. Starting with Adamin of McGill University (1), medical textbooks devoted major sections to inflammation, and today a number of textbooks and conference proceedings are entirely focused on the topic (2). The microcirculation is the major playground where the events in inflammation can be studied and analyzed. In fact, one of the founders of modern bioengineering, Benjamin W. Zweifach of the University of California, San Diego (UCSD), a physiologist and a pioneer in microvascular research (3, 4), set out to develop new quantitative approaches for the study of inflammation when he joined a group of engineers at the California Institute of Technology. His effort led to the formation of a formal alliance between engineering and medicine and the start of bioengineering as an organized academic activity at UCSD.

Although recognized for its classical medical signs of acute pain sensation, heat, redness, swelling, and eventual healing of the tissue with scar formation, from an engineering point of view, inflammation may be more appropriately referred to as an inflammatory cascade. It consists of a long chain of reactions and cellular activities that serve to repair a tissue in many circumstances of life, from a small skin cut or repair of tissue after birth to healing of the most severe burn injuries. The inflammatory cascade at the tissue and cellular level involves a sequence of events with dilation of the arterioles and venules, as well as increased blood vessel permeability and blood flow, followed in many cases by stasis and thrombosis, infiltration of leukocytes into the tissue, escape of plasma into the tissue, a breakdown of tissue by proteolytic activity and oxygen free radical formation, necrosis and apoptosis, removal by phagocytic cells, generation of new humoral mediators for cell growth, and regeneration of new functional and connective tissue. The latter stage of inflammation has been referred to as resolution of inflammation (5, 6) (Figure 1). An inflammatory cascade that does not reach resolution leads to organ dysfunction and eventually death. Just about every tissue has the ability to execute an inflammatory cascade. The inflammatory cascade is preprogrammed and stereotypic. It is the only known mechanism for tissue repair after injury and as such it is at the heart of medical thinking. Some of the most interesting cellular processes (e.g., chemotaxis, phagocytosis, mitosis, cell differentiation) are part of the inflammatory cascade.

The literature on inflammation is very rich and far beyond the scope of a single review. Many medical journals are devoted to the subject and many more list inflammation as one of their topics of interest. I focus here on selected microvascular, cellular, and molecular events and otherwise will refer to reviews. The discussion will be limited to an outline of some key events in the inflammatory cascade. There is a large literature on how the immune system (antibodies and cellular immunity) triggers an inflammatory reaction and there is another large clinical literature that deals with interventional studies against individual steps in inflammation. Without doubt, future articles in this series will need to address selected aspects of the inflammatory cascade.
INFLAMMATION AND DISEASE

Inflammation is present in patients with bacterial, viral, fungal or parasitic infections; in anaphylaxis; in environmental diseases (smoke inhalation, asbestos exposure, etc.); in rheumatoid arthritis, gout, autoimmune diseases, and intestinal diseases; and in endocrinological or autoimmune diseases; as well as in chronic diseases such as diabetes (see, for example, any textbook on internal medicine).

But in the past three decades, it has also become evident that a much larger variety of diseases have telltale cellular and molecular evidence for inflammation. These include chronic arterial and venous disease (7, 8), myocardial ischemia (9–11), acute cerebral stroke and Alzheimer’s chronic disease (12–17), and more recently arterial
hypertension (18) and cancer (19–21). For several decades there have been clinical
observations and recently also molecular evidence for inflammation in osteoarthritis
(22–24). A severe form of inflammation is observed in shock and multiorgan fail-
ure (25, 26), a condition with one of the highest mortalities. There are signs of
inflammation in patients with depression (27, 28) even without overt symptoms for
inflammation or just exposure to environmental risks (29). The list of diseases that
are associated with molecular markers of inflammation is large and growing. Since
introduction of effective and inexpensive measurements to detect signs of inflam-
mation in plasma of larger groups of patients [one of them is the c-reactive protein
(cRP) synthesized during inflammation in the liver together with other proteins like
fibrinogen], a wave of large-scale clinical studies provide supporting evidence for
the smaller experimental studies in the past (30–33). Clinical studies statistically link
inflammation to obesity (34, 35) and a lack of regular (moderate but not excessive)
exercise (36, 37). Inflammation is also a key problem in biomaterials dealing with
the events at the interface between a living tissue and a nonliving implant or a living
graft (38, 39) and in the design of blood substitutes (40). Therefore inflammation is
a central issue in tissue engineering. Today, inflammation has become the holy grail
for studies of human disease. Antiinflammatory treatments that have been shown to
be effective in one disease may turn out to be effective in another, thereby opening a
wider range of opportunities for intervention.

In the meantime, from a bioengineering point of view, no quantitative (i.e., pre-
dictive) model of the inflammatory cascade is available. But selected aspects have been
studied, although only within a narrow scope considering the cascade as a whole. Most
aspects of the inflammatory cascade are still at the beginning of a quantitative analysis,
and yet we are at one of the most important steps in the analysis, i.e., identification
of the tissues, cells, proteins, and genes that participate in the cascade. Medicine has
been deeply engaged; engineering science has to become involved as well. Tradi-
tional thinking of what constitutes a valid engineering analysis of well-defined field
equations and boundary value analysis, their prediction and experimental verification,
must be expanded. Identification of the players (among different cell types, thousands
of genes, proteins, lipids, and carbohydrates) has to become an integral and early part
of the engineering analysis. Identification of the molecular players constitutes sig-
ificant progress and can often lead to new ideas for possible interventions. There are
numerous opportunities for diagnostic and interventional designs. The complexity
of the inflammatory cascade is one of the great opportunities for biomedical engi-
neering.

EXAMPLES OF INFLAMMATION IN THE
MICROCIRCULATION

The various forms of inflammation differ in large part by their location in the tissue,
the organ where they occur, and the nature and the severity of the tissue injury (e.g.,
mechanical injury, infectious agent, chemical injury, burn injury, radiation, tissue
injury owing to lack of organ perfusion, and oxygen supply).
A Localized Endothelial Injury

A particularly localized form of tissue injury that permits experimental study of in vivo inflammation almost at the level of single cells is by means of a narrowly focused laser burn. Generation of a laser injury on microvascular endothelium leads to rapid growth of a platelet thrombus over the injury site by attachment of platelets to the injured endothelium and to each other with little entrapment of red cells. Depending on the size and intensity of the laser injury, the thrombus may grow to occlude the microvessel, but may also stop growing and detach from the injury site. Detachment may occur in form of fragmentation of the thrombus, for example, from its tip, but may also detach at the base of the thrombus where it is attached to the endothelium, and thus this is the region in the thrombus with the lowest shear stress. This evidence suggests that there is an enzymatic process that serves to cleave the thrombus over the endothelium. Upon release the thrombus is carried by the bloodstream in the circulation, smaller thrombi are broken up as they pass through the capillary network, and large aggregates may obstruct microvessels in which they become entrapped. The original burn site may be subject to renewed platelet adhesion and repetition of the cycle that start with a platelet thrombus grown followed by release in form of an embolus. In the early phase of this form of tissue injury a few cells are observed to migrate into the tissue (41–43).

A Tissue Burn

In contrast, if the laser burn is applied to a local tissue region in which entire cell groups are injured, a somewhat different cellular sequence of events is observed. If injury in the tissue is generated, one sees an elevation of microvascular permeability in the adjacent blood vessels even though they may not have been hit by the laser burn per se. Circulating leukocytes carried from central arteries that are unaffected by the laser burn attach to the endothelium and eventually migrate toward the site of the laser injury in a chemotactic process. Once leukocytes arrive at the laser injury, their migration is arrested and they accumulate along the periphery of the burn injury site. They phagocyte cell debris generated by the laser burn. In this injury model there is less evidence of platelet adhesion to the neighboring microvessels, unless the endothelium is actually injured. The leukocyte population initially consists of neutrophils but is eventually followed by monocytes that upon migration into the tissue differentiate into macrophages. In general, during the initial reaction the tissue injury generated by the laser tends to grow in size but is eventually replaced by new cells and even by growth of new blood vessels. The tissue that replaces the burned tissue in many—but not all—cases is less-functional connective tissue (e.g., extracellular matrix, fibroblast, mast cells). The role of stem cells in regeneration of functional tissue is largely unexplored in inflammation (44).

Ischemia

A different form of inflammation is observed in ischemia followed by reperfusion. Any prolonged reduction of the blood flow in the microcirculation is followed by a
period of reperfusion with typical signs of inflammation. Although cell injury may occur already during ischemia, reperfusion frequently enhances injury or even death of previously intact cells, the extent of which depends upon the duration and degree of blood flow reduction during ischemia. Arterioles dilate, the endothelium in capillaries and postcapillaries develops an elevated permeability, leukocytes adhere to the endothelium, individual capillaries may stop being perfused, tissue mast cells degranulate, blood flow may even reduce as reperfusion is continued, and organ and cell functions deteriorate to the point of complete failure. The tissue injury is enhanced if the reduction of blood flow is accompanied by elevation of the microvascular blood pressure (i.e., by an occlusion on the venous side of the circulation) as compared to an occlusion on the arterial side (with reduction of microvascular pressure during the occlusion) (45). Leukocytes also adhere to the endothelium and migrate into the adjacent tissue, but are less directed than in the case of a local laser injury. In more severe forms of ischemia/reperfusion, red cells may escape into the interstitial space, especially from microvessels with elevated blood pressure (such as a venous occlusion).

A similar form of inflammation can be generated by a number of proinflammatory mediators, such as generation of platelet-activating factor (PAF, a membrane fragment derived from phospholipids), the presence of complement fragments (complement plays an important role in antigen-antibody reactions and is proteolytically fragmented from a large initial complex into smaller bioactive peptides), cytokines (small signaling proteins without enzymatic activity), or nitric oxide blockade. The inflammation generated by such biochemical mediators does not require a reduction of blood flow but qualitatively leads to similar microvascular manifestation of the inflammatory cascade, as observed in ischemia/reperfusion.

Physiological Shock

A somewhat different inflammatory cascade can be observed in physiological shock (e.g., owing to a major blood loss, a major burn, or trauma). In shock, organs that are not involved in the initial injury may become victims of the inflammatory cascade. The intestine or the pancreas are especially effective in this respect. The intestine can generate a form of inflammation that spreads globally and causes injury in remote innocent bystander organs that are not involved in the initial events that led to inflammation in the intestine (e.g., by occlusion of the superior mesenteric artery). This particular form of inflammation is accompanied by entrapment of platelets and leukocytes in the microcirculation of remote organs (especially the lung and liver), generation of blood coagulation and widespread capillary occlusion, elevated permeability and tissue swelling, aggregation of red cells, reduction of central blood pressure, and cell apoptosis and parenchymal tissue failure in different organs (pulmonary, renal, hepatic, and central nervous organ dysfunctions). Consequently, the intestine is a source of inflammation in which multiple organs fail that were unaffected by the original injury. It is the way many people die. We discuss this extraordinarily important case of inflammation below.
Inflammation Generated by Arthus and Shwartzman Reaction

A specialized form of inflammation was observed by Arthus after repeated injections of horse serum into the skin of a rabbit. He noted that at first there was redness and swelling. With each subsequent injection the reaction became more intense and finally led to necrotic lesions. The reaction can be evoked by several different materials (bacterial infiltrates) and is accompanied by accumulation of leukocytes, degranulation, and apoptosis.

Shwartzman in 1927 described a related inflammatory skin reaction, which now bears his name (46). Injection of a filtrate of *Bacillus typhosus* in the skin by itself produced only a mild reaction. If the initial injection is followed 24 h later by an intravenous injection with the same bacterial filtrate, a hemorrhagic and often necrotic skin lesion is produced at the original injection site. The reaction can be produced with different bacterial filtrates and with more purified endotoxin. In fact, many inflammatory materials of larger molecular weight that do not diffuse significantly from the injection site produce a Shwartzman reaction. It requires the presence of neutrophil infiltration into the tissue, which is the consequence of the primary injection.

There are as many forms of inflammation as there are ways to injure living tissues; each plays out in its specific way. But all forms of inflammation under normal conditions end up with a repaired tissue, whereas inflammation that goes unchecked leads eventually to loss of organ function and even to the point of tissue necrosis if the affected tissue does not belong to the vital organs (e.g., as seen in skin ulcers).

Even newborns have the ability to mount an inflammatory reaction, although in some cases with attenuated level of inflammatory indicators (47, 48). Inflammation tends to become more extensive and aggravated with age (49).

CELLULAR MARKERS OF INFLAMMATION

Although there are many approaches to study inflammation in living tissues of acute or chronically instrumental models, the study of inflammation in man is still limited unless the inflammation is superficial and accessible by current microscopic techniques. There is an evolving technology using microbubbles that adhere to inflammatory sites and can be detected with whole-body imaging techniques (e.g., ultrasound) (50–53). These may facilitate future direct study of inflammatory markers in individual organs of patients.

In the past, the majority of studies of inflammation have relied on a collection of venous blood samples, or to a limited degree urine or lymph samples. Such samples will only be useful if the inflammation has reached a more advanced stage so that markers of inflammation become globally detectable. There are then two opportunities, testing either circulating cells and/or plasma.

Cell Activation Observed In Vitro

Early signs of inflammation can be detected by study of individual cells in the circulation, e.g., from a venous blood sample. A good candidate is circulating leukocytes
because they not only play a central role in inflammation but they are also readily available from patients and volunteers for experimental and patient studies. Two general approaches are possible: a study of the level of activation of circulating cells or collection of plasma from patients. This plasma can then be applied to naïve cells from nonsymptomatic volunteers without inflammation or to a cell culture previously not exposed to overt inflammatory stimuli. The two approaches give different information. A study of the level of activation of circulating cells has to depend on those cells that can actually be collected, for example, from an arm vein. But the circulating cells collected in this fashion are all cells that have the ability to circulate, and therefore they have low levels of cell activation. Fully activated leukocytes or platelets quickly become trapped in the next capillary network they enter and stop circulating. Thus a venous blood sample contains a subpopulation of cells with relatively low levels of activation and as such is not representative of the cells in general and is not participating in the local inflammation (54). In contrast, if plasma samples are used for analysis of cell activation, it is possible to determine whether there are inflammatory mediators present in plasma. Such evidence raises questions about the biochemical nature and source of humoral activators. Inflammatory mediators suspended in plasma have the ability to reach every organ, and they may play an important role in spreading cell activation and inflammatory reactions from one organ to the next.

**Pseudopod Formation**

One of the most visible signs of cell activation is cytoplasmic pseudopod formation (Figure 2). Pseudopods are local cytoplasmic projections generated by reorganization of actin and other cytoskeletal proteins that can reach several micrometers in size, have many different shapes, and are membrane covered. A microcirculation with low levels of inflammation has few leukocytes with pseudopods. Pseudopods are the essential element to give a cell the ability to spread and move on an adhesive substrate (55). Pseudopods are generated by actin polymerization (56), triggered typically by G protein–coupled receptor activation (for example, the formyl peptide receptor, the interleukin-8 receptor, and the platelet-activating receptor) (57) via a signaling pathway that includes phosphatidylinositol 3-kinase (PI3 kinase), phosphatidylinositol-P3 (PIP3), small guanosine triphosphatases (GTPases), Wiskott-Aldrich syndrome protein (WASp), and actin-related protein 2/3 (Arp2/3) as an actin-binding protein that determines the spreading directions of pseudopods (57, 58). Pseudopod projection is not necessarily Ca²⁺ ion dependent (59), and there exist signaling pathways that are PI3 kinase–independent if leukocytes are primed (exposed but without full stimulation) with insulin (60). Growth of pseudopods proceeds from the tip by diffusion of G-actin toward a thin actin polymerization layer underneath the cell membrane where actin monomers are inserted into the actin filaments, thereby pushing the membrane outward (61). Projection of pseudopods may occur whether a leukocyte is in free suspension or whether it is attached to endothelium or other substrates. Pseudopods are projected and retracted in a cyclic fashion and typically grow at a rate of approximately 10 μm/min. When exposed to specific agonists, their rate of projection depends on agonist concentration (62).
Figure 2
Scanning electron micrograph of a neutrophil in a nonactivated state (with red cell) (top panel) and a neutrophil after activation (bottom panel). The nonactivated cell has an overall spherical shape with small membrane folds. After activation, the cell projects characteristic pseudopods owing to actin polymerization. The process is frequently accompanied by expression of membrane adhesion molecules, degranulation, and oxygen free radical production. Bar length = 10 μm.

Pseudopods are essential elements for many inflammatory activities of circulating leukocytes, be it spreading on a substrate or over a bacterium during phagocytosis or projection during migration across the endothelium into the tissue. Different leukocyte types have different pseudopod shapes, some of them are sheet-like (lamellipodia) and others are finger-like (filipodia), and membrane-covered tethers formed at points of cell retraction. The mechanics of projecting pseudopods are dominated by the polymerization of actin filaments either at their tips underneath the membrane or along preexisting actin fibers at the point of the actin-related protein (ARP2/3), a process that permits spreading of the actin polymers into veil-like shapes (63). In contrast, the mechanics of tethers are determined largely by membrane tension (64). Even though pseudopods are mobile due to cyclic actin assembly and disassembly, at any instant of time pseudopods have cross-linked fibrous actin, with mechanical properties that are elastic with a minimal viscoelastic creep (65).
Pseudopod projections are rarely detected on endothelial cells in normal blood vessels. In contrast, lamellar-shaped pseudopods are observed in capillaries after blood pressure reduction (Figure 3). They frequently appear at the tight junctions between neighboring endothelial cells or are formed when endothelial cells in capillaries attach to themselves to form a tubular structure that makes up the capillary. The cytoplasmic projections have a thin sheet-like shape that may reach several micrometers into the lumen of microvessels. In capillaries with narrow vessel lumen, such cytoplasmic projections are sufficient to interrupt the flow of blood cells (66). Cytoplasmic pseudopods formed by endothelial cells are also observed when leukocytes migrate across the endothelium (see below), when endothelial cells phagocytose, or when they migrate during angiogenesis. Dewey and colleagues have introduced a direct technique with fluorescently labeled actin to visualize cytoplasmic actin fibers in endothelial cells and to study the turnover of actin in pseudopods (67, 68).

Platelets also undergo dramatic shape changes after activation. Besides transformation from their normal discoid shape into spheres (69), in a process that is temperature sensitive (70), they form cytoplasmic extensions that are finger shaped (filopodia).
and may reach several micrometers by both calcium-sensitive and Rho-associated kinase p160ROCK pathways. Filopodia contain actin bundles of long actin filaments that end with an actin-capping protein at their tip. They also form filipodia bundles that are abnormal in that they do not end at the filopodial tips but form actin loops that return to the cell body (71). The discoid shape of platelets in the passive state is maintained by a marginal band that consists of a single microtubule wound into a coil (72).

**Membrane Adhesion Molecules**

An important form of cell activation in the microcirculation is the expression of membrane adhesion molecules that facilitate the attachment of circulating cells to the endothelium and to tissue structures over which they migrate. Two general classes of adhesion molecules have been described in leukocytes, integrins, and selectins and their ligands. They are preexpressed in the plasma membrane and also in cytoplasmic granules, or may be newly synthesized upon stimulation. The topic is discussed in detail in several recent reviews (73, 74); therefore I highlight only a few selected topics important for microvascular inflammation. Initial leukocyte attachment to endothelial membranes is facilitated by selectins, a group of three mammalian lectins (P-, E-, L-selectin) capable of generating rapid and strong carbohydrate-protein bonds with their ligands (P-selectin glycoprotein ligand-1, PSGL-1; sialyl Lewis X; and many others). Leukocytes have L-selectin located preferentially on the tip of leukocyte microvilli regions of the membrane that make initial attachment with the endothelium (75). P-selectin in endothelial cells preexpressed in membrane bounds vesicles, the Weibel-Palade bodies, a set of membrane-covered granules in their cytoplasm (76) from which they can be released onto the plasma membrane (77), and therefore rapidly facilitate rolling attachment. P-selectin can also be induced by de novo protein synthesis, a much slower process. E-selectin is predominantly on endothelium and needs to be induced under inflammatory conditions. The lifetime of the bond between P-selectins and their counter-receptors increases with applied force but without a tendency for firm adhesion (78, 79), and vice versa, as the force is reduced the two molecules reduce the bond lifetime to the point that at low forces they detach altogether (80) (designated as catch bonds). Evans and his colleagues have identified the PSGL-1/P-selectin attachment to depend on metal-ion (Ca$^{2+}$) bonds and weaker hydrogen bonds (81).

In contrast, firm membrane adhesion and spreading of cells on the endothelium are facilitated by integrins, a family of heterodimers, and tyrosine kinase receptors that attach extracellular matrix adhesion sites to the structural proteins in the cell cytoplasm and also serve molecular signaling. Leukocyte adhesion associated with migration across the endothelium requires the engagement of integrins. The integrins used by leukocytes share a common β$\alpha$-chain (CD18) and four different α-chains (α$\i$ or CD11a; α$\m$, CD11b; α$\x$, CD11c; α$\u$, CD11d). One of the important integrins in the inflammatory reaction is CD11b/CD18 (also known as complement receptor 3 or Mac-1) expressed on neutrophils, monocytes and macrophages, and natural killer lymphocytes. Mac-1 is presynthesized (like P-selectin) and stored in...
cytoplasmic granules and transported to the cell surface upon stimulation or linking of L-selectin.

An interesting feature in inflammation is the fact that the integrin can be found in different stages: a passive stage, an activated stage, and even intermediate stages. Activation can be achieved by stimulation of the cells with humoral inflammatory mediators, but it can also be achieved by fluid mechanical stress (H. Shin & G.W. Schmid-Schönbein, unpublished results). In the activated stage, Mac-1 undergoes a conformational change in its molecular structure that exposes an active binding site (82).

Mac-1 can bind to a variety of ligands, an important one being the intercellular adhesion molecule 1 (ICAM-1) expressed on specific endothelial cells in the venules of the microcirculation (83). Arterioles and capillaries have less ICAM-1 expression. ICAM-1 is a member of the immunoglobulin superfamily. Its expression is regulated by transcription through mechanisms in the microcirculation that are largely unknown. Animals with genetic deletions of ICAM-1 exhibit significant reduction of inflammatory signals after challenge with endotoxin, ischemia/reperfusion, and others.

Platelets have, in part, similar and also their own membrane adhesion molecules, some of which are stored in granules and released upon stimulation (84). Platelet P-selectin in the plasma membrane surface serves as a cell adhesion receptor to interact with other cell receptors, including PSGL-1 and glycoprotein (GP) Iβ, a subgroup of the platelets membrane glycoprotein (GP) IIb-IX-V complex (85, 86). Platelets also utilize the glycoprotein (GP) IIb-IX-V complex to roll and slow down on a surface coated with von Willebrand factor (vWF), a protein that can be released from the Weibel-Palade bodies of activated endothelial cells (87, 88). They also use the GP IIb-IX-V complex to bind to subendothelial extracellular matrix proteins (88). Binding to collagen is facilitated by the platelet collagen receptors α2β1 and GPVI. These two molecules also play an important role in the activation of platelets (43). The integrin α2β1 binds platelets firmly to extracellular matrix proteins (collagen, fibronectin, laminin, among others) (89). Platelet adhesion to the endothelium in turn serves to facilitate adhesion of other cells to the endothelium or adhesion of platelets to other circulating cells (86, 90–93).

The pathways that lead to expression and activation of the adhesion molecules in inflammation have mostly been studied in acute situations, less in chronic conditions. The interactions are complex. They may be redundant, in part owing to the fact that specific adhesion molecules may only play a vital role under specific inflammatory stimulations.

**Oxygen Free Radical Formation**

A major hallmark of cell activation in the microcirculation is oxygen free radical formation (superoxide O2−, hydrogen peroxide H2O2, the hydroxyl radical, nitric oxide-related oxidants) (94–96). A number of techniques have been introduced that permit detection of free radical formation in vivo with fluorescent indicators, so that their location and time course can be studied. One of the earliest of these in vivo
Figure 4
Selected micrographs of the rat mesentery with visualization of oxyradical-dependent photoemission with ultrasensitive video intensifier microscopy during endothelium-granulocyte interaction in microvascular beds treated with platelet-activating factor (100 nM) (97). The photonic burst were detected with a chemiluminescence probe (luminol) and superimposed digitally on a bright field image of the same region of the mesentery (335). A: arteriole, V: venule. Reproduced with permission, courtesy of Dr. Makoto Suematsu.

Techniques was introduced by M. Suematsu and his colleagues at Keio University using an ultrasensitive photon counting technique in conjunction with fluorescent indicators that are reactive to individual free radical species (97). Stimulation of a microcirculation leads initially to superoxide formation that is closely colocalized with individual neutrophils and occurs in bursts (Figure 4). Stimulation over several minutes to hours leads to the formation of free radicals in more than the neutrophils. Multiple free radical species are detectable in the vicinity of the endothelium in venular locations where neutrophils migrate across the endothelium and into the interstitial space. A lower level of free radicals is formed in the interstitial space adjacent to the arterioles or capillaries where fewer or no leukocytes are present (98). One of the most toxic free radicals formed in vivo is the hydroxyl radical, which requires an iron-catalyzed reaction (Haber-Weiss reaction) for its formation. Therefore, the source of iron is an important consideration in understanding the oxygen free radical dynamics. A major source of iron is derived from hemoglobin (99, 100). Thus, while fresh hemorrhages into a tissue may in fact be protective against oxygen free radical damage, excessive iron in the body can lead to iron overloading and tissue damage.
radical-mediated parenchymal tissue injury—owing to the presence of scavenging molecules (like superoxide dismutase and catalase)—older red cells become highly cytotoxic to the microcirculation because they are depleted of such scavenging capability and at the same time may be a source of iron that catalyzes the Haber-Weiss reaction. Escape of aged red cells into the tissue causes enhanced parenchymal cell death (101).

**Degranulation**

The variety of granules and lysosomes carried by most cells in the microcirculation gives degranulation during inflammation a special significance. Degranulation of membrane-covered organelles requires cytoplasmic rearrangement and membrane fusion. Membrane proteins may be incorporated into the plasma membrane, or the presynthesized contents in the organelles may be released into the surrounding tissue. The broad variety of granule contents that can be released in inflammation leads to numerous signaling possibilities.

One of the most visible signs for cell degranulation during inflammation is observed in tissue mast cells, carriers of a prominent set of granules with histamine, bradykinin, tryptase, and arachidonic acid derivatives, each of which in turn serve to activate other microvascular cells (102). Mast cells can be degranulated by nitric oxide suppression via a superoxide-mediated mechanism that in turn generates leukocyte adhesion to the endothelium and triggers an inflammatory cascade with elevated permeability (103, 104). Mast cell degranulation also plays an important role in the initiation of inflammation in ischemia/reperfusion in an organ-dependent way that can generate microvascular dysfunction in the intestine but less in skeletal muscle (105). The process can be mediated by histamine (106) and PAF (107) and involves expression of P-selectin, likely owing to release and degranulation of Weibel-Palade bodies. The degranulation of Weibel-Palade bodies facilitates release of VWF, a molecule that serves to initiate coagulation and platelet adhesion (108, 109) and of interleukin-8 (IL-8), which in turn mediates inflammation (76). Consequently, degranulation of Weibel-Palade bodies and P-selectin expression on the endothelial surface facilitates neutrophil adhesion, which stimulates release of proteolytic granules, carriers of a wide variety of antibacterial agents and lysosomal enzymes (110, 111). Hypoxia (112) and activated platelets (90), in addition to other stimuli (thrombin, epinephrine, or histamine), will also stimulate secretion of Weibel-Palade bodies (76). Thus, there is an intricate network of cells, adhesion molecules, and humoral signaling molecules that serves to control the inflammatory cascade at this step. No quantitative engineering analysis exists.

**Endothelial Glycocalyx**

Recent evidence supports the notion that the glycocalyx, which grows by biosynthesis of glycoaminoglycans on endothelial cells, may form a layer, which if intact, has an antiinflammatory effect (113). The glycocalyx may reach a length that in some studies was reported to be several hundred nanometers (114) and clearly exceeds the size...
of the extracellular domain of adhesion molecules such as ICAM-1. Even though ICAM-1 is constitutively present on postcapillary venules, it serves only as an active binding site if the glycocalyx is enzymatically cleaved during inflammation or after ischemia/reperfusion (115).

**Plasma Markers for Inflammation**

The most direct and conclusive way to demonstrate the presence of inflammatory mediators in plasma is to mix the plasma with naïve cells (such as leukocytes, platelets, and endothelial cells) and measure markers of inflammation on those cells (such as pseudopod formation, free radical formation, etc., as discussed above). But this approach is less practical in large-scale clinical studies in which a large number of samples are under investigation. The ideal way is to detect directly the molecule responsible for the cell activation. But we do not know presently the specific molecule(s) that cause(s) cell activation during inflammation, and much of the current evidence suggests that there may be more than one inflammatory mediator present. Instead, it is necessary to rely on specific molecules produced during inflammation. There are a number of possible candidates. One class of molecules has been designated as acute-phase proteins (also designated acute-phase reactants). They are synthesized in the liver, neurons, lymphocytes, and monocytes during inflammation and released into the plasma. These molecules include fibrinogen, c-reactive protein (cRP), serum amyloid A, α1-acid glycoprotein, α-1 antichymotrypsin, α-1 antitrypsin, haptoglobins, ferritin, and others. The protein synthesis can be induced by cytokines (IL-1, IL-6). cRP and fibrinogen levels have been found to be useful clinical indicators that have low daily fluctuations (116, 117). An alternate approach is to measure the production of oxygen free radicals in fresh plasma (118).

**MICROVASCULAR MANIFESTATIONS OF CELL ACTIVATION**

**Endothelial Permeability and Microhemorrhage**

One of the earliest signs of inflammation in the microcirculation is an elevation of endothelial permeability. Continuous endothelium becomes leaky for just about all components contained in plasma, water, and ions, up to the size of large protein species. The mechanisms by which permeability is elevated involves transmembrane signaling and cytoplasmic reactions in the endothelial cells (119–121). A large number of biochemical inflammatory mediators cause an elevation of permeability, as does adhesion of leukocytes to the endothelium (122), or a lack of fluid shear stress acting on the endothelial membrane (123). The most prominent and earliest site of elevated permeability is in the postcapillary venules of the microcirculation, although arterioles also contribute to transport of plasma proteins into the tissue (124). Early forms of elevated permeability are accompanied by separation of VE-cadherins at intercellular junctions, a situation that leads to rapid pore formation at intercellular junctions (125) (Figure 5). The pores can close again by actin repolymerization (126).
Pore formation in endothelium can also be observed inside endothelial cells, especially if the cells are mechanically stretched (127, 128), as is the case when stasis is caused by occlusions on the venous side (45). Pores in or between endothelial cells are facilitated by actin depolymerization and can be shaped by membrane tension (129). McDonald and his colleagues also reported interesting pores that have membrane tethers (126).

In more advanced stages of inflammation, one frequently observes extravasation of red cells. The escape occurs across pores with a size that permits even transfer of red cells and build up of microvascular hemorrhages. The egress of the red cells is driven by convective flow, and their passage through pores is largely determined by
the red cell ratio of membrane surface and cell volume (130). In the skin, such cell extravasation leads to characteristic discolorations, which serve as clinical indicators for various diseases.

An important element in any consideration of elevated permeability in microvessels is the structure of the basement membrane. Increasing evidence suggests that the basement membrane is a mechanically strong structure that is able to carry most of the mechanical stress in the wall of microvessels in spite of the fact that it is thin compared to the thickness of the endothelium. Transfer of leukocytes across the vascular wall requires proteolytic breakdown of the basement membrane (131), but the exact proteolytic mechanism is still uncertain (132). In the case of neutrophils, this may be achieved by release of elastase and matrix metalloproteinases, especially by gelatinase, from their secondary granules after attachment of the neutrophil membrane to the basement membrane via integrins (133). Breaks in the basement membrane are likely repaired by the endothelial cells.

**Leukocyte Infiltration into the Microcirculation**

The accumulation of circulating neutrophils is one of the most visible signs of inflammation. To understand the interaction between leukocyte or platelets and the endothelium in the microcirculation, we need to recognize that this is a flow domain with low Reynolds number and virtual absence of inertial effects. Other than a mild radial migration of the highly flexible erythrocytes away from the endothelium and formation of a thin stochastic plasma-free zone in larger venules, stiffer cells such as platelets and leukocytes experience negligible radial dispersion unless they interact hydrodynamically with red cells in a velocity shear field.

In acute local inflammation, neutrophils (which exhibit few signs of activation while in the free circulation) accumulate in postcapillary venules (typically about 10 to 12 μm in diameter and the first site in the venular microcirculation with ICAM-1 expression) where they are forced by a specific hydrodynamic interaction with red cells to make membrane contact with the endothelium (Figure 6). The interaction is brought about because the larger and stiffer leukocytes move with a lower velocity than the red cells, leading to their accumulation upstream of leukocytes (134). In the absence of red cells, no leukocyte makes membrane attachment with the endothelium in postcapillary venules (135). Leukocytes may also be forced to make membrane attachment with the endothelium at confluent venular bifurcations (136). The neutrophils that are attached to the endothelium are subject to a mechanical moment that forces the cell to roll on the endothelium. The shear force distorts the cell into a teardrop shape during rolling (137). The centroid velocity of a typical neutrophil rolling on the endothelium is of the order of 1%–3% of the centerline red cell velocity. The rolling velocity varies nonlinearly with the applied shear rate (138, 139), it depends on the distribution and tethering of selectin to the cytoskeleton (78), and appears to reach a threshold that may be L-selectin-specific because it can be observed in cell-free systems (140). Neutrophil rolling on the endothelium in the narrow postcapillary venules is almost always present, with or without inflammation, owing to the inevitable interaction with red cells. Without inflammation, however, the neutrophils,
Figure 6
Schematic diagram displaying various stages of leukocyte interaction with the endothelium on the venous side of the microcirculation. The initial point of leukocyte attachment is in postcapillary venules (position 1) and at small convergent bifurcations (position 2) where the leukocytes are displaced by red cells to the endothelium. Depending on the presence and activity of endothelial membrane adhesion molecules, the leukocytes roll on the endothelium, stop rolling and responding to the fluid shear stress applied by the blood flow in the venule (position 3), spread on the endothelium, and eventually may actively squeeze between and/or through the endothelium and enzymatically break through the basement membrane to actively migrate into the adjacent connective tissue. Leukocyte attachment to the venules critically depends on the presence of the red cells.

as they enter larger venules, detach from the endothelium by random shear force interactions with the red cells. In contrast, in inflammation, the rolling interaction persists into the largest venules owing to the enhanced expression of selectins on the endothelium and the fact that in inflammation the red cells may exhibit enhanced membrane aggregation, and red cell aggregates displace smaller leukocytes on average.
toward the endothelium (141–144). Neutrophils roll on P-selectin, E-selectin (145, 146), and on their own L-selectin, and seem to involve a ligand protein [P-selectin glycoprotein ligand-1, (147)]. Monocyte rolling can also be mediated by selectins and their ligands or α4β1 integrin interacting with endothelial VCAM-1 (148).

In acute inflammation, the main adhesion molecule used for rolling is P-selectin on the endothelium, with PSGL-1 as the counter-receptor on the neutrophil (149, 150). While the cell rolls along the endothelium it is subject to increased activation with elevation of intracellular Ca2+ (151) through multiple mechanisms, including G protein–coupled receptors (such as the IL-8 receptor), the L-selectin/PSLG-1 interaction, attachment to E-selectin, and activation of CD18 (152).

Leukocyte rolling on the endothelium has received an extraordinary amount of attention, and a variety of biophysical models have been developed. Early models described the membrane adhesion process in the form of adhesion or peeling energy, and demonstrated that bond length and bond flexibility play an important role on the rolling motion (153). Using an energy balance between applied fluid mechanical energy, the energy dissipated in viscoelastic deformation in the cell cytoplasm to form a rapidly changing contact region during rolling, and given the membrane adhesion energy it is possible to make predictions of average rolling velocity (154–156). Dembo and coworkers established a quantitative relationship between applied force, adhesion molecule chemical rate constants, and transient and steady-state detachment velocities (157). Damiano and his colleagues proposed a model in which adhesion energy density varies inversely with instantaneous rolling velocity and directly with instantaneous deformation (137). Zhao developed a stochastic model of the micromechanics of cell rolling and provides an analytical method for treating experimental data and separating the contributions of temporal fluctuations and population heterogeneity of measured rolling velocities (158). Hammer and his coinvestigators proposed a sequence of models in which the adhesive dynamics were considered in the form of individual bonds, with bond formation and breakage as stochastic events, while maintaining a relationship between rolling, force, and kinetic constants (159, 160). They developed models for the fluctuation in the rolling velocity about the average velocity (161), rolling on E-selectin (162), models with microvilli (163), and models that take into consideration the hydrodynamic interaction between neighboring rolling leukocytes (164), as well as a number of elegant in vitro systems to study the bond interaction during rolling, a topic that is outside the scope of this review.

Leukocyte rolling is followed by more firm adhesion to the endothelium. The adhesion is facilitated by integrins and their counter receptors (e.g., ICAM-1, VCAM-1) (165–167). A large number of publications have addressed this issue because blockade of the adhesion serves to interrupt one of the key steps in the inflammatory cascade involving leukocytes, and therefore raises the hope to interrupt inflammation as a therapeutic intervention (168). Neutrophil adhesion to the endothelium is mediated by β2 integrins. They become activated and in turn activate an extensive intracellular molecular signaling network. On the apical surface of the endothelial cell, bound chemokines (e.g., MCP-1, MIP-1α/β) can activate leukocyte β2 integrins for tight adhesion to ICAM-1 and ICAM-2. I refer the reader to a previous review by Simon (73).
The site of leukocyte adhesion in the microcirculation is predominantly in post-capillaries and venules with less but still occasional involvement of the arterioles. Sites of leukocyte rolling and firm adhesion may be physically separated in the microcirculation but may also overlap. Little is known about this topographical aspect of leukocyte adhesion in a microvascular network. The firm adhesion of leukocytes is followed by pseudopod projection over the endothelium.

The transition to pseudopod formation in a venule is facilitated by stasis with absence of fluid shear stress (169). But adhesion and cytoplasmic spreading can also proceed in the presence of fluid flow in a microvessel if the leukocyte has been pre-stimulated with an inflammatory mediator or if production of the second messenger, cGMP, is suppressed by inhibition of nitric oxide (170). Thus, biochemical mediators not only control adhesion and migration on the endothelium but also the ability of a leukocyte to respond to fluid mechanical stress. It is a truly interdependent process.

Fluid shear stress can impede adhesion, as may be the case in noninflammatory conditions (169), but in the presence of inflammatory mediators that activate leukocytes, it can also promote adhesion to the endothelium (171). This diverse set of reactions to fluid shear stress is in line with the variety of shear stress responses observed in leukocytes that depend on cofactors (170, 172). Migration on the endothelium may persist, especially if the source of the inflammatory mediators is intravascular. The migration may be in the direction of the fluid flow or opposite to it.

Trapping of Leukocyte in Capillaries

In contrast to the peripheral microcirculation referred to above, where most leukocytes roll in venules, leukocytes in the lung accumulate in the capillaries (173). In the peripheral circulation, entrapment of leukocytes and plugging of capillaries occur predominantly during periods of inflammation and central leukocyte activation. There are two forms of leukocyte plugging in capillaries, transient and permanent plugging (Figure 7). Transient plugging occurs almost exclusively at the endings of the terminal arterioles (the smallest in the circulation), right at the entry into the capillaries (174). It is present with and without inflammation. It is caused by transient compression of leukocytes because passage of leukocytes through capillaries requires deformation of the usually spherical cells (with a diameter of between ~6.5 μm for an average lymphocyte to ~8 μm for neutrophils) into a compressed shape that fits into the cylindrical lumen of capillaries (~5 to 6 μm). The process requires compression of the viscoelastic cell cytoplasm by a mechanical stress that is provided by the squeeze pressure generated in a vessel with convergent lumen dimensions (175). As the cell approaches such a capillary entry region, typically at a bifurcation, its cell centroid velocity is rapidly reduced, and then it slowly creeps into the capillary lumen. At the instant that the leukocyte fits into the lumen dimension, it rapidly speeds up again and passes through the capillary (176). The typical entry time under physiological conditions is approximately 1 s (as compared to the more rapid passage of a red cell through the same entry region of approximately 10 ms) but the process is sensitive with regard to the cytoplasmic rheological properties of the leukocyte and the pressure drop across the capillary. There is no evidence for membrane attachment...
Figure 7
Schematic diagram displaying two cases of leukocyte capillary plugging at the bifurcation of terminal arterioles (with single smooth muscle coat) into a capillary (case A) and inside a capillary (case B). Case A shows the temporary plugging during the relatively slow deformation of a leukocyte into an elongated shape ready to fit into the capillary lumen. The leukocyte entry speed and deformation are determined by the pressure drop across the leukocyte, the viscoelastic properties of its cytoplasm, and the exact dimensions of the capillary lumen and the leukocyte. Activated leukocytes with stiffer cytoplasm tend to become more readily trapped in capillaries, both at the entry and inside the capillaries. Case B shows permanent plugging of leukocytes inside a capillary. Such permanent plugging leads to complete cessation of cell motion, accumulation of red cells, and eventually apoptosis of the endothelial cells.

between leukocyte and endothelium during transient plugging of leukocytes. The relatively low-pressure drop across the dense pulmonary capillary network makes initial leukocyte attachment to the endothelium sensitive with regard to cytoplasmic stiffness of leukocytes (e.g., pseudopod formation) and less dependent on adhesion molecules (173, 177).

In contrast, permanent plugging of capillaries is caused by entrapment of leukocytes in the lumen of a capillary and requires attachment of the leukocyte and capillary endothelial membrane. The specific adhesion molecules involved in permanent capillary plugging have not been determined. Permanent capillary plugging causes occlusion of the capillary lumen (9, 17), a phenomenon that is responsible for the capillary no-reflow phenomenon during periods of reperfusion following ischemia.
The leukocytes in such obstructed capillaries are deformed into cylindrical cell shapes that make broad membrane contact with the endothelial membrane. Just like transient occlusion, permanent occlusion by leukocytes is also sensitive with respect to their cytoplasmic properties (178). Depending on the capillary network density, obstruction of individual capillaries causes an increase in hemodynamic resistance in the microcirculation (179, 180) and an increase in capillary plugging enhances tissue apoptosis (181). Depletion of leukocytes from the circulation attenuates the no-reflow phenomenon in acute inflammation, reduces organ injury (182), and serves to maintain tissue function in most organs tested (182–184).

**Transendothelial Migration**

In most inflammatory conditions, the leukocytes migrate within minutes after attachment across and under the endothelium (185, 186) (Figure 8). The passageway across the endothelium has been uncertain ever since the first electron microscopic investigations of the phenomenon (187, 188). Much of the uncertainty has been focused around the issue of whether the leukocytes migrate through junctions between endothelial cells or through new pores inside individual endothelial cells. While a definitive analysis is not available today, much of the evidence points to both possibilities. The pathway selected by a neutrophil depends on the precondition of the endothelial cells. In the case of continuous endothelial cells preexposed to low levels of inflammatory mediators and to continuous fluid shear, the cell cytoplasm is relatively thick and openings in the endothelium have been observed predominantly between endothelial cells (paracellular) utilizing a PECAM-1 (a transmembrane protein and member of the immunoglobulin superfamily) (189, 190) interaction between the monocyte and the endothelial cells, followed by similar homophilic adhesion via CD99. Openings appear to involve lateral diffusion of interendothelial adhesion molecules (VE-cadherins) (Figure 9) and opening of pores with actin depolymerization. In contrast, endothelial cells that have a thin cytoplasm or endothelial cells with fenestrations can have open pores inside (transcellular) the cells, especially if they are subject to lateral stretch. In either case, leukocytes readily project pseudopods inside either such pores. Neutrophils have the ability to migrate across pores, whether made of cells or of artificial materials. In the pulmonary microcirculation, neutrophils frequently migrate across the endothelium at tricellular endothelial junctions (191).

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**Figure 8**

Transmission electron micrograph of venule in rat mesentery after 1 h stimulation with f-Met-Leu-Phe. Different stages of the transendothelial migration by neutrophils (N) are visible, from initial attachment with minimal membrane contact, to spreading and projection of pseudopods through the endothelium (case B), to spreading of the endothelial cell over the body of the transmigrating neutrophil (case A), as well as spreading of neutrophils on the endothelial basement membrane and underlying connective tissue. There are some platelets (P) in the vicinity of the site of transendothelial migration but this is not a requirement for transendothelial migration. No extravasation of erythrocytes (E) is detectable.
In acute inflammatory conditions, neutrophils may persist on the basement membrane, form a contact area, and spread their cytoplasm (Figure 8). This evidence clearly supports the hypothesis that the basement membrane is a mechanical barrier for leukocyte migration. Breakage through the endothelial basement membrane is a proteolytic process (192–196). It is interesting to note that most studies of the acute inflammatory response have observed leukocyte migration (neutrophils, monocytes, eosinophils, T-lymphocytes), whereas there are few studies that have reported platelet migration across the endothelium in inflammation.

Neutrophils spread their pseudopods on the basement membrane underneath the endothelium and slip across the pore, often aided by a contraction ring (a local contraction around the cell body that travels over the cell body) (65), while at the same time the endothelial cell may project its own cytoplasm over the part of the leukocyte body that is still inside the vessel lumen. This gives the appearance of a cup shape by the endothelial cell (197). The neutrophil activates the myosin light chain kinase activity and cAMP-protein kinase A activity, possibly by sliding ICAM-1 past CD18 attachment sites (198). During passage the neutrophil and endothelial cell membranes remain attached to each other. Studies with plasma protein labels point to the fact that in the process of transendothelial migration, the seal between the two cell membranes may remain tight without leakage of plasma or there may be

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**Figure 9**

Bright field and fluorescent images of the time course of a monocyte (red label) migrating across interendothelial junctions displayed by vascular endothelial (VE)-cadherin (green fluorescence). The VE-cadherin separates at the points where the monocyte crosses the endothelium and reseals the interendothelial VE-cadherin after passage of the monocyte (201). Courtesy of Dr. Francis W Luscinskas.
transient plasma leakage depending on the particular choice of chemotactic agent (199). The elevation of microvascular permeability during neutrophil transmigration involves the kinetics of the focal adhesion kinase in the endothelial cells, suggesting that contact with neutrophils leads to a cytoplasmic motion of the endothelial cells (200). During transendothelial migration between endothelial cells under physiological flow conditions, monocytes induce focal loss in the staining of the interendothelial adhesion molecules VE-cadherin, alpha-catenin, beta-catenin, and plakoglobin (201), suggesting that these adhesion molecules move laterally after dislodgement from the underlying actin matrix and thereby facilitate interendothelial pore formation during local actin depolymerization.

Upon breakage through the basement membrane, the leukocytes migrate in a random pattern into the interstitial space with a direction that leads, on average, away from the endothelium (202, 203) (Figure 10). Migration in the interstitium requires attachment of neutrophils to extracellular collagen fibers via a mechanism that involves $\beta_1$ integrins (204). It has been proposed from studies on reconstituted extracellular matrix proteins (fibrin) that $\beta_1$ integrins also serve as regulators for control of neutrophil migration in a fashion that depends on the particular choice of the chemotactic agent (205, 206). After penetration of the endothelial basement membrane, monocytes migrate through the extracellular matrix of the tissues where they may differentiate into tissue macrophages and/or migrate to sites of inflammation. The migrating leukocytes displace perivascular cells, such as pericytes or fibroblasts (207).

Additionally, monocytes in the tissues may migrate in the direction of the lymphatics and thereby return back into the bloodstream, both of which involve basal to apical (reverse) transendothelial migration, possibly mediated by tissue factor and p-glycoprotein (148).

It is also interesting to note that even though the neutrophils are one of the first cell types to respond to an inflammatory signal, they may not necessarily participate in large numbers in a local process of attachment and transendothelial migration, and instead the majority of neutrophils continues to circulate (208).

**Monocyte and Lymphocyte Infiltration**

In inflammation the first wave of neutrophil migration into the tissue is followed by a second wave of monocytes (cells that are much less frequent in the circulation). This wave follows typically several hours after the initial inflammatory insult, but it persists in chronic forms of inflammation when neutrophils may not be present anymore. The monocyte infiltration is thus frequently observed in chronic inflammatory conditions, be it in large vessels during atherosclerosis [an extensive literature exists on this topic (7, 209, 210)] or in capillaries during chronic retinopathy (211). Monocytes also utilize the $\beta_1$ and $\beta_2$ integrin to make firm membrane attachment. Upon migration into the tissue, they differentiate into larger macrophages and phagocytose cell debris. They carry a set of scavenger receptors (212) that permit binding of tissue degradation products, such as oxidized low-density lipoprotein. They also synthesize and release a number of growth factors that in the microcirculation lead to formation of a new set of
Time-lapse record of a neutrophil (arrow) migrating across a venule (bottom of each panel) into the interstitial tissue of the mesentery after topical application of the f-Met-Leu-Phe. The cell migrates, on average, away from the vessel wall, but is unaffected by the adjacent tissue mast cell. M: mast cell, E: endothelial cell, L: venular lumen. From Reference 185.
capillary blood vessels that can be observed to grow around clusters of macrophages and give a coiled capillary appearance (211). The monocytes may accumulate cell debris- or lipid-filled intracellular organelles in their cell cytoplasm, giving them a morphological appearance of foam cells (213).

The mechanisms for attraction of monocytes to the lesion in arterial disease have attracted intense interest. A family (with several subfamilies) of small, nonenzymatic proteins, lymphokines, were identified that have characteristic cysteine inserts/repeats (C, CC, CXC, and CXXXC) (214). One of these is the monocyte-chemoattractant protein 1 (MCP-1), which can be synthesized by a number of cells. Deletion of this cytokine or its receptors leads to reduction of atherosclerotic lesions in mice that are otherwise susceptible to atherosclerosis (215). But in addition to MCP-1, other chemokines have been implicated to serve as chemoattractant for monocytes (reviewed in 209), but no conclusive analysis exists that would implicate any single signaling molecule as the cause for the progression of monocyte infiltration in atherosclerosis. Chemokines also influence other cell activities and may be one of the sets of signaling molecules involved in the resolution of inflammation rather than generation of inflammation.

Inflammatory sites are infiltrated by several other cell types. The thymus-derived lymphocytes (T-lymphocytes) and some of their subtypes may accumulate in a chronically inflamed site. These cells usually circulate and use a pathway through the lymphatics, which includes migration across a specialized endothelium in lymph nodes and in Peyer's patches (a specialized lymphoid tissue on the intestinal wall), and they are regularly observed in the spleen. T-lymphocytes infiltrate tissue without overt signs of inflammation but also migrate into almost any site of inflammation, also utilizing selectins to initially adhere to the endothelium. The use of animals with specific gene deletions has opened a new level of analysis into this extensive topic closely associated with specific immunity [see, e.g., reviews on selectins and integrins in T-lymphocyte adhesion (216, 217), the specific antigen mediated T-lymphocyte activation (218), T-lymphocyte/monocyte interaction in chronic rheumatoid arthritis (219) and in chronic pulmonary inflammation (220)]. Other cell types that may infiltrate inflammatory sites are fibroblasts, smooth muscle cells, and mast cells.

**Microvascular Cell Death**

Inflammation is accompanied by both programmed (apoptotic) and necrotic cell death, with and without attraction of inflammatory cells, such as neutrophils. A variety of useful indicators have been introduced to detect cell death during inflammation in a living microcirculation. The extent of cell death depends on the biochemical nature and the level of the inflammatory stimulus. In acute ischemia and reperfusion, most cell death of parenchymal tissue cells occurs during the period of reperfusion (Figure 11).

The presence of neutrophils per se does not necessarily lead to cell death. For example, in the mesentery, after stimulation with a single inflammatory stimulus at concentrations that lead to extensive neutrophil attachment to postcapillary venules and migration into the mesentery tissue, no enhancement of parenchymal cell death
Typical time course of cell death in skeletal muscle (as detected by the life-death indicator propidium iodine) (top panel), plugging of capillaries by leukocytes (middle panel), and adhesion of leukocytes to postcapillary venules (bottom panel) during inflammation generated by ischemia and followed by reperfusion. Ischemia (I group) by itself causes cell death, but there is a dramatic enhancement of cell death if the ischemic period is followed by reperfusion (I/R group). Entrapment of leukocytes in capillaries and venules is enhanced during the course of the reperfusion. Adapted from Reference 222.
can be detected (202, 221). The lack of parenchymal cell death is present over several hours irrespective of the particular choice of inflammatory mediator that is used to stimulate the inflammatory reaction (the synthetic tripeptide f-Met-Leu-Phe; PAF; complement fragment C3a; tumor necrosis factor-α, TNFα). In contrast, if a combination of two inflammatory mediators is applied at the same time irrespective of their particular choice, then besides infiltration of leukocytes into the tissue, extensive parenchymal cell death occurs, spatially correlated with the neutrophil accumulation.

The cell death observed under acute inflammatory conditions depends also on physiological and metabolic activities. In skeletal muscle with a mixture of oxidative and glycolytic muscle fibers, the first cells that are subject to cell death are fibers with low oxidative metabolism, and therefore low levels of oxygen free radical scavengers. Muscle fibers with high oxidative metabolism have a number of enzymes that scavenge oxygen free radicals and therefore experience lower levels of cell death. In rat skeletal muscle, the endothelial cells xanthine oxidase is a major source of oxygen free radicals that kills the glycolytic muscle fibers (222).

Even though a number of mechanisms are well recognized to produce cell apoptosis in vitro (via death receptors: Fas and TNFα receptor 1, reactive oxygen free radicals, a lack of integrin binding, and others), the molecular mechanisms that actually cause cell death in a living tissue in inflammation are less explored. The ability to produce or inhibit apoptosis depends on cofactors. For example in endothelial cells, application of fluid shear stress prior to exposure to hydrogen peroxide or TNFα serves to prevent apoptosis (223, 224).

Besides parenchymal cells, cells that infiltrate into the tissue during inflammation are also subject to apoptosis. Neutrophils have a natural average lifespan of less than half a day, whereas mature peripheral T-lymphocytes have an estimated lifetime of 1–2 months. Interestingly, neutrophils at inflammatory sites have a delayed apoptosis (225) that seems to depend on the presence of a growth factor (granulocyte/macrophage colony-stimulating factor) released from activated endothelial cells. In the presence of proapoptotic signals, such as tumor necrosis factor, Mac-1 engagement accelerates apoptosis (226). Apoptotic neutrophils in the circulation are removed by the spleen, or after migration into the peripheral microcirculation, by macrophage and fibroblast phagocytosis (227, 228).

**Capillary Restructuring Under Inflammation**

Capillary and microvascular restructuring may occur at any stage of inflammation. Many different shape and microvascular network changes can be observed. A typical event, depending on the nature and duration of the inflammatory stimulus, is permanent restructuring of the endothelium to the point that capillaries are converted and enlarged into venules (229). Capillaries may disappear altogether, a process referred to as rarefaction (230) and largely due to endothelial apoptosis (231). Resolution of inflammation is associated with angiogenesis that may also go through several phases. For example, inside a blood clot, new vessels will grow into the blood clot from preexisting channels that are formed only inside the blood clot. The initial channels are highly irregular in shape and may have high network density but have no
endothelial cells. Once endothelial cells start to grow into the newly formed channels, these channels assume a morphology more typical for microvessels with narrow diameters. The in-growth of the initial channels takes days; formation of a more complete tissue with intact arterioles, nerves, and lymphatics requires several weeks (232). This is an area important for further investigation because clinical interventions against inflammation need to be evaluated in light of this repair process.

**TRIGGER MECHANISMS FOR INFLAMMATION**

An important consideration to control the level of inflammation is to identify molecular mechanisms that can trigger an inflammatory reaction. To achieve this, it is necessary to detect inflammation at an early stage. There are a large number of mechanisms to activate cells and stimulate an inflammatory cascade. It is convenient to classify mechanisms for cell activation into several general categories (233):

(a) **Positive feedback mechanisms**: There exists a class of inflammatory reactions that are mediated by direct action of plasma inflammatory stimulators. A list of mediators proposed in the past include endotoxins, oxygen free radicals (234), PAF (235), cytokines (a family of small molecular signaling proteins, e.g., TNF-α, IL-1, IL-8) (236, 237), polypeptides (such as bradykinin, complement fragments (238)), coagulation and fibrinolytic factors (239, 240), thrombin (241, 242), leukotrienes (243), histamine (106), and oxidized LDL (244, 245). The list of inflammatory mediators is long indeed, and may in part be triggered by trauma or by bacterial, viral, or fungal sources.

(b) **Negative feedback mechanisms**: An alternative pathway for cell upregulation in the microcirculation is by depletion of antiinflammatory factors. This list is shorter and includes nitric oxide (246), adenosine (247), adrenal glucocorticoids, and selected cytokines (e.g., IL-10) (248). The reaction of these mediators is nonlinear and depends on cofactors and concentrations.

(c) **Contact activation**: A specific form of cell activation by membrane contact has been proposed in the form of juxtacrine activation (249). A nonactivated endothelial cell may be stimulated during membrane contact by an activated leukocyte and vice versa, e.g., by oxygen free radical production in the membrane contact region between the cells and by formation of PAF and other bioactive lipids.

(d) **Activation by mechanotransduction**: Alternative forms of cell activation owing to either a shift to unphysiologically low or high fluid shear stresses acting on the endothelium or a shift in the oxygen supply to the tissue (169, 250). Fluid shear serves as a control mechanism for various forms of cell activation and the expression of antiinflammatory and proinflammatory genes. Inflammatory stimulators of the sort listed above can influence the fluid shear response via a cGMP mediated mechanism (170) (see below).

(e) **Activation by hyperglycemic/hyperosmolar conditions**: A form of inflammation that may be relevant to diabetes (251).

(f) **Activation by physical transients**: Transients of gas (like oxygen, carbon dioxide, etc.) concentrations and temperature transients have the ability to stimulate
cell activation irrespective of the direction of the transient (up or down) but
dependent on the magnitude of the transient (252–256).

Over the lifetime of a person, it is likely that several—if not all—of these mech-
anisms may at one time or the other stimulate inflammation. The challenge is to
identify dominating mechanisms. In the following, I discuss a source of inflammation
in physiological shock, one of the conditions with the highest level of inflammation
and also one of the conditions with highest mortality.

THE SOURCE OF INFLAMMATORY
MEDIATORS IN SHOCK

Inflammatory Mediators in Plasma

Plasma of animals (and patients), after intestinal ischemia and reperfusion as well as
in shock conditions, contains factors that exhibit a variety of biological activities,
including activation of circulating leukocytes, depression of T-lymphocyte prolifera-
tion, depression of cardiac muscle activity (the myocardial depression factor), carcino-
genic activity (clastogenic activity), leukocyte activating factor, and others (257–262).
There are many reports in the literature of pancreatic enzymes releasing active fac-
tors affecting heart tissue (257), with the hypothesis that the myocardial depression
factor is released from ischemic pancreas during shock. Recently, it was observed that
a chemotactic factor is released from self-digested spleen (263). Thus, what we regard
to be an inflammatory factor in the microcirculation during ischemia, for a number
of parenchymal cells may be a depressing factor. But none of these terms are entirely
adequate to describe the pancreatic protease-derived factor(s) encountered, consid-
ering the fact that one or more of the factors lead to direct cell death irrespective of
the presence or absence of leukocytes (264).

Traditionally, the most studied candidates for inflammatory mediators were bac-
teria in the intestine. Bacterial products in the form of lipopolysaccharide (endotoxin,
from the cell wall of bacteria) derived from gram-negative pathogenic bacteria (E. coli, Salmonella, Shigella, Pseudomonas, Neisseria, Haemophilus, and other pathogens, in
contrast to Lactobacillus, Bifidobacteria, and Clostridium difficile, probiotics that support
digestion) can produce a strong inflammatory reaction (265) that can be as lethal as
hemorrhagic shock (266). There is an extensive literature on this topic (267). One
can find strong inflammatory mediators in the plasma, but their biochemical nature
and source have remained uncertain. The endotoxin and bacterial translocation hy-
pothesis states that endotoxin or whole intestinal bacteria may be transported from
the lumen of the intestine across the epithelial mucosal (brush border) barrier into
the wall of the intestine, where they are absorbed by the lymphatics or blood vessels
and carried back into the central circulation and produce inflammation (268). But
this hypothesis is not supported by conclusive evidence (269, 270).

We have recently obtained evidence that inflammatory and even cytotoxic medi-
ators can also be derived from digested foods in the intestine (271). Fully char-
acterized bioactive peptides from milk and casein have been described as having a
wide range of functions (e.g., antimicrobial, immunostimulatory, immunosuppressive,
antihypertensive, opioid-like, and mineral binding) (272–275). Cytotoxic peptides have been identified in trypsin digests of casein (276, 277). Consequently, the lumenal content of the intestine is a complex toxic mixture of mediators and factors that are derived from digested foodstuff and the autolytic breakdown of the pancreatic enzymes themselves.

Pancreatic Enzymes and the Production of Inflammatory Mediators

It has been known for a long time that the intestine plays a central role in shock. The collective set of pancreatic enzymes has the ability to digest virtually all biological molecules except cellulose. This ability is one of the main requirements for normal digestion. Digestive enzymes are discharged from the pancreas in the form ofzymogens and are activated by enterokinases in the duodenal segments of the upper ileum (278). From there on, the digestive enzymes in the ileum are fully activated as part of normal digestion until they are degraded and/or auto-degrade as they pass through the lumen of the ileum. Under most circumstances, the digestive enzymes are present in high concentrations in the entire ileum, cecum, and large intestine (279). Even though food proteins, lipids, and complex carbohydrates are digested as part of normal nutrition, digestion of the intestinal tissue itself appears to be largely prevented. The mucosal barrier formed by the villi in the intestine minimizes self-digestion by the activated pancreatic enzymes in the intestine. Two major mechanisms are known to prevent transport of digestive enzymes into the wall of the intestine: (a) the tight barrier formed by mucosal epithelium and (b) mucus secretion from goblet cells that lead to a net outward transport away from the brush border barrier. The epithelial cell lining is usually impermeable to digestive pancreatic enzymes (with a barrier permeability range below the size of digestive enzymes) (280–282). However, under ischemic conditions, the usually tight epithelial barrier is compromised and higher-molecular-weight materials may penetrate the interepithelial gaps and enter into the interstitial space of the intestinal wall (270, 283–288).

The New Autodigestion Hypothesis

If pancreatic digestive enzymes should penetrate through the mucosal epithelial brush border, could this lead to production of inflammatory mediators? We explored this question in Reference 289. To identify a possible source of inflammatory mediators, tissues from different organs were homogenized and incubated for 2 h at 37 °C and centrifuged. The ability of the supernatant from these organs to activate was tested on naive leukocytes. The experiments yielded a surprising result. All organ homogenates produced only low levels of cell activation factors, except for a dramatically elevated level of cell activation induced by homogenate from the pancreas (Figure 12). Similar high levels of cell activation were detected independent of the species used (pig, rat, mouse pancreas) (290). A mixture of organ homogenates that by themselves produce only low levels of inflammation with selected pancreatic enzymes (serine proteases, lipases) produces high levels of inflammatory mediators. This evidence suggests that
Figure 12
Neutrophil death (detected by propidium iodide labeling) after exposure to homogenates of fresh rat tissues from different organs. While several organs generate low levels of cell death (and activation), the pancreas homogenate generates high levels of cell death. The cell death is due to generation of inflammatory mediators by pancreatic digestive enzymes (serine proteases, lipases) and can be generated in a similar fashion if digestive enzymes are added to other organs. These results indicated a unique role for digestive enzymes as sources for generation of humoral inflammatory and cytotoxic mediators (291). Courtesy of Steve Waldo.

The production of inflammatory mediators by pancreatic digestive enzyme is not unique to the pancreas but instead depends on where in the tissue and in the circulation pancreatic enzymes are transported and activated. A key organ to consider in this respect is the intestine, where all digestive enzymes are fully activated and present in high concentrations.

The supernatant from pancreatic homogenate not only produces upregulation of leukocytes but is highly cytotoxic and causes high mortality (289, 291). Infusion of a pancreatic homogenate derived from one third of a rat pancreas into an adult rat causes death within 6 min, on average. Superfusion of whole pancreatic extract on the mesentery microcirculation of normal rats, without exposure to shock, produces signs of inflammatory reaction. This includes rolling of leukocytes on postcapillary endothelium, adhesion, migration into the mesentery interstitial tissue, production of peroxide by microvascular endothelium, mast cell degranulation, and cell death, as detected by the life/death indicator propidium iodide (105, 222, 292–294). Serine protease inhibitors block the production of cytotoxic mediators produced by pancreatic homogenate.

As indicated, the pancreas may not only be a major source for inflammatory mediators but all organs may become a source for inflammatory mediators in the presence of pancreatic or other tissue enzymes, especially serine proteases and lipases (289).
This observation is especially relevant with respect to the intestine. If the intestine is carefully rinsed of digestive enzyme, it produces relatively low levels of inflammatory mediators after homogenization. In contrast, if the intestine (or any other organ) is incubated with pancreatic enzymes, such as trypsin, chymotrypsin, elastase, or a combination thereof, its homogenate causes inflammatory cell activation at a level that is comparable to that of the pancreas (291). In light of the fact that the intestine has most of the activated digestive enzymes, the critical issue in the production of inflammatory mediators is the distribution of these enzymes within the interstitial space of the intestinal wall. Thus an important aspect in understanding the production of inflammatory mediators produced by self-digestion of autologous tissue structures is to study in the future the transport and activation of the digestive enzymes across the intestinal barrier.

**Pancreatic Enzyme Blockade in the Lumen of the Intestine**

One of the experiments that serve to examine the role of pancreatic digestive enzymes in acute ischemia is to rinse the initial intestinal contents and block pancreatic enzymes in the lumen of the intestine with an inhibitor [e.g., with the synthetic serine protease/lipase inhibitor 6-amidino-2-naphthyl p-guanyldibenzoate dimethanesulfate, ANGD (295)] before and during ischemia of the small intestine (296). Such an intervention almost completely inhibits activation of circulating leukocytes during the ischemia and reperfusion, and prevents the appearance of inflammatory mediators in portal venous and systemic artery plasma. All early symptoms of organ injury are attenuated. While the mean arterial blood pressure in the controls on average falls below 40% of a normal mean arterial pressure after two hours of reperfusion, mean arterial blood pressure on average remains at 84% of preischemic values in the ANGD group. Digestive enzyme blockade in the intestinal lumen preserves pulse pressures close to control values; attenuates the morphological damage to the intestinal wall and the accumulation of leukocytes in the intestine, the liver, and lung; and it prevents formation of lung edema as an important early sign of organ failure. Several protease inhibitors, if applied into the lumen of the intestine, also block the production of inflammatory mediators in the intestine (297, 298). After blockade of the production of inflammatory mediators in the intestine, peripheral organs are also protected against inflammatory reactions (299). The intestinal tissue produces only low levels of activators in the absence of pancreatic enzymes, while in the presence of digestive enzymes, powerful inflammatory mediators are produced in a concentrated and time-dependent fashion. This protection against inflammation has been observed in several species (279) and confirmed in a shock model that consists of a combination of trauma and hemorrhagic shock (300). Protection against inflammation is seen even with partial blockade of digestive enzymes without complete lavage of the small intestine (279).

Furthermore, we also have been able to demonstrate protection against microvascular inflammation after blockade of digestive enzymes and in the presence of endotoxin (301). These results indicate that even though endotoxin can serve as an inflammatory mediator, its effect is significantly amplified by the action of digestive.
enzymes because endotoxin per se serves to elevate the permeability of the intestinal barrier, so that self-digestion is initiated.

TISSUE MECHANISMS TO REDUCE INFLAMMATION

Preconditioning

In contrast to the Arthus and Shwartzmann reactions that enhance inflammation (see above), there are mechanisms to reduce the level of inflammation in a fashion that depends on tissue pretreatment. These are some of the most interesting phenomena associated with inflammation but are not well understood.

An interesting phenomenon described by Jennings and his associates at Duke University is designated ischemic preconditioning. Multiple interruption of the blood flow to an organ for short periods of time (on the order of 1 min) serves to reduce the level of inflammation and organ dysfunction compared with control ischemia without such temporary blood flow interruption (302, 303). Such preconditioning reduces the development of inflammation after ischemia. Reduction of inflammation by preconditioning can be generated in different organs and can also be simulated by short exposure to other inflammatory challenges (304–306).

Tolerance

A strong level of protection against inflammation can be achieved by chronic pre-treatment with an inflammatory mediator for a period of several days, such as mild trauma or sublethal endotoxin administration (307, 308). Pretreatment dramatically reduces signs of inflammation in ischemia or in shock and is referred to as tolerance (309, 310). Tolerance can be achieved by a variety of different stimuli, but the pre-treatment needs to be maintained. If it is stopped, the tolerance vanishes after several days. The protection associated with tolerance has been attributed to production of glucocorticoids and suppression of the inducible form of NOS (311).

FLUID SHEAR STRESS CONTROL OF INFLAMMATION

Endothelial Cells

There is an increasing body of evidence to suggest that inflammation is under the control of fluid shear stress in the absence of biochemical agonists. By now, all cells in the microcirculation have been shown to be controlled by fluid shear. In addition to the elongation of the endothelial cells in the direction of flow, the endothelial cells under steady shear increase the thickness of their cytoplasm and develop a set of actin bundles (actin stress fibers) with firm adhesion to their substrate via focal adhesion sites. But in addition to these morphological responses, endothelial cells also produce a variety of other responses when exposed to shear stress or a tensile stretch. These responses depend on the details of the stress history that is imposed on the cells and on the level of attachment to surrounding cells and to the substrate. The topic is large and has been previously reviewed (312–316).
Physiological levels of fluid shear stress (e.g., steady shear at 10 dyn/cm² for several hours) applied to endothelial cells transforms the cells into a state in which expression of antiinflammatory genes (such as eNOS, COX-2, and Mn-SOD) and signs of inflammation or thrombosis are reduced (317, 318). Gene-chip analysis confirms that a number of proinflammatory genes are upregulated by laminar shear stress over one day (319) and also when the endothelial cells are located close to smooth muscle cells (320). Laminar shear stress reduces cell attachment to the endothelium and the susceptibility of the endothelial cells to proinflammatory mediators (321, 322). In contrast, unsteady shear stress, especially if accompanied by reversal of the shear direction or by cyclic strain, induces proinflammatory genes (323).

Shear stress controls a wide variety of genes, and therefore it is of interest to identify the transcription factors and DNA binding sites that are sensitive to mechanical stress. Shear stress regulation appears to occur by binding of certain transcription factors, such as NFκB and Egr-1, to shear stress response elements (SSREs) (324) that are present in the promoters of biomechanically inducible genes. The Kruppel-like factor 2, a member of the zinc finger family transcription factors, which regulate cellular differentiation and tissue development, is induced by laminar shear stress in endothelial cell cultures and has a distinct antiinflammatory effect (325).

**Leukocytes**

Leukocytes also respond to fluid shear stress, but in a different fashion. The leukocyte membrane is subject to a different fluid stress field than the membrane of an endothelial cell, and in fact the shear stress leukocytes experience in the circulation depends on whether they are in free suspension or attached to the endothelium (326). The response depends on the prior activity of the cell. When neutrophils migrate on a substrate with cyclic projection of pseudopods, fluid shear stress leads to retraction of these pseudopods (169) (Figure 13). Pseudopod retraction is also observed in monocytes. In contrast, when the neutrophils are maintained in a deactivated state prior to shear, they exhibit a reversed response and project pseudopods (172). Pseudopod retraction is associated with downregulation of small GTPase Rac1 and -2 (327). This behavior is in line with the enhanced migration of stimulated lymphocytes under fluid shear (171). Yap & Kamm observed transient neutrophil activation upon entry into a narrow fluid channel (328, 329). Zhelev and his colleagues showed pseudopod formation after mechanical extension of a membrane tether on a neutrophil (56). Thus, clearly, the activity of a major class of inflammatory cells is under the control of biomechanical stresses.

Pseudopod retraction occurs at levels of fluid shear that are typical for the microcirculation, but the stresses are too small to evoke a passive viscoelastic response. In addition to retraction of pseudopods, the leukocytes also show other responses to fluid shear. Neutrophil adhesion to each other (homotypic interaction) is under the control of fluid shear (73). Neutrophils shed CD18 on their membrane by proteolytic cleavage with cathepsin B derived from their granules (330), and they produce oxygen free radicals. Pseudopod projection in free suspension is sensitive with respect to fluid shear stress (rather than shear rate) but depends on the presence of red cells (331). When
neutrophils adhere via $\beta_2$ integrins to their substrate they respond readily to fluid shear, whereas during attachment to $\beta_1$ integrins the response is attenuated (332).

The question arises, How do leukocytes respond to fluid shear stress in the presence of inflammatory mediators? If neutrophils are subjected to increasing concentrations of inflammatory mediators (e.g., PAF or tripeptide f-Met-Leu-Phe), a progressive reduction of the fluid shear response is observed (170). The reduction of the response can be reversed by the second messenger, cGMP, controlled by nitric oxide. This mechanism facilitates an enhanced leukocyte response to fluid shear stress if the cell is adhered to an endothelial cell that is still synthesizing nitric oxide as compared to an endothelial cell in inflammation that has enhanced levels of superoxide and less nitric oxide.

In light of the fact that the fluid shear stress response is quite specific for individual cell types, we recently explored the question, What particular mechanisms may be involved in the mechanosensing of fluid shear stress on the cell membrane? A number of structures have been proposed (ion channels, the glycocalyx, the bilipid layer, and others), but no proposal has been advanced that provides a hypothesis that is in line with the high specificity actually observed among different cell types after exposure to fluid shear stress. Chien and his collaborators have observed, in the case of the endothelial cell, that the vascular endothelial growth factor receptor 2 is activated by fluid shear stress together with the same signaling pathways that are activated by an agonist to this receptor (VEGFA) (333). Thus, this preliminary evidence suggests that one of the actual sensors for mechanical shear stress may be the G protein—coupled receptor or tyrosine receptors in the membrane. Such receptors are unique...
to each cell and may provide the high specificity associated with the fluid shear stress response. We tested this hypothesis directly in the case of the neutrophil, focusing on the formyl peptide receptor (FPR), a receptor with the highest constitutive activity in this particular cell. Deletion of the FPR receptor with silencing RNA (siRNA) and generation of a cell without this receptor lead to abolishment of the fluid shear response. In contrast, transfection and expression of the receptor in a cell line without other receptors that respond to fluid shear serve to generate a cell with a single FPR population. Such cells respond to fluid shear (334). Thus, this initial evidence suggests that the humoral signaling pathways and the biomechanical signaling pathways overlap in inflammation, so that the state of receptor activation depends on biomechanical stress and on the mediators synthesized and released.

Even though shear stress serves to control inflammatory signals in cell cultures and in several in vivo models, and even though there is a nonuniform velocity field around the risk sites for inflammation at arterial bifurcations, there is no conclusive evidence that atherosclerosis is caused by fluid shear stress abnormalities at those sites. The same anatomic risk for shear stress–induced inflammation at arterial bifurcations exists at younger ages or during periods of time when arteries have no overt signs of inflammation. Instead it is necessary to look at cofactors in patients. Patients may have other trigger mechanisms for inflammation, such as infections, diabetes, or an intestinal source for inflammatory mediators, to name just a few. These risk factors generate their own proinflammatory state on which fluid shear stress is acting as an antiinflammatory stimulus. It is a different situation compared to fluid shear stress acting on noninflamed endothelium.

CONCLUSION

The study of inflammation is a key to understanding the origin and progression of disease. While the inflammatory cascade is used during a lifetime as a tissue repair mechanism, detection of signs of inflammation is now starting to be recognized as a warning sign, indicating that the tissue is engaged in a repair that may or may not come to a resolution. If it does not come to a resolution, cell death inevitably follows.

Some forms of interventions against inflammation have now been attempted at several stages of the cascade, but with mixed results. This is not surprising if we consider the facts that the inflammatory cascade serves primarily as a repair function and that we do not know in many cases what caused the inflammation in the first place. To achieve more control over the inflammatory cascade, it is essential to better understand the actual trigger mechanisms that cause inflammation instead of interfering only with subsequent events in the cascade.

In addition to traditional sources of inflammation, we have identified a fundamental mechanism for inflammation that is associated with the digestive system. The digestive enzymes, an integral part of nutrient supply in the intestine, are present over a lifetime and have the ability to digest any tissue with generation of powerful inflammatory mediators. Escape of the digestive enzymes from the lumen into the wall of the intestine is prevented by the mucosal barrier in the intestine. Any breach of this barrier leads to self-digestion and generation of powerful inflammatory...
mediators. This process has been shown to play a central role in experimental forms of shock with high levels of inflammation.

Study of inflammation is one of the great problems of modern medicine and it is most suitable to bioengineering analysis. Every new insight derived from the engineering analysis will open the door to new and improved interventions.

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