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Inflammation: a common pathway in cardiovascular diseases

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This primer of inflammation biology introduces the cast of characters under consideration in subsequent contributions to this volume—a cast that has mushroomed in recent years. It describes the mechanisms of innate and acquired immunity, and the key players in the inflammatory response, explicating and disentangling the connections between them—the better to argue its case that the entire spectrum of inflammatory diseases represents host defenses gone awry. A number of cardiovascular conditions, some exotic, but many common, involve an important component of inflammation, representing host defense mechanisms that can cause disease. The recognition of the central role of inflammatory pathways in circulatory pathophysiology furnishes a new dimension to understanding even the most familiar and clinically compelling cardiovascular diseases, notably atherosclerosis and acute myocardial infarction, each of which is discussed in detail. In addition, these inflammatory aspects of cardiovascular diseases may provide, in some cases, new therapeutic opportunities to forestall the development or the consequences of various cardiovascular conditions. Seemingly far afield at the outset, contemporary inflammation biology has concrete clinical ramifications for the practitioner. Learning to redirect inappropriate inflammatory responses may help us to improve patient outcomes in years to come.

Keywords: inflammation; cardiovascular disease; inflammatory factor; immune response; cytokine; host defense; atherosclerosis; acute myocardial infarction; treatment

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The dedication of this issue of *Dialogues in Cardiovascular Medicine* to inflammation in cardiovascular diseases acknowledges the growing appreciation of the central role of inflammatory pathways in circulatory pathophysiology. This introductory essay aims to provide a general background to the biology of inflammation. The discussion will serve as a roadmap and glossary of inflammation that will facilitate placing this topic into the clinical context of cardiovascular diseases. In addition, this essay will illustrate how inflammatory pathways contribute to two common and clinically compelling cardiovascular diseases. The first example, atherosclerosis, affects the blood vessels. The second, acute myocardial infarction, affects the heart itself.

INFLAMMATION IS INTERTWINED WITH THE VASCULATURE

The ancients recognized the importance of inflammation in pathophysiology. Inflammation has features easily recognizable at the macroscopic level grossly apparent on physical examination. The Latin terminology handed down from Celsus in classical times described the cardinal features of inflammation: *rubor, tumor, calor,* and *dolor. Rubor* (redness) arises from increased local blood flow due to vasodilatation. *Calor* (heat) also likely reflects increased regional blood flow and vasodilatation. *Dolor* (pain) results from local release of inflammatory mediators that stimulate nociceptors in local nerves. *Tumor* (swelling) reflects tissue edema and often accompanies the redness and heat at sites of inflammation. Localized increases in vascular permeability, part and parcel of the inflammatory process, contribute to the local edema manifest as swelling. Aside from pain, all of these classic signs of inflammation relate to perturbations in the vasculature. Increased blood flow, vasodilatation, and increased vascular permeability characteristically accompany sites of inflammation. Considered in this context, the

SELECTED ABBREVIATIONS

aFGF	acid fibroblast growth factor
AGE	advanced glycation end product
APC	antigen-presenting cell
bFGF	basic fibroblast growth factor
COX-2	cyclooxygenase (isoenzyme)
CRP	C-reactive protein
ICAM-1	intercellular adhesion molecule–1
ICE	interleukin-1β–converting enzyme
IFN-γ	interferon gamma
IL-1, IL-6, IL-8	interleukin-1, interleukin-6, interleukin-8
MCP-1	macrophage chemoattractant protein–1
M-CSF	macrophage-colony stimulating factor
MMP	matrix metalloproteinase
NF-κB	nuclear factor kappa B
NO	nitric oxide
PAF	platelet-activating factor
PAI-1	plasminogen activator inhibitor–1
PDGF	platelet-derived growth factor
PPAR	peroxysome proliferation activation receptors
TLR	toll-like receptor
TNF	tumor necrosis factor
VCAM-1	vascular cell adhesion molecule–1
VEGF	vascular endothelial growth factor
VLA-4	very-late antigen–4

general pathology of inflammation falls within the province of cardiovascular pathophysiology in its most fundamental aspects.

Although known to the ancients, knowledge on the cellular basis of inflammation required advances in microscopy and tissue analysis made in the 19th century. The commercial development of aniline dyes in the textile industry stimulated the development of synthetic organic chemistry in the 1800s. The scientific "spinoff" of this commercially driven research and development included the application of newly synthesized dyes to provide contrast of stained tissues during microscopic examination. This technological advance spurred the development of cellular pathology embodied in the work of Rudolph Virchow. Armed only with stained tissue sections and keen inductive reasoning, the cellular pathologists of the 19th century worked out the involvement of leukocytes in inflammatory reactions in tissues.[1]

The 19th century also witnessed the birth of experimental pathology. The investigation of inflammatory phenomena figured prominently in the early development of this science. Elie Metchnikoff, summering in Messina, Italy, observed the engulfment by the leukocytes of marine invertebrates of foreign bodies, a process he called phagocytosis.[2] These observations and Metchnikoff's astute interpretations led to the recognition of the role of leukocytes and host defenses against foreign invaders. As the germ theory of disease developed in the latter part of the 19th century, the concept of foreign bodies extended to microbial invaders. To this day, much of the biology of inflammation consists of exchanges of messages among various classes of leukocytes and with parenchymal cells.

The molecular nature of the messages exchanged among cells during inflammatory reactions developed largely during the 20th century. During the first two thirds of the 20th century, much work focused on the purification and elucidation of the structures of low-molecular-weight mediators of inflammation. Molecules such as histamine, related to a simple amino acid, exemplified "autacoids" involved in inflammatory responses. Histamine can cause profound changes in vascular biology, including increased permeability of microvessels. Histamine also elicits the contraction of smooth muscle cells, manifest as vasoconstriction of some beds and bronchoconstriction in the airways. Small-molecule lipid mediators characterized during this period included the prostaglandins, the leukotrienes, the lipoxins, and platelet-activating factor (PAF). Some peptide mediators of inflammation of higher molecular weight also underwent purification and characterization, including bradykinin.

In the latter third of the 20th century, the study of protein mediators of inflammation and immunity burgeoned. Advances in protein biochemistry, and eventually molecular biology, led to the identification of the cytokines, now considered major messengers in host defenses and inflammatory reactions. Originally considered products of "professional" inflammatory cells such as leukocytes, traditional concepts viewed "peripheral" cells such as those of the vasculature or parenchymal cells of various organs as responders to signals elaborated by leukocytes. More recently, we have come to appreciate that many cell types, notably including vascular endothelial and smooth muscle cells, can produce as well as respond to various cytokines.[3] Thus, our understanding of the complexity of the networks involved in inflammatory signaling has grown considerably as scientific knowledge has advanced.

PHAGOCYTES: THE FIRST LINE OF HOST DEFENSES

In the more than a century following Metchnikoff's definition of phagocytosis, appreciation of the importance of this process has only grown as we have filled in the cellular and molecular mechanisms. The granulocyte—or polymorphonuclear leukocyte—embodies the phagocyte par excellence involved in acute inflammatory reactions. In acute bacterial infections, chemotactic peptides of microbial origin such as formyl-methionyl-leucyl-phenylalanine (f-met-leu-phe) or anaphylatoxins (see below) beckoned granulocytes from the vasculature to the nidus of infection. Activated by these bacterial products and others such as endotoxin (lipopolysaccharides manufactured by certain bacteria), the polymorphonuclear leukocyte degranulates, releasing enzymes that can attack the invaders. Granulocytes also manufacture enzymes that produce molecules involved in killing microbial invaders. Various oxidases generate superoxide anion (O_2^-), an example of a bactericidal reactive oxygen species. The granulocyte also releases myeloperoxidase, an enzyme that produces hypochlorous acid (HOCl). Hypochlorous acid, more commonly known as laundry bleach, can also kill bacteria. Although bacterial products may directly initiate the recruitment of granulocytes during infection, endogenous mechanisms soon join in.

Bacterial endotoxin can stimulate the expression on the surface of vascular endothelial cells of an adhesion molecule that binds selectively to a ligand on granulocytes.[4] E-selectin, not normally expressed by resting endothelial cells, arises rapidly on endothelial cells stimulated with bacterial lipopolysaccharide or certain cytokines. E-selectin binds to sialyl Lewis X molecules on the granulocyte surface. This interaction captures the polymorphonuclear leukocyte flowing through postcapillary venules and mediates a rolling of the granulocyte on the endothelial surface.[5] Other members of the selectin family may serve similar roles in slowing the progress of the polymorphonuclear leukocyte through the microcirculation. Tarrying in venules due to selectin-mediated rolling, the leukocyte can then form firmer interactions by binding to members of another family of adhesion molecules expressed by inflamed endothelial cells: members of the immunoglobulin G (IgG) superfamily.[6] These molecules, exemplified by intercellular adhesion molecule–1 (ICAM-1), arrest the rolling leukocyte, the next step in the recruitment process. Once adherent, the granulocyte receives chemoattractant signals from beyond the endothelial layer to trigger transmigration of the adherent leukocyte so that it can penetrate into the target tissue. The bacterial-derived chemoattractants, such as f-met-leu-phe, promote this directed migration. In addition, host cytokines such as interleukin-8 (IL-8) manufactured locally in response to bacterial products such as endotoxin can unite with bacterially derived chemoattractants to call the polymorphonuclear leukocyte into the tissue invaded by bacteria.[7,8] This schema of a local inflammatory stimulus (the bacteria) and recruitment of leukocytes by adherence transmigration followed by activation of the effector mechanisms of the leukocyte as summarized above applies not only to acute inflammation due to a bacterial infection, but serves as a general paradigm for localized inflammatory responses.

Of course, bacterial invasion sometimes spreads beyond the local tissue. Septicemia, or blood-borne infection, elicits a systemic or generalized host-defense reaction with profound cardiovascular consequences. Bacterial endotoxemia wreaks havoc with homeostasis of blood coagulation.[9] Endotoxin induces endothelial expression of the potent procoagulant molecule tissue factor.[10] It also augments production of inhibitors of fibrinolysis such as plasminogen-activator inhibitor–1 (PAI-1).[11] Such changes can give rise to disseminated intravascular coagulation, one of the dread consequences of disseminated Gram-negative infection. Circulating endotoxin leads to widespread release of cytokines, such as IL-1 and tumor necrosis factor (TNF) (see below). These cytokines can induce fever, which in turn causes tachycardia. In addition, the small molecule nitric oxide (NO), produced by endothelial cells stimulated with bacterial endotoxin as well as deriving from other inflamed cells including leukocytes, can promote vasodilatation and hypotension.[12] A hyperkinetic state ensues with low resistance and high cardiac output characteristic of septic shock. This picture, all too commonly encountered clinically, represents an extreme case on a systemic or generalized scale of the acute inflammatory response to a bacterial invader.

Not all bacterial infections play out on a scale of hours to days. Chronic microbial infections represent an example of inflammatory responses that endure longer. While some of the cells and messengers in the dramatis personae of chronic inflammatory responses differ from those described above in the context of acute responses, many of the fundamental principles remain the same. Consider, for example, the classic chronic bacterial disease, tuberculosis. The pathological hallmark of infection with the tubercle bacillus is a granuloma rather than an abscess populated by polymorphonuclear leukocytes. In the granuloma, the

mononuclear phagocyte, rather than the polymorphonuclear leukocyte, takes center stage. Derived from blood monocytes, the tissue macrophage becomes the key effector cell in chronic inflammatory responses. The tubercle granuloma can last for years as opposed to the abscess populated by polymorphonuclear leukocytes in a pyogenic bacterial infection. The granuloma represents the result of the interaction of the parenchymal tissue of the infected organ with the macrophages. This pathologic process plays out in a similar fashion in atheroma, an example of a special case of granuloma formation considered below.

As alluded to above, recruitment of monocytes to sites of chronic infection or inflammation recapitulate on a slower scale the accumulation of granulocytes to foci of acute inflammation. In the case of the monocyte, adhesion molecules of the IgG superfamily, such as vascular cell adhesion molecule–1 (VCAM-1), interact with integrin molecules on the mononuclear cell, such as very late antigen–4 (VLA-4). Once adherent to the endothelial cell, endogenous chemoattractant molecules direct the migration of the mononuclear phagocyte into the inflamed tissue. While IL-8 favors recruitment of granulocytes, members of another chemokine family, the CXC chemokines, selectively recruit monocytes. Macrophage chemoattractant protein–1 (MCP-1) represents an example of a mononuclear phagocyte chemoattractant that helps to recruit these cells to sites of formation of granuloma for other chronic inflammatory processes.

Granulocytes, the typical effector cells of acute inflammatory responses, have eponymous granules packed with preformed mediators. When recruited to sites of acute inflammation, they degranulate, rapidly produce a reactive oxygen species through the "respiratory burst," and die, often by apoptosis.[13] At sites of chronic inflammation, the life history of the mononuclear phagocyte differs considerably. The blood monocyte, recruited to a site of chronic inflammation, differentiates into a macrophage. A variety of endogenous stimuli favor the transition of the blood monocyte into a tissue macrophage involved in chronic inflammatory responses. Molecules such as macrophage-colony stimulating factor (M-CSF) and activators of protein kinase–C promote monocyte differentiation. Equipped for prolonged combat rather than a short battle with acute invaders, the macrophage effector mechanisms typically outlast those of granulocytes. Macrophages have a well-developed phagolysosome system that is well suited for combating chronic invaders. For example, the macrophage can engulf particulate matter such as the tubercle bacillus and transport the engulfed particle to the lysosome, an intracellular compartment filled with digestive enzymes. Often in concert with phagocytosis, the macrophage elaborates reactive oxygen species, including superoxide anion (O_2^-), NO, and hypochlorous acid.[12] The macrophage appears capable of producing these small-molecule effectors of host defenses on a more prolonged timescale than the granulocyte. The mononuclear phagocyte excels at producing higher-molecular-weight mediators as well. The macrophage, when activated, can produce large quantities of cytokines (see below). In addition, the tissue macrophage produces abundant proteases involved in tissue remodeling, a property of particular importance in cardiovascular pathology (see below).[14,15] Pus comprised of a mixture of live, dying, and dead granulocytes typifies abscesses at sites of acute inflammation. In granuloma, however, rather than committing suicide, macrophages may fuse, forming giant cells whose palisade of nuclei classically characterizes the tuberculous granuloma. Prolonged persistence of the phagocytic cell predominates at sites of chronic, as opposed to acute, inflammatory responses.

THE INNATE IMMUNE RESPONSE

The two general pathways of inflammation described above represent two limbs of host defense mechanisms now often described as "innate immunity." The term innate denotes instant readiness to cope with invaders, circumventing the need for an instructional period, as the acquired (antigen-specific) immune response requires (see below). The types of structures on invaders that trigger the innate immune response include molecules with shared structural features, such as Gram-negative endotoxin. The structures that recognize these relatively conserved structures have been called "pattern-recognition receptors." Families of pattern recognition receptors include scavenger receptors (first characterized in the context of atherosclerosis, see below),[16] and a recently characterized family of transmembrane molecules known as toll-like receptors (TLR).[17] Innate immunity represents a first line of defense against invaders capable of rapid deployment. Innate immunity plays out first not only within the organism, but also arises earlier during phylogeny. The innate immune response represents a more primitive form of host defense present in invertebrates and independent of the more selective recognition involved in adaptive immunity, a more recent development during evolution.[18] In addition to the leukocytic effector cells of acute and chronic inflammation described above, the complement system contributes importantly to innate immunity.[19,20]

Cytokine	Abbreviation(s)	Important functions
Interleukin-1	IL-1	Endogenous pyrogen, T cell coactivator, proinflammatory activation of vascular cells
Tumor necrosis factor	TNF	As above, autocrine regulator of myocardiocytes
Gamma interferon	IFN-γ	Macrophage activator, inducer of histocompatibility antigens, prototype Th1 cytokine
Interleukin-10	IL-10	Often anti-inflammatory, prototype Th2 cytokine
Macrophage-colony stimulating factor	M-CSF	Macrophage activator and comitogen
Monocyte chemoattractant protein–1	MCP-1	Macrophage chemoattractant
CD40 ligand	CD40L, CD154, gp39	As IL-1, but also activates caspase-1 and induces macrophage tissue factor expression
Interleukin-18	IL-18	Gamma interferon induction

Table I. *Examples of cytokines involved in cardiovascular inflammation.*

The alternative pathway of complement activation recognizes common patterns (for example, de-sialylated glycoconjugates on the surface of erythrocytes). Activation of complement by the alternative pathway leads to formation of the terminal membrane attack complex that can effectively punch holes in invading microbial cells. In this way, complement can kill bacterial and other invaders and, when unleashed inappropriately, host cells as well.

CYTOKINES: OMNIPRESENT ORGANIZERS OF THE INFLAMMATORY RESPONSE

As noted above, for most of the 20th century, attention focused on small molecules as mediators of inflammation. Over the last quarter century, we have become acquainted with large families of protein mediators of inflammation and immunity known collectively as cytokines.[21] These cytokines fall into many categories. Originally considered exclusively as products of leukocytes, many initially bore the name "interleukin." As our knowledge of the biology of cytokines has increased, so has our appreciation that many cells can elaborate these multifunctional mediators. Hence, more recently recognized families of cytokines often bear other names. Many of the cytokines categorized early on derive from mononuclear phagocytes, classifying them as monokines. Others, produced by T lymphocytes, bore the name "lymphokine." Chemoattractant cytokines fall into families known as chemokines.[8] A related class of protein mediators known as colony-stimulating factors has overlapping characteristics with classic cytokines (eg, interleukin-3). Therefore, discussions of cytokines often include colony-stimulating factors. The following paragraphs will introduce briefly certain of the prototypical cytokines to illustrate the properties of these important mediators. *Table I* shows examples of cytokines important in cardiovascular pathology and often cited in the cardiovascular literature.

INTERLEUKIN-1: THE PROTOTYPICAL MONOKINE

As is typical, the protein now known as interleukin-1 (IL-1) represented a convergence of various activities found to reside in a small family of closely-related proteins after years of parallel work in various laboratories.[22] The molecular characterization of IL-1 arose from the identification, purification, and eventually the cDNA cloning of the active principle in mediating fever known as endogenous pyrogen. The example of IL-1 illustrates many features common to cytokines. Seldom produced by resting cells, many cell types produce this protein inducibly in response to inflammatory stimuli, such as bacterial endotoxin. Initially synthesized as a 33 000 dalton precursor, interleukin-1β (IL-1β), the predominant secreted form of this cytokine, requires processing to attain its full biological activity. An enzyme known as interleukin-1β–converting enzyme, or ICE, effects this conversion.[23] Interestingly, ICE represented the prototype of a new family of proteinases known as caspases, so named because they are cysteinyl proteinases that cleave at aspartyl residues (asp) in their substrates. The other isoform of IL-1, known as interleukin-1α (IL-1α), often remains associated with the cell surface and does not require proteolytic processing for biological activity.

IL-1 binds to selective transmembrane receptors. The signaling receptor for IL-1β feeds into the same intracellular pathway as the pattern-recognition receptors,

TLRs, alluded to above. IL-1β engagement of its signaling receptor ultimately causes activation of a transcription factor known as nuclear factor kappa B (NF-κB).[24] NF-κB, when activated, binds to cognate sequences in the promoter regions of the genes encoding a wide variety of inflammatory effectors. Thus, IL-1β action unleashes a coordinate program of inflammatory mechanisms in many leukocytes and other host cells. In this way, IL-1β acts as a master regulator of the innate immune response, evoking the elaboration of many other cytokines, including augmentation of its own gene expression.[25] Like many potent biological pathways, IL-1β has an endogenous inhibitor, a structural homolog known as IL-1 receptor antagonist. This endogenous molecule competes for the signaling receptor of IL-1β, limiting its actions. Itself induced in response to inflammatory signals, the IL-1β receptor antagonist constitutes a negative feedback loop poised to prevent untrammeled amplification of this potent proinflammatory pathway.

TUMOR NECROSIS FACTOR–α: A DEATH-LINKED CYTOKINE

Tumor necrosis factor–α (TNF-α), a close cousin of IL-1β, exhibits augmented production and spectral and biological activities similar to those due to IL-1. Like IL-1β, TNF-α has several surface receptors. The active signaling receptor, however, elicits activation not only of NF-κB, but also of the pathway that causes programmed cell death, or apoptosis.[26] Thus, in addition to the proinflammatory action that TNF-α has in common with IL-1β, it links to the control of cell death.

INTERLEUKIN-6: A "MESSENGER" CYTOKINE

A prominent target gene for activation by TNF-α and IL-1β, many peripheral cells, including vascular smooth muscle, can secrete copious quantities of interleukin-6 (IL-6) when stimulated by the primary proinflammatory cytokines IL-1 or TNF. IL-6 activates B cells and promotes their maturation, an important aspect of antibody production and acquired immunity (see below). Of great interest in cardiovascular pathology, IL-6 serves as a link between the acute inflammatory response mediated by the primary proinflammatory cytokines and the systemic reaction to acute inflammation, known as the "acute-phase response."[8,27] IL-6 alters the pattern of protein synthesis by hepatocytes. After encountering IL-6, the liver shifts new protein synthesis from "housekeeping" proteins such as albumen toward production of acute-phase reactants, which serve as readily sampled systemic markers of inflammation. The acute phase reactants may also participate in inflammatory responses involved in cardiovascular pathology. For example, C-reactive protein (CRP), a classic acute-phase reactant, has garnered considerable interest recently as a prognostic or predictive indicator of cardiovascular risk.[28] In addition, CRP can activate complement and thus actively participate in innate immunity. Serum amyloid A, another prominent acute-phase reactant, may increase hundreds of times in the plasma of patients with inflammatory states. Serum amyloid A can bind to high-density lipoprotein (HDL), impairing some of the salutary properties of this lipoprotein molecule, thus potentially increasing coronary risk prospectively.[29] Another acute-phase reactant, fibrinogen, directly participates in blood coagulation. In this manner, the acute-phase response may link to a thrombotic diathesis of considerable import in cardiovascular diseases. IL-6, as an instigator of the acute-phase response, can in this manner serve as an important link between inflammation and cardiovascular events.

INTERFERON GAMMA: THE PROTOTYPICAL LYMPHOKINE

Immune interferon, or interferon gamma (IFN-γ), contrasts with the aforementioned cytokines inasmuch as a much smaller gamut of cells produce this cytokine. Classically considered exclusively a product of activated T lymphocytes, even after exhaustive investigations only a few cell types have proven capable of producing IFN-γ. As might be expected of a cytokine derived from a cell type prominent in adaptive immunity, IFN-γ enhances antigen presentation by augmenting the expression of major histocompatibility complex antigens important for recognition of foreign antigens (see below).[30] However, IFN-γ also stimulates mononuclear phagocytes, among other aspects of the innate immune response. Nonetheless, the range of actions produced by IFN-γ shows considerably more selectivity than that of IL-1 or TNF, illustrating that all cytokines act promiscuously.

MACROPHAGE CHEMOATTRACTANT PROTEIN–1: A TYPICAL CHEMOATTRACTANT CHEMOKINE

Several families of chemokines exist. Specialists use a shorthand based on the spacing of conserved cysteine residues in the amino acid sequence of the chemokines to classify these various families. MCP-1 falls into the family of CXC chemokines, indicating that an unspecified (X) amino acid residue lies between neighboring

cysteine residues.[8] Chemokines typically bind to specific membrane receptors grouped into similar families based on their ligand. The MCP-1 receptor, CXC-R2, belong to the large family of heptahelical, or 7-membrane spanning G-protein–coupled receptors. Thus, the signaling mechanism of chemokines does not generally overlap with those of the primary proinflammatory cytokines. As the spectrum of receptors on various classes of cells varies, so does the specificity of the targets for chemoattraction of various chemokines. MCP-1, as mentioned above, binds selectively to cells of the mononuclear family. Mice lacking the receptor for MCP-1 show impaired recruitment of mononuclear phagocytes to sites of chronic inflammation.

THE LYMPHOCYTES: MAJOR MEDIATORS OF ACQUIRED IMMUNITY

Thus far, our discussion of inflammatory responses has focused on innate or "ready-made" immunity. Higher organisms have added to the repertoire of host defenses a complex surveillance and effector system based on highly-variable and fine structural determinants known as acquired immunity. This name also conveys the idea that acquisition of this form of immunity requires time, rather than being prefabricated as in the case of innate immunity. The T lymphocyte orchestrates acquired immunity, which has both humoral (antibody-mediated) and cellular limbs.

GENERATION OF THE ACQUIRED IMMUNE RESPONSE: THE HELPER T CELL

A subset of T cells known as helper T cells, usually bearing the CD4 determinant, initiates most acquired immune responses by recognizing antigen via engagement of a selective cell surface receptor. The recognition of antigens by the helper T cell requires presentation on the surface of a cooperating cell, known as the antigen-presenting cell, or APC. The T cell receptor does not recognize the foreign antigen in isolation, but requires presentation in the context of a structure on the surface of the APC, the class II major histocompatibility antigen. Each individual bears its own mix of these highly polymorphic molecules that serve an essential role in generating the immune response. Incidentally, histocompatibility antigens bear this name because they themselves are capable of generating an immune response to organs transplanted from different members of a species, hence the term histocompatibility antigen. The engagement of the T cell's receptor for antigen causes the T cell to secrete IFN-γ, which raises the level of class II histocompatibility antigens on the surface of potential antigen-presenting cells, priming them to contribute to the generation of the acquired immune response. Engagement of the antigen receptor also causes the T cell to augment its production of a lymphokine known as IL-2, originally termed T-cell growth factor. The T cell activated by antigen also increases its expression of the cell surface that binds IL-2. Thus, the T cell that has encountered foreign antigen in the context of its own class II molecule can undergo self-perpetuated clonal expansion, producing and responding to its own growth factor, an example of an autocrine growth control loop. Expanding the population of antigen-specific T cells amplifies the response to a given foreign antigen, for example a virus.

The term "helper T cell" refers to the ability of the CD4 cell, when activated, to promote development of a full-blown immune response, both humoral and cellular. Stimulation of the humoral immune response involves the expression on the surface of the activated T cell of CD154, also known as CD40 ligand.[31] This surface molecule binds to a cognate receptor on the B lymphocyte, the antigen-producing cell, causing it to mature into a plasma cell capable of secreting large quantities of antibody directed against specific antigens. The IL-2 secreted by the activated T cell serves not only in autocrine growth, but also in "paracrine growth," stimulating the proliferation of CD8 cells, also known as killer or cytotoxic T cells, the efferent limb of the cellular immune response.

CYTOLYTIC T CELLS: EFFECTORS OF THE CELLULAR IMMUNE RESPONSE

Stimulated by IL-2 secreted by the activated helper T cell, CD8 cells proliferate and become activated themselves. The CD8 cell recognizes foreign antigen in the context of class I MHC (major histocompatibility complex) molecules. While helper T cells recognize antigen presented on the surface of antigen-presenting cells in the context of self–class II MHC, the cytolytic T cell recognizes antigen presented in the context of class I molecules on the surface cells infected with viruses or, in the case of transplanted organs, the foreign cell itself. Recognition of the foreign antigen causes the cytolytic T cell to secrete a molecule known as perforin, which resembles the terminal membrane attack complex component of complement, and likewise can poke holes in the membrane of the cell under attack.[32] In addition, digestive enzymes, such as granzyme, arise from the activated killer T cell. The cytolytic T cell, usually bearing the CD8 antigen on its surface, can also express

Fas, a TNF-like molecule, on its surface. Fas engages Fas-ligand on the cell under attack. Fas ligation, like TNF, causes activation of the cell-death cascade. The Fas pathway thus represents yet another mechanism by which the CD8-positive T cell can kill a cell that harbors a viral infection or otherwise presents foreign antigen.

B CELLS: A SOURCE OF ANTIBODY

As described above, the effector limb of cellular immunity requires contact between the cytolytic T cell and its target. Humoral responses mediated by antibody, soluble molecules, do not require such cell-cell contact. The B cell, in receipt of "help" from T cells and aided by the "messenger" cytokine IL-6, becomes a plasma cell specialized in secreting large amounts of antibody.

Antibody can serve several roles in host defenses. By binding to determinants on the surface of microbial invaders, antibody molecules can "fix" complement through the classic pathway, leading to lysis of the invading cell by mechanisms already discussed. Antibody coating the surface of bacteria also can engage the Fc-receptors on the surface of phagocytes, targeting them for engulfment and destruction. This function of antibody, known as opsonization, was likened by the Irish playwright George Bernard Shaw to putting butter on a slice of bread before eating it.[33] Antibody can also neutralize invaders such as viruses simply by binding them tightly and preventing their access to target cells.

INFLAMMATORY DISEASES: HOST DEFENSES GONE AWRY

The preceding sections have outlined a series of host defense mechanisms ranging from primitive to highly-developed and specific, all focused on defending the organism from invading pathogens and foreign bodies. These defense mechanisms each serve important roles in health and homeostasis. Individuals with congenital or acquired deficiencies in each of these major pathways of host defenses exhibit heightened susceptibility to different types of disease. As impaired antibody responses predispose to pyogenic bacterial diseases, consider the case of the increased incidence of pneumococcal disease in splenectomized patients. Individuals with impaired cellular immunity have difficulty eliminating viruses and intracellular bacteria, as in the case of the acquired immune deficiency syndrome (AIDS). Individuals with mutations that impair their ability to make superoxide anion show susceptibility to pyogenic infections, particularly *Staphylococcus aureus.*

However, these powerful host defense mechanisms can cause disease when expressed inappropriately or in excess. The entire spectrum of inflammatory diseases represents host defenses gone awry. The humoral and cellular immune responses, appropriately directed against foreign invaders, become pathological when turned against self, as in the case of the autoimmune diseases. Many autoimmune diseases cause vasculitis, placing them within the spectrum of cardiovascular diseases. Acute allograft rejection and the chronic vasculopathy often seen in transplanted organs represent another undesired consequence of the otherwise salubrious host-immune response. In some cases, microbial invaders may elicit an immune response that cross-reacts with an endogenous host structure. Such antigenic mimicry may account for the association of rheumatic heart disease with streptococcal infections. Recent evidence inculpates antigenic mimicry between *Chlamydia pneumoniae* and a determinant of cardiac myosin as a mechanism of autoimmune cardiomyopathy. Cholesterol emboli may activate complement, provoking an inflammatory response with often dramatic clinical consequences. *Table II* lists a number of cardiovascular conditions, some exotic, but many common, that involve an important component of inflammation, representing host defense mechanisms that nonetheless can cause disease. The following sections will consider examples of some common cardiovascular diseases that specifically illustrate this principle.

ATHEROSCLEROSIS: A CHRONIC CARDIOVASCULAR INFLAMMATORY DISEASE

In the past, most considered atherosclerosis a type of lipid storage disease caused by excessive cholesterol accumulating in arteries in bland pools of extracellular lipid. Recent work, however, has heightened interest in the inflammatory aspects of atherosclerosis, not only at a fundamental level, but also in relation to the clinic. Actually, atherosclerosis involves inflammation at all stages, ranging from the earliest steps in atheroma formation, straight through to the ultimate clinical complications of this common affliction.

INFLAMMATION AND THE INITIATION OF ATHEROSCLEROSIS

The first steps in atherogenesis recapitulate host defenses against microbial pathogens as explicated above. However, in this caricature of a normal host defense pathway, lipids appear to play an important role as a trigger. Lipoproteins enter the arterial intima, where

Disease	Example of inflammatory process in pathogenesis
Atherosclerosis	Fibroproliferation in response to macrophage-derived mediators
Unstable angina pectoris	Inflammation characterized by elevated C-reactive protein without infarction
Atheroma disruption	Macrophage-induced collagenolysis, T-cell inhibition of collagen synthesis
Vascular thrombosis	Favored by acute-phase reactants fibrinogen, plasminogen inhibitor
Myocardial infarction	Phagocyte ingestion of necrotic cells and subsequent tissue repair
Myocarditis	Leukocyte infiltration and cytokine activation of cardiomyocytes and endothelium
Abdominal aortic aneurysm	Prominent inflammation of the adventitia as well as intima-media
Cholesterol emboli syndrome	Complement activation
Cardiovascular complications of lupus erythematosus, scleroderma, rheumatoid arthritis, etc	Vasculitis
Rheumatic heart disease	Pancarditis
Transplantation rejection	Killer-T-cell–mediated myocardiocytolysis
Allograft vasculopathy	CD4-mediated fibroproliferative response to foreign antigens
"No-reflow"	Leukocyte sludging due to activated endothelium
Restenosis post–arterial intervention	Activation of vascular cells and leukocyte recruitment provoked by injury
Infective endocarditis	Complement activation by immune complexes contribute to complications

Table II. *Examples of cardiovascular diseases involving inflammation.*

they can undergo modification by oxidation, forming oxidized forms of lipids that can incite inflammation.[34] Another type of modification of lipoproteins arises by chemical condensation with glucose residues and subsequent chemical reactions that yield advanced glycation end products (AGEs).[35] Hyperglycemia such as that encountered in diabetics accelerates formation of AGEs. AGE-modified proteins can also stimulate inflammation. Recent evidence has renewed interest in the possibility that infectious agents themselves may participate in atherogenesis.[36] In this case, viral or bacterial pathogens may trigger the localized inflammatory response inculpated in the initiation of atherosclerosis. Modified lipids, glycated proteins, and infectious agents and their products alike can elicit the expression of proinflammatory cytokines from cells resident in the arterial wall, including endothelium and vascular smooth muscle cells.[37] The proinflammatory cytokines thus produced can elicit the expression on the endothelial surface of adhesion molecules, such as VCAM-1, specialized in the recruitment of mononuclear cells. VCAM-1 binds just the subclasses of leukocytes found in early atheroma, monocyte/macrophages and T lymphocytes. Experiments in animals with defective VCAM-1 molecules show reduced atherosclerosis in response to hypercholesterolemia, supporting a role for this adhesion molecule in lesion formation.[38]

Once adherent, the mononuclear cell enters the artery wall in response to chemoattractant stimuli. In the case of tissue microbial invasion, postcapillary venules typically serve as the portal of entry of the leukocyte into the affected tissue. In the case of atherosclerosis, the artery wall itself being the site of the inflammatory response, chemoattractants such as MCP-1 cause the leukocyte to enter the arterial intima. Once resident within the intimal layer, the monocyte undergoes differentiation into a macrophage. In the context of atherogenesis, the macrophage takes on a special phenotype, the lipid-laden foam cell. Engorgement with lipid cannot occur by binding of LDL particles to the classic LDL-receptor. Cholesterol loading rapidly reduces the expression of the LDL-receptor. This autoregulation prevents foam cell formation by this mechanism. In atheroma, macrophages express a variety of "scavenger receptors" that evade this regulatory step, continue to be expressed despite cellular lipid accumulation, and permit foam cell formation by facilitating entry of modified lipoprotein particles into the phagocyte.[39] While resting monocytes express only low levels of scavenger receptors, after exposure to certain inflammatory mediators found in atheroma such as M-CSF, macrophages express higher levels of these receptors that facilitate foam cell formation.[37] Mutations in various scavenger receptors limit evolution of fatty lesions in hypercholes-

terolemic mice.[40,41] Leukocyte adhesion, chemoattraction, and activation thus occur within the nascent atheroma, replicating the steps in a typical inflammatory response. The foam cell–rich fatty streak represents the first stage of atheroma formation.

INFLAMMATION AND EVOLUTION OF THE ATHEROMATOUS PLAQUE

If inflammatory stimulation persists, fatty streaks can progress to more complicated forms of atherosclerosis, such as the fibro-fatty plaque. Fibrogenesis results from elaboration by arterial smooth muscle cells of extracellular matrix macromolecules, including collagens, elastin, and various proteoglycan molecules. Smooth muscle cells in the plaque arise from precursors resident in the intima in humans. Lesional smooth muscle cells may also arise by migration of medial smooth muscle cells into the intima, across the demarcating internal elastic lamina. In some types of arterial pathology, bone marrow–derived precursors may also give rise to smooth muscle cells involved in fibrous lesion formation.[42] A variety of peptide growth factors can stimulate smooth muscle migration and proliferation as well as regulate their biosynthesis of extracellular matrix macromolecules that form the fibrous part of complex atherosclerotic plaques. Protein mediators that stimulate smooth muscle migration and division include platelet-derived growth factor (PDGF) and basic fibroblast growth factor (bFGF).[21] Although named growth factors, in practical terms these proteins could just as well be called cytokines, as no strict difference separates these two classic categories of biological mediators.

Cytokines such as IL-1 or TNF can augment the production of growth factors such as PDGF or bFGF by smooth muscle cells, providing a direct link between inflammation and the control of growth of vascular smooth muscle cells. PDGF in vivo probably promotes smooth muscle migration to a greater extent than proliferation. PDGF can also augment collagen production by vascular smooth muscle cells, consistent with its role in lesion evolution.

Atherosclerotic lesions also contain considerable numbers of T lymphocytes.[43] These T cells bear markers of activation. Atherosclerotic lesions contain IFN-γ, a typical product of activated T cells. Smooth muscle cells and macrophages in human lesions also express class II histocompatibility antigens, an indicator of stimulation by IFN-γ. The reader can surmise from the foregoing summary of the cellular immune response that the atherosclerotic lesion thus contains all of the cells involved in acquired immunity. Antigens found in atherosclerotic plaques, such as modified lipoproteins and heat shock proteins (produced by cells in the atherosclerotic lesion), can act as antigens, stimulating an ongoing cellular immune response in the atheroma. In addition to innate immunity (activated macrophages) and the afferent limb of cellular immunity mediated by helper T cells, cell death mediated by Fas ligation and/or cytolytic T cells may also contribute to apoptosis of smooth muscle cells and macrophages, as now clearly demonstrated in advanced human atherosclerotic lesions.[44] In illustrating the basic biology of chronic immune responses, this essay invoked the example of the granuloma engendered by infection with the tubercle bacillus. In many ways, the atheroma resembles a specialized form of granuloma, noncaseating, but with a lipid core. Much of the cellular biology and pathophysiological response in the atheroma resemble those in the infectious granuloma. In addition, many of the molecular mediators, the cytokines and growth factors, participate in both processes. Thus, atheroma, far from being a bland accumulation of lipids, rather resembles a smoldering chronic inflammatory response, another example of host defenses gone awry.

INFLAMMATION AND THE ACUTE COMPLICATIONS OF ATHEROSCLEROSIS

Atheromata seldom cause acute clinical manifestations because of their obstructive, space-occupying properties. Such substantial stenoses may cause stable angina pectoris, but most acute coronary syndromes result from thrombosis complicating plaques. In fact, many acute coronary syndromes result not from highly stenotic lesions, but from lesions that may cause lesser degrees of stenosis. Clinical data supporting this view have emerged from angiograms performed following lysis of the culprit clot by thrombolytic therapy. A substantial minority of lesions that precipitate acute myocardial infarction produce stenoses of less that 50% once the occlusive thrombus has undergone lysis. Serial angiographic studies also indicate that the culprit lesion of acute myocardial infarction often showed modest degrees of stenosis on antecedent angiograms. Other serial angiographic studies have shown that a substantial number of human atherosclerotic plaques in coronary arteries evolve rapidly and discontinuously, rather than slowly and smoothly in time.

We now recognize that most thrombotic complications of atherosclerosis that cause acute events result from the physical disruption in the atherosclerotic plaque.

One form of disruption, superficial erosion, may involve concomitant inflammation, although opinions on this point differ. However, all observers agree that a fracture of the fibrous cap, the most common cause of fatal acute myocardial infarction in humans, arises at sites of heightened inflammatory responses.[45,46] Analysis of twenty culprit lesions of fatal acute myocardial infarctions showed that T lymphocytes and macrophages predominated at sites of clinical plaque rupture, causing fatal thrombi. Smooth muscle cells and macrophages in these zones of fatal plaque disruption showed expression of the class II histocompatibility antigen HLA-DR. This result supports a role for activated T cells and macrophages in clinically significant plaque disruption. Interruption in collagen synthesis by smooth muscle cells caused by the T cell product IFN-γ (the inducer of class II histocompatibility molecules) may account for weakness and fragility of the plaque's fibrous cap at places of rupture. Autopsy studies have shown that the presence of T lymphocytes correlates inversely with indices of the synthesis of fibrillar forms of collagen, the extracellular matrix macromolecule that lends strength to the plaque's fibrous cap.

Weakening the fibrous cap results not only from decreased collagen synthesis, but from augmented collagen degradation as well. Activated macrophages secrete several types of proteinases that can attack the extracellular matrix molecules responsible for the integrity of the plaque's fibrous cap. Macrophages exposed to inflammatory cytokines step up their production of collagenases of the matrix metalloproteinase (MMP) family and lysosomal enzymes capable of dissolving arterial extracellular matrix macromolecules as well. These findings provide a firm foundation for involvement of inflammation in weakening of the plaque's fibrous cap and plaque disruption and thrombosis.

Once the fibrous cap fails, blood coagulation factors undergo activation by contact with tissue factor expressed by macrophages in the plaque's lipid core.[10] The expression of the tissue factor gene requires activation by inflammatory mediators. As discussed above in the context of Gram-negative sepsis, bacterial lipopolysaccharide can induce tissue factor expression in human monocyte/macrophages. T cells, found adjacent to macrophages in fatally disrupted human atherosclerotic plaques, can activate tissue factor expression on macrophages by producing CD154 (CD40 ligand).[47] Once again, pathways first unraveled in the context of cellular immune responses appear to participate importantly in aspects of atherogenesis, this time regulating the thrombogenicity of the plaque's lipid core.

ATHEROSCLEROSIS AND INFLAMMATION: THERAPEUTIC IMPLICATIONS

The foregoing discussion has illustrated the pivotal role of inflammation in all phases of atherosclerosis, lesion initiation, progression, and complication. This central role of inflammation and immunity in atherogenesis suggests that anti-inflammatory therapies might have a role in the management of this ubiquitous disease. Indeed, we have advanced the notion that lipid-lowering therapy exerts its benefit in atherosclerosis in part by acting as a specific anti-inflammatory intervention directed at the relevant instigation stimulus in this disease. Experimental studies have validated the concept that lipid lowering causes inflammation associated with atherosclerosis to subside.[48] Human observations have shown decreases in inflammatory markers such as CRP with lipid lowering. While much of this benefit probably accrues due to lipid lowering itself, some of the pharmacologic agents used in lipid management may have direct effects independent of their hypolipidemic actions. For example, statins may possess so-called "pleiotropic" effects. By interfering with intracellular signaling pathways, statins may interrupt certain inflammatory pathways. However, many in vitro studies of pleiotropic actions of statins employ concentrations not likely achievable under clinical circumstances. Other classes of agents used to manage atherosclerotic risk factors, including the fibric acid derivatives and thiazolidinediones (such as the insulin-sensitizing "glitazone" drugs), may have direct anti-inflammatory effects mediated by binding to nuclear receptors known as peroxysome proliferation activation receptors (PPARs).[49] Once again, although based on substantial in vitro evidence, the clinical relevance of these nonlipid-dependent effects of PPAR agonists remains speculative at a clinical level.

Some have advocated the use of nonspecific anti-inflammatory drugs in the treatment of atherosclerosis. Atherosclerotic lesions do express the "inflammatory" isoenzyme of cyclooxygenase (COX-2).[50] This enzyme catalyzes the production of prostaglandins possibly involved in vascular pathophysiology. However, as these agents interrupt production of the vasodilatory and antiplatelet aggregatory prostanoid prostacyclin, inhibition of COX-2 as cardiovascular therapy will require careful clinical evaluation. The strategy of identifying the proximal triggers of inflammatory responses and directing therapy at the triggers rather than distal effector pathways of inflammation appears more attractive.

ACUTE MYOCARDIAL INFARCTION: AN INFLAMMATORY CARDIAC DISEASE

The previous section discussed how inflammation can set the stage for acute coronary syndromes in concluding myocardial infarction. However, myocardial infarction itself unleashes an inflammatory response at the level of the ventricular myocardium. Tissue injury can stimulate the innate immune response. Tissue necrosis elicits recruitment, first of the leukocyte emblematic of the acute inflammatory response, the granulocyte. The inflammatory response mediated by granulocytes in infarcting myocardium may actually extend the injury. These specific granules of neutrophils contain a form of collagenase (MMP-8) that can cleave interstitial collagen in the myocardium, favoring expansion of the infarct zone, the first step in myocardial remodeling. Such infarct expansion correlates with worsened clinical outcome. The reactive oxygen species released by the activated neutrophil can heighten local tissue injury in the infarcting myocardium. In addition, endothelial damage following on neutrophil activation can contribute to the "no-reflow" phenomenon and microvascular dysfunction that currently represent an obstacle to reperfusion therapies. Strategies that limit neutrophil accumulation following coronary ligation can alleviate some of the consequences of reperfusion injury.

Within days after acute myocardial infarction, the acute inflammatory response gives way to a more chronic reaction. Macrophages supplant polymorphonuclear leukocytes as the principal inflammatory cell type. The macrophages also contribute to tissue remodeling. Production of interstitial collagenases and other proteolytic enzymes can accentuate tissue remodeling initiated by granulocyte-derived proteinases. Administration of inhibitors of matrix metalloproteinases can limit left ventricular remodeling following experimental coronary ligation. Targeted disruption of the gene encoding MMP-9 can likewise limit infarct expansion.

The macrophage in the infarct can phagocytize dead cardiac myocytes and their debris. The macrophage also releases fibrogenic mediators that elicit tissue repair. Granulation tissue, comprised of stromal cells proliferating in response to protein growth factors migrating into the infarcted zone in response to these mediators, leads to scar formation replacing zones of coagulation necrosis of cardiac myocytes.

Angiogenesis also characterizes granulation tissue, replacing infarcted myocardium. Inflammatory mediators released by leukocytes infiltrating the infarcted zone may promote the production of angiogenic peptides such as acidic fibroblast growth factor (aFGF), bFGF, and vascular endothelial growth factor (VEGF). Thus, the "mopping up" operation affected by the chronic inflammatory cells may also promote collateral growth as part of the normal reparative mechanism in the aftermath of an acute myocardial infarction.

The tissue injury of acute myocardial infarction elicits an acute-phase response. CRP and serum amyloid A levels in peripheral blood in the throws of an acute coronary event correlate with prognosis. Thus, the degree of the inflammatory response mounted in response to an ischemic insult to the myocardium can have considerable clinical consequences.

The exposure to immune cells of antigens usually contained within cells due to acute ischemic injury can elicit immune responses as well. Antimyosin antibodies can instigate autoimmune myocarditis in experimental models. The postpericardiotomy syndrome (Dressler's) may represent an autoimmune response engendered by myocardial injury. The above examples illustrate how inflammatory responses participate in many aspects of acute myocardial infarction and its clinical complications.

CONCLUSION

This primer of inflammation biology introduces the cast of characters under consideration in subsequent contributions to this issue. The general pathway of inflammation plays out in most cardiovascular diseases of clinical import. The recognition of the role of inflammatory processes in cardiovascular diseases furnishes a new dimension to the understanding of their pathophysiology. In addition, the inflammatory aspects of cardiovascular diseases may provide, in some cases, new therapeutic opportunities to forestall the development or the consequences of various cardiovascular conditions. Seemingly far afield at the outset, contemporary inflammation biology has concrete clinical ramifications for the practitioner. Learning to redirect inappropriate inflammatory responses may help us to improve patient outcomes in years to come.

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THREE KEY QUESTIONS

The highly complex array of inflammatory processes and mediators discussed above clearly fall into two categories: that of *friend,* when, according to plan, they ward off external or internal aggressions, or *foe*, when they succeed too well (much to our distress as in the case of allograft rejection), become overactive (as in the case of autoimmune disorders), fail (as with AIDS), or do only part of the job (as in the case of the chronic tubercle granuloma or atheroma). If we now look at inflammation from the viewpoint of the clinical cardiologist, then we see that they can be exploited as *markers* of the disease process or as possible *targets* for therapeutic intervention. The topic of inflammation and coronary artery disease undoubtedly gives rise to far more questions than the format of *Dialogues* allows, but for the purpose of this issue we have identified three key questions relating to daily practical concerns of the clinician dealing with atheroma and myocardial infarction. In all three answers, C-reactive protein (CRP) holds center stage, since, despite lingering debate, clinical applications appear to be just around the corner. Cornelis Kluft is asked: **"What are the practical consequences of the important role of inflammation in cardiovascular disease and the use of C-reactive protein as a risk marker?"** and in his reply sees CRP as now close to being formally recognized in primary prevention of vascular events when inflammation is present, in the face of "normal" lipid levels. Attilio Maseri, in response to the question: **"Which tests should the clinician use today to assess arterial inflammation in patients?"** advocates, for his part, a wait-and-see approach, advising us to restrict the measurement of CRP to clinical research until we are more certain of its value. Finally, John Danesh explores one aspect of the debate alluded to above: **"Are markers of inflammation elevated in patients at risk for atherosclerosis because of vascular or extravascular inflammation?"** He singles out several possible confounding factors that may have muddled—though certainly not made void—the issue of the value of CRP. Thus, though practical applications of our growing knowledge of inflammation are close at hand, there certainly is need for further experimentation. And since CRP is but one of the host of inflammatory mediators pregnant with potential clinical applications, *Dialogues* is sure to report on the rapid advances in the field in a not too-distant future!

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What are the practical consequences of the recognition of the important role of inflammation in cardiovascular disease and the use of C-reactive protein as a risk marker?

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The risk of cardiovascular events associated with increased inflammation has been discovered and recognized thanks to a readily available marker: C-reactive protein (CRP). The use of CRP has made it possible to carry out a rapid evaluation of epidemiological studies and randomized controlled trials, building up evidence for its usefulness in clinical practice. Use of CRP to determine individuals at risk due to increased inflammation, but with lipid levels not requiring therapy, is now close to being implemented in primary prevention. Treatment options are available. Currently, CRP is the parameter of risk of choice; further research on vascular inflammation may change or refine the approach.

Keywords: inflammation; diagnostic strategy; C-reactive protein; cardiovascular disease; atherosclerosis; risk prediction

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In humans, vascular walls are subjected to chronic insults and the progressive development of atherosclerosis from a very early age.[1] Classically, clinicians have mainly been concerned with lipid infiltration and deposition. However, this focus of attention has changed with the recent discovery that inflammation played a pivotal role in cardiovascular disease (CVD), was individually different, and signaled a major risk for vascular events. Ever since findings from the European Concerted Action on Thrombosis (ECAT) study demonstrated that a chronically elevated blood level of the inflammation marker C-reactive protein (CRP) was a risk marker independent of cholesterol, it became clear that risk prediction of events was at least equal, and sometimes better, with CRP than with lipid levels.[2,3] The easy availability of CRP as a marker of inflammation gave rise to an upsurge of epidemiological and intervention studies, which resulted in an explosion of publications since the first disclosures of ECAT in 1994,[4] thereby increasing our awareness of the importance of the inflammatory process and of developing means to control it.

SELECTED ABBREVIATIONS AND ACRONYMS

Apo-E	apolipoprotein E
CABG	coronary artery bypass grafting
CRP	C-reactive protein
CVD	cardiovascular disease
DALI	Diabetes Atorvastatin Lipid Intervention (study)
ECAT	European Concerted Action on Thrombosis
ICAM-1	intercellular cell adhesion molecule–1
IL-6	interleukin 6
IL-18	interleukin 18
MIRACL	Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering (trial)
PTCA	percutaneous transluminal coronary angioplasty
SAA	serum amyloid A
sPLA2	secretory phospholipase A_2
TNF-α	tumor necrosis factor–α

UNDERSTANDING THE MECHANISM

The importance of inflammation in terms of risk of stochastic events such as myocardial infarction remains unclear if we merely look at the chronic

inflammatory nature of the atherosclerotic process. Two other essential aspects must be taken into consideration: first, events take place whether the underlying stenosis is moderate or severe; second, the role of inflammation is enhanced in the presence of plaque instability and thrombogenicity. Only then is it possible to understand, on the one hand, the relationship between CRP levels and the degree or speed of the chronic atherosclerosis process and, for instance, intima-media thickness or restenosis, as well as, on the other hand, that between elevated levels of CRP and specific inflammation related to plaque instability, thrombogenicity, and events. CRP should therefore be viewed as a partially independent risk marker of the risk of infarction in the presence of low-grade stenosis.

Furthermore, the thrombotic nature of cardiovascular events or infarctions suggests that inflammation results in an increase in the risk of clotting. Indeed, local accumulation of tissue factor during the inflammatory process appears to result in a major risk of events when plaques are rupturing.[5]

Thus, the clinician's principal concern in terms of prevention and treatment of cardiovascular events is both to control the specific inflammation related to events and prevent clotting,[5] while control of the atherosclerotic process is a more long-term aim.

Most available studies using animal models have focused on the progression or regression of atherogenesis and atherosclerosis, and only few have looked at events. Inflammation and thrombosis require different animal models than those based on lipid abnormalities (eg, apolipoprotein E [Apo E] knockout and Apo E3 Leiden).[6,7] A change in focus is expected here, in light of the fact that although monogenetic lipid disorders lead to atherosclerosis, this does not automatically result in events.

In addition to vascular inflammation, related to plaque instability, rupture, and thrombogenicity, another inflammatory mechanism, the response in acute situations, plays an important role in the severity of events and will be discussed below in the Perspectives section.

Inflammation poses a dilemma in terms of prevention and cure.[5] Since inflammation is a mechanism of tissue repair and remodeling, its inhibition is hardly indicated! Nevertheless, the fact that inflammation becomes chronic in atherosclerosis and is excessive before events points to an inadequate process of removal of the trigger or regulation of inflammation. The exact nature of the inflammatory trigger is unknown and may vary among individuals, but probably involves accumulated and modified lipids, glycated molecules, infectious agents, immunogenic factors, cell remnants, etc. Reducing these triggers may reduce inflammation, providing a possible, indirect explanation for the effects of lipid-lowering, antioxidant, and antidiabetic treatments.

Although direct, general anti-inflammatory treatments may have an important role in reducing risk, this could be at the expense of proper healing. This suggests that such an approach can only be temporarily applied. It appears more logical to seek to control specific aspects of inflammation, such as excessive or dysregulated inflammation, and inhibit the sequels of the inflammatory process, such as coagulation and complement activation, in high-risk individuals. Thus, although drugs with pleiotropic anti-inflammatory effects such as statins and fibrates may be beneficial in high-risk individuals, they should only be given after due consideration to asymptomatic individuals, as preventive treatment.

TOWARD PRACTICAL APPLICATIONS

Although implementation of the recently acquired knowledge about inflammation and CRP still requires further study, one can nevertheless assume that a change in paradigm is under way, which will take us from the classic predominant lipid approach to CVD to one including inflammation. Confirmation that a patient-group at risk for CVD can be identified among individuals with "normal" lipid levels hitherto not considered as requiring treatment, should come as a genuine "eye-opening experience" for many physicians,[8] all the more since effective, risk-reducing treatments for such individuals are already available (eg, aspirin, statins).[8,9] Logically, this should lead to an increase in the number of individuals at high risk that will be identified and treated, and thus a corresponding increase in health care costs, but which should be offset by a potentially important gain in health.

We now turn our attention to four practical applications of the recent knowledge about inflammation, for which implementation appears to be "just around the corner": (i) use of CRP to identify individuals at increased risk of cardiovascular events or unstable atherosclerosis and thrombogenic plaques; (ii) identification of sources of vascular and other inflammation and trauma as potential risk determinants for cardiovascular events, in the wake of the increased awareness of inflammation; (iii) rationalization of treatment following identification of un-

derlying mechanisms suggested by increased CRP values; and (iv) the foreseeable trend toward increased use of treatments with a significant impact on inflammation.

Prognostic value of CRP

A relationship between high CRP levels and elevated risk of cardiovascular events both in the general population and in CVD patients has been repeatedly demonstrated.[10]

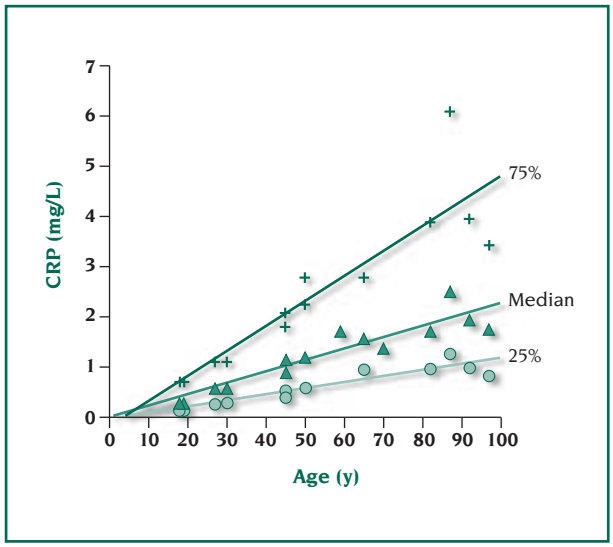

Figure 1.
Relationship between age and C-reactive protein (CRP) levels. Median CRP values, and thresholds for quartiles 1 and 3 obtained from control groups of population studies.[10,11] Values are positioned at the mean age of the groups. Lines are obtained by linear curve fitting.

Translation of these epidemiological findings into treatment options (eg, use of aspirin and statins[8,9]) requires, among others things, that CRP levels can be reliably determined in the individual subject, and that a consensus about cutoff levels is obtained.

That individual levels can be adequately measured has been clearly documented.[11] However, special care should be given to eliminate the possibility of temporary trauma- or infection-related increases in CRP.[11] Consensus on cutoff values has not been formally achieved yet. As there are differences in absolute risk as well as in CRP levels with age, this means that selection of age-independent cutoff values is a major decision. *Figure 1* illustrates the role of age as a major determinant of the increase in CRP values, as reported by various studies.[10,11]

In terms of primary prevention, there are two possible options for basing management guidelines on CRP values.[11] One is to define a single threshold value.[12] In practice, this implies that the finding of a low value, below the cutoff point, can be interpreted as reflecting absence of risk, but that a value above the cutoff point will require confirmation from a second measurement, approximately 2 weeks later.[12] The other option is to define three categories (low-, medium-, and high-risk), which, whatever the category, requires measurement of at least two blood samples taken 2 or more weeks apart. These two options are described in *Table I (page 80).*

A previous recommendation to enhance the specificity of CRP testing for cardiovascular disease was to combine indication for its measurement with that of cholesterol. It has to be realized that a subject may show a serum cholesterol level or a cholesterol/HDL ratio that does not constitute an indication for treatment, but a CRP value that does. Furthermore, as obesity and insulin resistance may be accompanied by elevated CRP, these situations may be further ground for a more frequent assessment of CRP.

CRP measurement is now widely practiced in the US in middle-aged individuals, and attempts to include it in the guidelines for risk assessment in primary prevention are close to being successful. Current evidence is mainly in support of primary prevention, identifying a new category of subjects with "normal" cholesterol, but elevated CRP, who stand to benefit from treatment with statins or aspirin,[13] while a high CRP value in individuals at intermediate risk should provide added impetus for recommending treatment and lifestyle improvement.

Increased CRP is also an independent marker of risk in stable CVD patients and in patients with acute coronary syndromes, but this has resulted in no change in the current therapeutic approach other than increased awareness of its justification. Specific treatments for the control of inflammation and intensified anticoagulation modalities are here a hot topic for future evaluation of therapeutic efficacy in this context. In terms of health economics, projections in Germany and Italy indicate that, in the age-groups 45 to 65 years, a CRP threshold of 3 mg/L would increase the number of individuals for treatment in primary prevention; however, the number of life-years thus saved would well be worth the added cost.[14]

It should be noted that no thorough evaluation has been done about the value of adding CRP data to the risk algorithms of Framingham or Procam, which are advocated by many European countries and have been recently refined.

A. General: select persons in a stable condition and confirm this by asking questions 1 and 2

- **Question 1 about possible outliers:** Did you have in the last 2 weeks or do you have now: an infection; common flu; fever; a vaccination or immunization; backache or headache; medical or dental treatment.
- **Question 2 about possible modifiers:** Did you, in the last 2 weeks, start or stop or significantly change: dietary habits (weight loss), smoking (only start); use of oral contraceptives or hormone replacement therapy; alcohol consumption; strenuous exercise; use of multiple vitamins; medication eg, statins, fibrates, triglitazones, aspirin, antibiotics, treatment for bronchial complaints.

B. Blood sampling scheme for two strategies

Strategy 1	Strategy 2
Criterion for classification: One threshold positioned above the median	Criterion for classification: Subdivision into tertiles
Take first sample and determine CRP • If below decision threshold accept • If above threshold, repeat sampling ≈2 weeks later • If above 10 mg/L discard as outlier	Take first sample
Take second sample and determine CRP • Accept mean if two values are <100% different (otherwise third sample) • Treat as first sample if CRP was >10 mg/L in first sample	Take second sample ≈2 weeks later Determine CRP in both samples • Accept mean if two values are <100% different; otherwise, third sample • Discard values as outliers when >10 mg/L, unless duplicates indicate it to be genuine

For details see reference 11. With a single threshold positioned above the median the analytical criterion of CVi/CVg <0.5 is met for the lower class with a single blood sample and can be met for the upper class by applying the average of two blood samples. For a tertile distribution for all classes, a mean of two samples is required to approach the analytical criterion.

Table I. Approach to the laboratory assessment of C-reactive protein (CRP) in primary prevention.

Inflammation as a determinant of risk

Increased risk has been shown to correlate with the winter season (when infections are more prevalent than during the summer). Acute myocardial infarction during vital exhaustion is well known. The relationship between inflammatory conditions (eg, respiratory infections, arthritis, etc) and risk of CVD requires closer investigation. Though trials with antibiotics are inconclusive, their use during infection may appear to be of benefit. Awareness of the importance of inflammation should now translate into intervention trials in individuals affected by various forms of inflammation in order to provide concrete evidence of the benefit of treatment.

Elevated CRP and therapeutic implications

Just as elevated cholesterol was recognized as a cause of CVD and triggered the development of lipid-lowering treatments, high levels of CRP will undoubtedly lead to the development of treatments to control inflammation. Specific inhibition of CRP and of complement is already under investigation.[15,16] Likewise, since inflammation also appears to enhance clotting mechanisms, specific anticoagulant measures are indicated.[5] Meanwhile, current cardiovascular treatments have been evaluated for their potential anti-inflammatory effects, and such effects have been clearly identified with statins, fibrates, angiotensin-converting enzyme (ACE) inhibitors, and triglitazones, involving various mechanisms.[5]

As stated above, it is preferable that specific aspects of inflammation be controlled without inhibiting all inflammatory processes indiscriminately. Alternatively, general anti-inflammatory treatments may be applied temporarily, only long enough to "cool down" the excessive inflammation and allow endogenous control to be restored. For instance, high-dose statin treatment may find a rationale here for temporary application.[17]

Another avenue to explore in patients with elevated CRP is the more aggressive use of treatments able to reduce the accumulation or persistence of inflammatory triggers (eg, cholesterol deposits, modified low-density lipoproteins [LDL], autoantibodies). The effects of such treatments on the inflammatory processes need, of course, to be monitored. However, whether CRP will prove a marker of choice in this respect as well is not at all certain (see Yeh and Willerson,[18] and, below, Perspectives section).[19]

Myocardial infarction: the anti-inflammatory option

It is to be expected that the anti-inflammatory properties of current treatments will become part of marketing strategy. At present the anti-

inflammatory properties of statins, fibrates, triglitazones, and ACE-inhibitors have been documented and their mechanisms are under investigation.[5] Aspirin appears to be effective in reducing the risk of myocardial infarction in individuals with elevated CRP.[9] The effect of lifestyle modifications (eg, alcohol use, physical activity, and weight reduction) on inflammation markers has also been evaluated.[20] Estrogens have been shown to exert a negative effect by increasing CRP.[21] Since no such increase is observed with transdermal 17β-estradiol,[22] this route of administration is arguably safer. High-dose atorvastatin elicits a much greater decrease in CRP than the conventional dose, independently of its effects on cholesterol, which supports its use in high-risk (high CRP) patients.[23]

Many of these developments are still in their infancy as they focus only on the effects on CRP. However, as pointed out below, CRP may not be an adequate surrogate end point, and documentation of anti-inflammatory effect requires demonstration of similarity of risk and intervention mechanism, or, alternatively, selection of more proximate disease risk markers such as direct markers stemming from the vascular process itself.[19]

PERSPECTIVES

Finding other inflammatory risk markers for identification of risk

The case for CRP as a risk marker of choice is quite well documented. However, CRP is by no means the only candidate. Other inflammation markers have been consistently identified as risk markers in prospective studies. These include markers originating in the liver (serum amyloid A [SAA], secretory phospholipase A_2 [sPLA2], fibrinogen), cytokines (tumor necrosis factor–α [TNF-α] interleukin-6 [IL-6], interleukin 18 [IL-18]), and vascular factors (serum intercellular adhesion molecule–1 [sICAM-1], von Willebrand factor, P-selectin). However, their value in the individual subject is not corroborated. For instance, in the ECAT trial, the groups with the highest quintiles of CRP and SAA overlap by only 24%. Similarly, in the non–insulin-dependent diabetes mellitus (NIDDM) population of the Diabetes Atorvastatin Lipid Intervention (DALI) study, the overlap of elevated CRP and SAA is only 21% (unpublished observation). In the DALI study, only 19% of individuals were in the top quartile of all factors, including CRP, SAA, sPLA2, fibrinogen, and IL-6 (unpublished observation).

There are three possible approaches to remedy this situation: (i) select individuals with several markers (for instance CRP and fibrinogen) in the highest group or above a threshold—this I refer to as "consistent" inflammation; (ii) include, in addition to individuals with elevated CRP, individuals with other elevated markers (for instance fibrinogen), but whose CRP values are below the threshold—which I refer to as "all" inflammation; or (iii) consider only those individuals with elevated CRP—ie, CRP as the only specific marker. *Figure 2* illustrates these three strategies. As suggested by the data above, the category of consistent inflammation is probably around 20% to 25% of that of a single marker and can be reasonably approached by the combination of two markers.

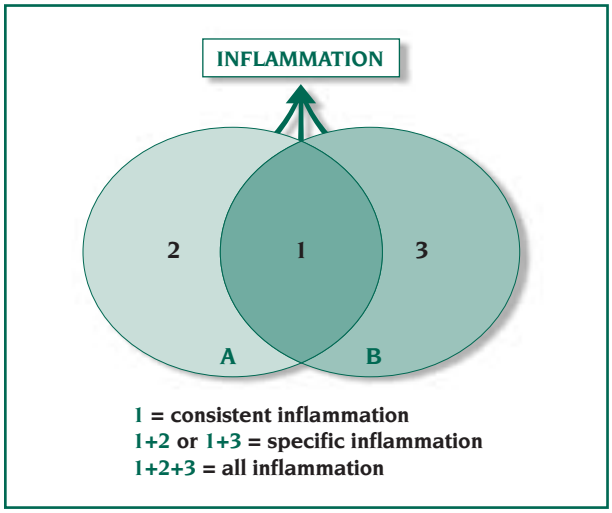

Figure 2.
Strategies for use of inflammation markers (for purposes of illustration, two markers A and B are considered). Collection of individuals (in circles) with values of inflammation marker A or B in the highest category or above a threshold. Area 2 is the group in which only marker A is elevated; area 3, same, for marker B; area 1 is the group in which both markers are elevated.

We have no clue today what the strategy of the future will be. Meanwhile, the simplicity of the CRP assay argues for continuing to base the assessment of inflammation on this parameter. However, this should not impede the search for other diagnostic strategies, which will likely also include specific vascular markers.

Surrogate end points

A surrogate end point fulfills other criteria than a risk marker. Clinical trials should document substantial quantitative changes in the surrogate end point following intervention, which should correlate with actual risk reduction. Furthermore, the mechanism of the change should be plausible. In this respect, CRP poses a problem inasmuch as it is

a hepatic protein whose synthesis is not only sensitive to the disease process, but also to other effects. Similar limitations apply to the other hepatic markers, fibrinogen, SAA, and sPLA2. Therapeutic intervention may possibly have a direct effect on the hepatic synthesis of these markers unrelated to the effect on inflammation. This has been recently documented in the case of fibrates, which have a direct influence on CRP synthesis.[23]

Furthermore, reports that fibrinogen and CRP do not always show concordant changes after therapeutic intervention should caution against using CRP as a surrogate end point without further evidence. For example, it has been shown that statins reduce CRP, but not fibrinogen, and that estrogens increase CRP, but not fibrinogen. It is thus advisable to include more proximate parameters as candidate surrogate end points, such as "long" pentraxin (PTX3), pregnancy-associated plasma protein–A (PAPP-A), oxidized low-density lipo-protein (ox-LDL), or s-ICAM-1 derived from the vessel wall.

Response theory

An interesting hypothesis regarding the relationship between elevated CRP and the risk of cardiovascular events states that chronic CRP levels are indicative of the acute phase response potential. Thus, Liuzzo et al[24] reported that IL-6 and CRP responses to percutaneous transluminal coronary angioplasty (PTCA) and angiography were stronger when levels of CRP were higher. This has been shown to be related to a more frequent/strong dislocation of nuclear factor kappa B (NF-κB) to the nucleus in circulating monocytes, indicative of a preactivation condition.[25] Extensive infarctions are well known to be associated with higher levels of CRP and acute-phase proteins.

It is tempting to speculate that there is not necessarily only a stronger response of CRP to more extensive trauma, but that higher CRP is (also) a causal factor with respect to the size of the infarction. CRP has been shown to a player in the acute process by activating complement and increasing infarct size in animals.[26,27] Furthermore, inhibition of complement reduces infarct size. sPLA2 is also involved in this process.[26]

High chronic CRP therefore appears to herald an increase in acute-phase response and may thus strongly correlate with the risk of events, by increasing severity. We thus clearly need to reconsider the roles of chronic inflammatory processes in determining the response pattern in acute situations.

Recent data on statin treatment during myocardial infarction indicate that this treatment is able to reduce infarct size.[27] This might be related to the possibility that statins dampen the inflammatory response. Further experimentation is necessary to confirm this action and explore other potentially useful interventions. At stake is a new approach to reducing the acute inflammatory response and the risk of deleterious consequences in many acute situations such as PTCA, coronary artery bypass grafting (CABG), and myocardial infarction. The recent Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering (MIRACL) trial showed that statin treatment was able to reduce recurrent ischemic events during the first 16 weeks after the onset of an acute coronary syndrome.[28]

CONCLUSIONS

The discovery of CRP's role as a risk predictor has opened up new perspectives for intervention, enabling more individuals to benefit from already available treatments. It has resulted in the definition of a new category of at-risk patients, that of middle-aged individuals with "normal" lipid levels, but elevated CRP. Use of CRP as a criterion in the selection of treatment is now close to formal practical implementation in primary prevention.

The fact that cardiovascular events are no longer considered the inevitable consequence of lipid disorders only, but also of inflammation, has led to a change in paradigm with far-reaching consequences. Inflammation has now become a target of intervention as well as a focus for the development of new treatments. For now, CRP remains the risk variable of choice, but we should avoid oversimplifications. Other markers may supplement CRP in future, and better understanding of how inflammatory processes are involved in cardiovascular disease is likely to result in further advances in risk analysis and treatments. Use of CRP as a surrogate end point must still await further confirmation.

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Which tests should the physician use today to assess arterial inflammation in patients?

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The hypothesis that atherosclerosis is largely an inflammatory disease is barely a decade old. As a result, even for basic research, relationships are still hazy between coronary artery inflammation and ischemic heart disease. Not only is inflammatory risk multifactorial, but the two major end points of ischemic heart disease—chronic atherosclerosis and acute coronary syndromes—differ in their pathogenesis: 70% of myocardial infarcts follow the sudden occlusion of an artery with no previous flow-limiting stenosis. Prognostic tests using systemic markers of inflammation, such as C-reactive protein, have yielded some striking correlations, epidemiologically and clinically, notably over the short term in unstable patients, but it is still premature and simplistic to expect them to reflect the general risk of two only partially related end points.

Keywords: inflammation; acute coronary syndrome; ischemic heart disease; coronary artery disease; myocardial infarction; atherosclerosis; CRP, inflammatory marker

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Dialogues Cardiovasc Med. 2003;8:84-89

The immediate thought that comes to mind is that the very question posed in the title of this chapter is premature: one would be at a loss, today, to cite definite practical applications. Indeed, the relationship between arterial inflammation, acute coronary syndromes (ACS), and routinely available tests is still fuzzy, because the interest in inflammation as a pathogenetic component of ischemic heart disease (IHD) has developed only recently. This development took place in three distinct phases. First, the hypothesis that atherosclerosis is largely an inflammatory disease, originally derived from animal studies, became established only recently.[1] Second, the observation that systemic markers of inflammation, such as C-reactive protein (CRP), are often elevated in patients with ACS, independently of myocardial ischemia and necrosis, and are correlated with short-term prognosis, was first reported only in 1994.[2] Finally, the observation that values of CRP within the normal range in healthy men and women are correlated with long term incidence of infarction (at 2, 4, 6 years), was first reported in 1997[3] and 2000,[4] respectively. The growing interest in the inflammatory component was largely stimulated by this latter observation, and by reports of an association between elevated CRP levels and various end points in large groups of patients with known IHD. However, it is still unknown whether these three aspects of inflammation reflect the same common underlying pathogenetic mechanisms.

THE QUEST FOR SIMPLE ANSWERS

The acquisition of new knowledge does not necessarily makes things more comprehensible and often adds novel complexities. Yet, when confronted with a pressing issue, such as predicting future adverse events, there is a natural inclination to seek and to accept generalizations.

There may be multiple potential chronic and acute components of inflammatory risk in a complex syndrome such as IHD; therefore, risk should be considered from the perspective of two major end points that appear to be only partially related: (i) the chronic development of atherosclerosis, which can proceed over periods of years; and (ii) the occasional triggers of acute coronary syndromes *(Figure 1)*. Prognostic tests may not reflect the risk of these two end points equally. Efforts to

SELECTED ABBREVIATIONS AND ACRONYMS

ACS	acute coronary syndromes
CRP	C-reactive protein
IHD	ischemic heart disease
MI	myocardial infarction

diagnose arterial inflammation in clinical practice imply a preliminary knowledge of its chronic or acute nature, of its potential relationship with plaque composition and geometry, and with systemic markers of inflammation, as well the availability of effective treatment strategies.

THE MULTIPLE COMPONENTS OF INFLAMMATORY RISK

Arterial inflammation may be a chronic component of the atherosclerotic process or a superimposed acute component, which could trigger some, but not necessarily all, ACS. To clarify this point, it is useful to examine the relationship between coronary atherosclerosis, ACS, and arterial inflammation, as well as between systemic markers of inflammation, atherosclerosis, ACS, and short-term and long-term risk prediction.

Atherosclerosis, ACS, and arterial inflammation

Chronic atherosclerosis and ACS

Atherosclerosis is the result of the local chronic inflammatory and immune response of the arterial wall to a variety of physical, chemical, infectious, immunological, and toxic stimuli, acting alone and in combination over periods of years or in bouts of weeks and months. The prevalence of atherogenic stimuli can vary according to environmental and genetic conditions, and they are not necessarily the same in all populations and all individuals; they can wax, wane, heal, and recur repeatedly during the lifetime of any given person. Finally, the local vascular response to atherogenic stimuli is determined by individual predisposing and protective genetic and environmental factors, as well as by the interactions of the vessel wall with blood components. Thus, some atherosclerotic plaques may be chronically inflamed during their development or during phases of activation, others may be totally healed.

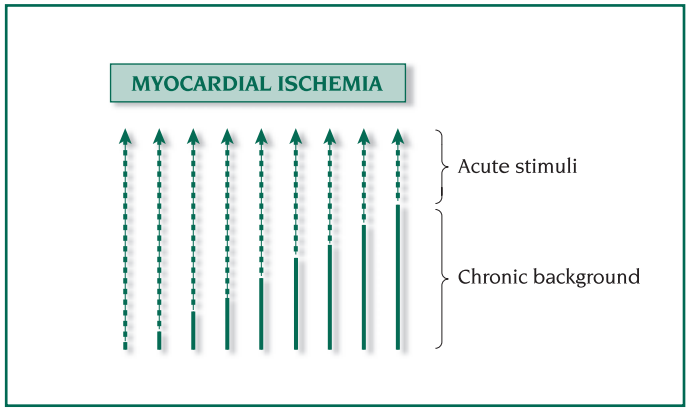

Figure 1. *Coronary atherosclerosis and ischemic events. Variable severity of coronary atherosclerosis (indicated by solid, continuous vertical lines) may result from various types of atherogenic stimuli and the local vascular and blood response. Occasionally, acute ischemic stimuli (indicated by dashed vertical lines) may suddenly reduce regional coronary blood flow, transiently or persistently, through coronary constriction, thrombosis, or both. Weaker stimuli may be sufficient to cause ischemia in the presence of severe atherosclerosis. Flow-limiting stenoses can reduce coronary flow reserve and cause ischemia when the increase in myocardial demand is excessive, but may remain stable for years. Conversely, those coronary arteries, which, in about 70% of cases, cause myocardial infarction on becoming suddenly occluded, only exhibit a mild, moderate flow-limiting stenosis.*

During the lifetime of an individual ACS develop very seldom or never, and when they do develop, it is against a background of chronic atherosclerosis of multiple origins and very variable severity. Most frequently, they occur suddenly and dramatically, and patients present with unstable angina, unheralded myocardial infarction (MI), or sudden ischemic death, as an inaugural clinical manifestation of IHD. Such initial, unheralded manifestations of ACS are explained by the observation that, in about 70% of cases, MI results from the sudden occlusion of a coronary artery in which there was no previous flow-limiting stenosis.[5] Conversely, patients presenting with uncomplicated, long-standing chronic stable angina usually have more extensive and severe coronary atherosclerosis than patients presenting with acute MI or with unstable angina as their very first clinical manifestation of IHD.[6,7] Finally, postmortem studies demonstrate that, past the age of 50, coronary atherosclerosis is extensive in most individuals who died of noncardiac causes in the absence of evidence of IHD.[8]

Thus, severity and extension of atherosclerosis cannot explain by themselves the occasional, sudden development of ACS.

Distinctive coronary and clinical features of ACS

Acute coronary alterations in ACS are typically characterized by thrombosis *(Figure 2, page 86).* At postmortem, most thrombi are found to be composed of platelets or of platelets and fibrin. This indicates that these thrombi were formed very gradually in flowing blood in response to weak thrombogenic stimuli, since platelets, of which there are only 400 000 per μm^3 of blood, take time to accumulate. Often, thrombi are composed of multiple layers of different ages, suggesting the recurrence of repeated bursts

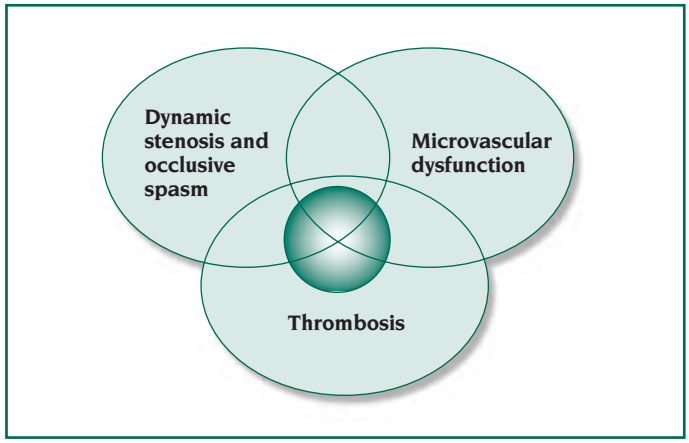

Figure 2. *Mechanism of acute coronary occlusion. Sudden interruption of coronary blood flow causing acute myocardial infarction (MI) may result from a variable combination of thrombosis, dynamic stenoses, occlusive spasm, and microvascular dysfunction, most often occurring in the absence of flow-limiting stenoses. Of these, thrombosis is the most easily visible component and most effective therapeutic target. The triggers of thrombosis may be multiple and the final outcome is strongly modulated by the local coronary constrictor response and the systemic hemostatic equilibrium. A specific, rational prevention of MI can only be based on precise knowledge of the various potential triggers of coronary thrombosis and vasoconstriction, which manifest only very occasionally, in relation with the severity of atherosclerosis and individual systemic thrombotic response.*

of weak thrombogenic stimuli. Less frequently, red thrombi, rich in fibrin and red cells, are found at the site of a disrupted plaque with a large central pultaceous core, suggestive of very strong thrombogenic stimuli with very rapid thrombus formation. Beneath fresh coronary thrombi, about 70% of plaques are ruptured or fissured. This means that about 30% have no fissure, thus endothelial erosions are postulated. Finally, some thrombosed plaques have no central pultaceous core.[9] Conversely, fissured plaques are found in 10% to 20% of patients with atherosclerosis dying of noncardiac causes.[10]*

The spectrum of clinical presentation of ACS is quite variable. At one end of the spectrum are patients who present with totally unheralded MI occurring like a bolt from the blue, without a single warning episode of angina, and not followed by recurrent instability. At the other end of the spectrum are patients who develop MI after a period of unstable angina of several days or weeks, and who often develop postinfarction angina and/or reinfarction. In these two extreme groups of patients the trigger of instability may not be the same.

Thus waxing, waning, and persistence of an acute inflammatory process would be compatible with the clinical fluctuations of instability, whereas the purely mechanical rupture of a thrombogenic plaque, or isolated strong, combined thrombogenic or spasmogenic stimuli, would be more plausible triggers for MI, occurring suddenly and unheralded, and not followed by recurrent instability.

Chronic and acute arterial inflammation

Inflammatory cells infiltrates together with activated smooth muscle cells expressing HLA-DR antigens are commonly observed, beneath fresh coronary thrombi, both in plaques with and without fissures and in plaques without a central lipid core.[9] Local and temporal variations of such inflammatory activity offer a handy explanation for the waxing, waning, and recurrence of thrombogenic stimuli, particularly in patients with recurrent or persistent clinical instability. Inflammatory activation of metalloproteases can weaken the fibrous cap of lipid-rich plaques, favoring their rupture. Under these circumstances, thrombus growth would be related to the size and thrombogenicity of the fissured plaque, as well as to the number and activation of exposed inflammatory cells. Alternatively, inflammation of the endothelium can modify its physiological vasodilator and antithrombotic properties (production of endothelium-derived relaxing factors [EDRFs], prostacyclin, t-PA [tissue plasminogen activator], and heparan sulfates), resulting in a pathologic vasoconstrictor and prothrombotic blood–vessel wall interface (no production of vasodilator and antithrombotic mediators, production of endothelin, plasminogen-activator inhibitor–1 (PAI-1), tissue factor, and leukocyte and platelet adhesion molecules). These changes are powerful local inducers of platelet and leukocyte adhesion, capable of inducing the initiation and growth of a platelet-rich thrombus, even in the absence of endothelial denudation (which, in experimental animals, is rapidly repaired without thrombus growth).

However, inflammatory cells infiltrates are also commonly found in patients with stable coronary disease. This latter finding led to the notion of "unstable plaques in stable patients."[11] Indeed, at postmortem, only quantitative, but no qualitative, differences in inflammatory markers were generally found between coronary plaques of patients with

*For a comprehensive review, see: Maseri A. Ischemic heart disease. In: *The Variable Chronic Atherosclerotic Background.* New York, NY: Churchill Livingstone; 1995. 1995(chap 8):193-235.

ACS and those of stable patients.[12] These findings are not surprising considering that atherosclerosis is largely a chronic inflammatory process. Thus, arterial inflammation, although very plausible at first glance, is such a common component of chronic atherosclerosis that, by itself, cannot explain the very occasional development of ACS. Also in vivo, evidence of coronary plaque inflammation was obtained by a thermistor catheter in 20% of stable patients compared with 40% and 67% of patients with unstable angina or with acute infarction.[13] The presence of inflammation in atherosclerotic plaques detected on the basis of temperature heterogenicity was also observed in about one third of carotid endarterectomy specimens.[14] Thus, although in vivo inflammation, detected by plaque temperature differences, is more frequently found in ACS, it is too common in stable patients to be by itself a plausible explanation for instability.

A superimposed, *acute* instability, possibly involving simultaneously multiple plaques and coronary branches, appears to be a more likely mechanism. In patients with unstable angina and elevated CRP levels, the hypothesis of extensive acute coronary inflammation is supported by a selective transcoronary decrease in neutrophil myeloperoxidase content, indicative of a significant leukocyte activation and degranulation across the coronary vascular bed. Such inflammatory activation appears to be confined to the coronary bed of unstable patients, as no neutrophil activation is detectable in the femoral vascular bed. It does not appear to be related to coronary atherosclerosis or recurrent ischemia, since it is not observed in patients with chronic stable angina and multivessel coronary disease or in patients with active variant angina.[15] The hypothesis of extensive coronary inflammation is supported by recent growing evidence of multiple complicated plaques[16,17] and multiple fissured plaques[18] involving different coronary branches in ACS. The number of "active" coronary plaque appears to be correlated with systemic levels of CRP.[17,19] Multiple fissured and thrombosed coronary plaques were also found at postmortem in earlier studies, but not commented upon.[20,21]

Thus, acute coronary arterial inflammation may often be related to a transiently "inflamed" patient rather than to a single inflamed plaque. Such acute, extensive coronary inflammation appears to be correlated with elevated systemic CRP levels.

Systemic inflammation and short-term and long-term risk prediction

Elevated systemic inflammatory markers, typically represented by CRP, are found in about 70% of patients with Brauwnwald Class IIIB unstable angina, even in the absence of elevated troponin levels, and are associated with an increased risk of infarction in the short term. The short-term predictive value of elevated CRP values in unstable angina is supported by the observation that they are also elevated in nearly 100% of patients with acute MI preceded by unstable angina, but in less than 50% of patients with totally unheralded MI (not preceded by unstable angina), and in only about 15% of those with chronic stable angina and documented severe coronary atherosclerosis or with active variant angina.[2,22,23] In unselected patients submitted to coronary arteriography, only a very weak correlation was found between the severity of coronary atherosclerosis and CRP levels.[24] During the first year, following hospital discharge after ACS, the average risk of MI or recurrent instability decreases exponentially. Persistent CRP elevation appears to identify those patients who are likely to develop recurrent instability or infarction; conversely, CRP values within the normal range predict event-free survival.[22,25]

The correlation between elevated CRP levels and short-term prognosis may be limited to unstable patients. The mechanisms through which elevated CRP levels in unselected patients with IHD have a long-term adverse prognostic value for total and cardiovascular mortality may not necessarily be related to arterial inflammation, since mortality is an end point resulting from a number of adverse factors potentially influenced directly by high CRP values themselves or indirectly by certain primary determinants of CRP elevation.

The mechanisms through which, in normal individuals, CRP values within the normal range have a long-term predictive value remain unclear. As the predictive levels are within the normal range, they are more likely to be markers of enhanced CRP responsiveness to inflammatory stimuli[26] rather than indicative of ongoing inflammation. This would be consistent with the enhanced CRP response observed in unstable patients following coronary angioplasty[27] and MI,[23] which is possibly related to monocyte hyperreactivity.[28] The hypothesis that the mechanisms responsible for the short-term predictive value of elevated CRP levels in unstable patients may not be the same as those responsible for the long-term predictive value of CRP levels within the normal range in normal subjects is supported by the different effect of aspirin in these two groups. The predictive value of CRP in normal individuals randomized to prophylactic

aspirin is lost,[3] as if MIs predicted by CRP in normals were prevented by aspirin. This finding is at variance with the short-term predictive value of elevated CRP levels in unstable patients in spite of aspirin treatment.

Thus, the enhanced systemic inflammatory responsiveness observed in unstable angina and in most cases of MI preceded by unstable angina, but which is observed in less than 50% of patients with MI not preceded by unstable angina, could be a marker of extensive acute coronary inflammation. However, acute coronary inflammation does not necessarily play a role in the long-term prognostic value of CRP levels within the normal range in normal individuals.

IMPLICATIONS FOR CLINICAL PRACTICE

The development of techniques capable of identifying vulnerable coronary plaques in patients is attracting growing attention.[13,29-31] However, success in achieving this goal requires more precise information on the actual causes of anatomic and functional plaque vulnerability and on chronic and acute inflammation, in order to select not only the most appropriate diagnostic technique for their detection, but also the most appropriate timing.

As discussed above, coronary plaques may be "vulnerable" or likely to suddenly cause ACS through a variety of mechanisms that may have different prevalences, for example in unstable angina and in MI not preceded by unstable angina.

At one end of the ACS spectrum, in unstable angina, progression toward infarction may be related to the intensity of acute local inflammatory processes, with or without plaque rupture of. Such acute inflammation may be extensive in coronary arteries and correlated with systemic inflammation. At the other end of the spectrum, a plaque with a central large thrombogenic lipid core and a thin fibrous cap may rupture, even in the absence of a local acute inflammatory process, as a result of mechanical stress and strain or of local spasm.[32] Such lipid-rich plaques may be multiple even in stable patients and do not appear to be more common in ACS, as documented by postmortem studies.[33,34] Finally, the effects of these thrombogenic stimuli in determining the coronary occlusion that causes MI is significantly modulated by the coronary vasomotor response and by the thrombogenicity of the blood.

Thus, the assessment of systemic inflammatory markers is beginning to find some application in clinical practice, but, for the time being, the direct assessment of arterial inflammation remains confined to the realm of clinical research.

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Are markers of inflammation elevated in patients at risk for atherosclerosis because of vascular or extravascular inflammation?

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Several prospective blood-based epidemiological studies have investigated various "inflammatory" factors as potential determinants of coronary heart disease in the general population. However, their value has been limited due to inadequate sample size, selective publication, and, most importantly, potential "confounding" by causative risk factors. These concerns can be addressed by larger studies with serial measurements and by large-scale case-control studies integrating information on these "inflammatory" markers with measurements of their genetic determinants. This should shed light on the existence of independent associations between particular "inflammatory" factors and coronary heart disease and whether the stimuli for any perturbations are endovascular in origin, extravascular, both, or neither.

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Circulating concentrations of several plasma components can fluctuate substantially during acute responses to tissue damage or infection.[1] Plasma concentrations of C-reactive protein and serum amyloid A protein can each rise 10 000-fold; plasma concentrations of fibrinogen, von Willebrand factor, ferritin, and the leukocyte count can each increase several-fold; and the concentration of serum albumin can fall by about 20%.[1] As discussed elsewhere in this issue, these plasma alterations appear to reflect, at least in part, the impact of molecular cascades mediated by proinflammatory cytokines on the liver and on other tissues.[2]

In recent years such "acute-phase reactants" have been studied as potential markers of more subtle and persistent systemic alterations, which may be loosely called low-grade inflammation. If the sharp short-term fluctuations are ignored, then long-term circulating concentrations of these factors show a similar year-to-year consistency within individuals to levels of some extensively studied risk factors, such as blood cholesterol concentration and blood pressure *(Table I).*[3-5] Advances in laboratory assays have allowed detection of subtle variations in several "inflammatory" factors (such as C-reactive protein and serum amyloid A protein) that would not previously have been no-

Table I. *Self-correlation coefficients for circulating values of selected inflammatory molecules and certain established vascular risk factors.*

	*Approximate self-correlation coefficient**
"Inflammatory" factors	
Albumin	0.6
C-reactive protein	0.6
Fibrinogen	0.7
Leukocyte count	0.6
von Willebrand factor	0.6
Classic risk factors	
Blood pressure	0.7
Total blood cholesterol	0.6

* Correlation coefficient between two measurements of the same factor taken about 5 years apart in the same individual.[3-5]

ticed in general populations. Moreover, as several of these factors are stable in long-frozen blood, they can be measured fairly reliably in banked serum samples. These favorable biological characteristics have encouraged many blood-based epidemiological investigations of inflammatory factors and coronary heart disease, providing an approach that complements pathological studies of the vessel wall *(Table II)*.[3,5-13]

The purpose of this article is to: (i) highlight the strengths and limitations of blood-based observational epidemiology in investigating inflammatory hypotheses of coronary heart disease; (ii) review the available prospective observational epidemiological evidence on several inflammatory factors and coronary heart disease in general populations; and (iii) discuss the potential vascular and extravascular sources of inflammation that could account for the observed plasma alterations in these markers of inflammation in studies of coronary heart disease

Type of factor/examples	*No. of incident CHD cases*	*Risk ratio**
Acute-phase reactants		
Leukocyte count[3]	6000	1.4 (1.3-1.5)
Albumin[3]	3700	1.5 (1.3-1.7)†
C-reative protein[7]	2000	2.0 (1.6-2.5)
Ferritin[8]	600	1.0 (0.8-1.3)
Serum amyloid A protein[7]	600	1.6 (1.1-2.2)
Hemostatic factors		
Fibrinogen[3]	3000	1.8 (1.6-2.0)
von Willebrand factor[5]	1500	1.5 (1.1-2.0)
Cell adhesion molecules		
ICAM-1[9]	1400	1.2 (1.0-1.6)
VCAM-1[9]	1300	1.0 (0.8-1.3)
P-selectin[9]	800	1.1 (0.7-1.4)
E-selectin[9]	800	1.2 (0.9-1.6)
Chronic infections		
Mixed strains of *H pylori*[10]	3500	1.2 (0.9-1.5)
C pneumoniae IgG titers[11]	3000	1.2 (1.0-1.4)
C pneumoniae IgA titers[12]	2300	1.2 (1.0-1.5)
Cytomegalovirus[13]	700	0.9 (0.7-1.2)
Cytotoxic strains of *H pylori*[13]	600	1.3 (0.9-1.9)

*Top third vs bottom third, seropositive vs negative.
†Bottom third vs top third.

Table II. *Literature-based meta-analyses of selected factors studied in long-term prospective studies of general populations.*

Abbreviations: *ICAM-1, intercellular adhesion molecule–1; IgG, immunoglobulin G; VCAM-1, vascular cell adhesion molecule–1.*

STRENGTHS AND LIMITATIONS OF BLOOD-BASED OBSERVATIONAL EPIDEMIOLOGY

Much of the available epidemiological evidence on circulating inflammatory markers and coronary heart disease derives from long-term prospective cohort studies of middle-aged individuals in approximately general populations. In such studies, individuals who develop coronary heart disease after entry ("cases") are typically compared with an appropriate subset of those who remain disease-free ("controls"). The use of such "nested" case-control studies in prospective cohorts with stored blood samples provides an efficient and rapid way to test many hypotheses and also retains the ability of prospective studies to reduce biases related to the selection of controls and should limit the influence of disease on the factors being investigated.

So far, however, individual prospective studies of inflammatory factors have typically involved only a few hundred cases of coronary heart disease and only a few hundred controls. Due to their inherent statistical uncertainties, studies of such scale are prone to false-negative and false-positive results. The impact of random error can, moreover, be compounded by unduly data-dependent analyses and selective reporting, such as when: (i) analytical cutoff values are chosen only after an exploration of the data has shown which seemed to be most strongly related to coronary heart disease; (ii) prominence is given to extreme findings in selected subgroups based on sparse data; (iii) results are preferentially reported just for those few factors (out of the many measured) that show extreme associations; and (iv) journals preferentially publish striking findings.[14] Hence, appropriate review of the available reports of particular "inflammatory" factors in coronary heart disease in

a literature-based meta-analysis should provide a better preliminary indication of their potential relevance than can individual studies involving just a few hundred cases and just a few hundred controls *(Table II).* For example, such meta-analyses of published data on coronary heart disease have helped refute inappropriate claims of strongly positive associations, such as for soluble adhesion molecules[9] and serum ferritin,[8] and have helped renew interest following inappropriate claims of null associations in relatively small, prominently published studies of serum albumin.[3]

However, although such literature-based meta-analyses can help limit certain biases related to random error and selective publication, they are less effective at reducing the impact of other potential biases. For example, most prospective studies have related risk of coronary heart disease to measurements of "inflammatory" (and other) factors taken at just a baseline survey. Lack of serial assessments can lead to substantial underestimation of any association with coronary heart disease due to within-individual fluctuations of inflammatory factors over time *(Table 1).*[4] For example, it is now recognized that the effects of some known vascular risk factors on coronary heart disease, such as blood pressure and blood cholesterol, have been underestimated by about 50% in long-term prospective studies due to failure to make allowances for this "regression dilution."[4] Conversely, residual biases related to "confounding" by causative risk factors may have resulted in exaggerated (or even spurious) estimates of the independent relevance of suspected risk factors to coronary heart disease.[15] For example, most published studies of inflammatory factors have reported statistical adjustment for only baseline values of some possible confounding factors (such as smoking, blood pressure, and blood lipids). Residual biases are, therefore, likely, both because baseline values of some confounders may provide inaccurate measurements of their long-term "usual" values (thereby resulting in incomplete statistical adjustments *[Table I]*), and because some possible confounders may not have been measured at all, such as the extent of preexisting atherosclerosis. As discussed in the next section, such potential biases limit the interpretation of the available prospective observational studies of "inflammatory" factors and coronary heart disease.

EVIDENCE FROM PROSPECTIVE OBSERVATIONAL STUDIES IN GENERAL POPULATIONS

In recent years, several dozen long-term prospective studies of coronary heart disease in approximately general populations have reported on associations with a number of circulating "inflammatory" molecules. Studies of some of these factors have been reviewed in literature-based meta-analyses, and these quantitative reviews have generally been updated to the year 2000. To provide standard comparisons, *Table II* compares the risk of coronary heart disease in those with plasma concentrations for the relevant "inflammatory" factor in the top third of the distribution of the population compared with those in the bottom third (or, for serum albumin, bottom third versus top third). *Table II* suggests that the risk ratios for coronary heart disease of most of these factors appear to be less than twofold, and are less extreme than the corresponding risk ratios for previously established risk factors such as smoking, blood pressure, and low-density lipoprotein cholesterol.[16-18] Because these risk ratios are not very extreme, many of them might be largely or wholly accounted for by confounding, suggesting that more detailed investigation of the effects of adjusting for potential confounding factors is essential.

For example, the British Regional Heart Study involved more cases of coronary heart disease than has any prospective study thus far of C-reactive protein and serum amyloid A protein.[7] It reported that baseline values of four plasma markers of low-grade inflammation (C-reactive protein, amyloid A protein, serum albumin, leukocyte count) were associated with one another as well as with future coronary heart disease risk in about 500 cases and about 1000 controls. Although these findings suggest that some low-grade inflammatory processes may be relevant to coronary heart disease, it is still uncertain whether these markers are causes or consequences of arterial damage (or of coronary heart disease itself) or just markers of established risk factors. In this study, the odds ratio for coronary heart disease in people with raised C-reactive protein values was reduced from 3.5 (χ^2_1=70) to 2.1 (χ^2_1=25) after adjustment for baseline values of some established risk factors. This substantial reduction in the apparent strength of the association (as indicated by the two-thirds reduction in the relevant χ^2 statistic) suggests that more exact adjustment based on serial measurements of these (and other) potential confounding factors might produce a greater reduction. Such uncertainties about the existence of any independent overall association between C-reactive protein and coronary heart disease make it particularly difficult to interpret various claims derived from subgroup analyses in relatively small studies

(such as the impact of C-reactive protein on disease risk in the presence of some known or suspected risk factors[19,20]).

Epidemiological uncertainties also apply to the interpretation of evidence on leukocyte count and on low serum albumin, for each of which it has been suggested that associations reported with coronary heart disease reflect, at least partly, confounding by classic risk factors (particularly cigarette smoking in the case of leukocyte count) and/or residual differences in socioeconomic status.[3] Associations between coronary heart disease and serum amyloid A protein have been less extensively investigated than several other acute-phase reactants. Indeed, the first report on serum amyloid A protein from a long-term prospective study was published in only 1997, and it may be that some of the studies published since would not have been if they had observed less striking results.[7] As regards coronary heart disease and plasma fibrinogen (which, like von Willebrand factor, has important hemostatic activities), the possibility of residual confounding in previous observational studies has been suggested by a meta-analysis showing no material association between the risk of coronary heart disease and genotypes associated with modest, but definite, increases in plasma fibrinogen.[21] In this comparison of 8350 cases and 17 248 controls, the combined risk ratio for coronary heart disease was just 1.00 (95% confidence interval [CI] 0.95-1.05) per higher-fibrinogen allele, whereas on the basis of the prospective observational studies of plasma fibrinogen it might have been expected to be about 1.20.[21] Since such genetic comparisons effectively "randomize" individuals at conception, in a Mendelian fashion, to higher or lower long-term plasma fibrinogen concentrations (which should eliminate any confounding), this finding is consistent with a lack of an important causal association between plasma fibrinogen concentration and coronary heart disease.[22]

POTENTIAL EXPLANATIONS FOR REPORTED ASSOCIATIONS

Despite the uncertainties about the independence of epidemiological associations reported between various "inflammatory" markers and coronary heart disease, a number of suggestions have been made about potential biological mechanisms to account for any causal associations that might be established. Apart from the case of plasma fibrinogen (which is the main hemostatic protein in plasma, as well as an acute-phase reactant), there is, as yet, no strong evidence for a role of any "inflammatory" factor as a direct mediator of vascular damage,[23,24] but additional experimental research is clearly needed. As moderately strong cross-sectional associations of several "inflammatory" factors with one another in apparently healthy people of circulating values have been reported,[7] it has been suggested that there is some underlying process related to "inflammation"—rather than any particular factor per se—that is fundamentally relevant to coronary heart disease. If so, what factors might be responsible for such inflammation?

A number of suggestions have been made, ranging from factors within the arterial wall, such as oxidized low-density lipoprotein and local infection by agents such as *Chlamydia pneumoniae* or cytomegalovirus, to specific factors outside the circulation such as chronic gastric infection with *Helicobacter pylori* or infective agents associated with periodontal disease, to systemic processes such as subclinical kidney dysfunction and "metabolic syndromes."[13,25] In general, the evidence for these hypotheses is, as yet, both relatively sparse and weak, as there is uncertainty about whether any of these suspected factors are themselves truly associated with risk of coronary heart disease and, further, about whether any of these factors are also associated with circulating levels of various relevant "inflammatory" factors.

For example, although endovascular markers of *C pneumoniae* infection (such as DNA, antigens, or elementary bodies) are reported about ten times more commonly in atherosclerotic lesions than in control vascular tissue, it remains uncertain whether local chlamydial infection is a cause or consequence of atheroma.[13] The latter possibility is suggested by observations in meta-analyses of long-term prospective seroepidemiological studies (which should be less liable to reverse association than are retrospective pathology-based studies of endovascular markers), which show no clear excess of high concentrations of *C pneumoniae*–specific IgG[11] or IgA[12] antibodies in people who subsequently develop coronary heart disease. Seroepidemiological investigations of other specific persistent infective agents (such as *H pylori* and cytomegalovirus) and coronary heart disease have been similarly inconclusive, and the larger such studies have generally failed to confirm the existence of associations between markers of chronic infection and circulating "inflammatory" factors.[10,11]

Some female sex hormones are produced in adipose tissue, a site for the production of certain proinflammatory cytokines, such as interleukin 6, that stimulate the liver to make several plasma acute-phase

reactants. The idea that estrogens might promote low-grade inflammation has been suggested by a randomized trial that reported sustained increases in plasma concentrations of C-reactive protein in women treated with hormone therapy regimens.[26] However, high levels of estrogens alone would not satisfactorily explain a role for low-grade inflammation in men with coronary heart disease. A partial genetic basis for low-grade inflammation has been suggested by a study of several hundred apparently healthy twins in whom monozygotic pairs had closer correlations of circulating concentrations of C-reactive protein and of amyloid A protein than did dizygotic pairs.[27]

EVIDENCE FROM RANDOMIZED INTERVENTION STUDIES

Despite incomplete knowledge about the factors responsible for persistent plasma alterations of "inflammatory" factors (and the uncertain nature of epidemiological associations reported between these factors and coronary heart disease), several interventions have been proposed to prevent coronary heart disease by their presumed "anti-inflammatory" actions. One report has suggested that the vascular protective effects of aspirin increase with increasing baseline plasma concentrations of C-reactive protein,[28] but that claim is not statistically convincing and is not supported by large syntheses of randomized trials that have reliably excluded much greater vascular benefits in patients taking higher ("anti-inflammatory") dosages of aspirin than in those taking lower dosages.[29] Small trials of macrolides (antibiotics with anti-chlamydial and, possibly, anti-inflammatory effects) have suggested reductions in certain plasma markers of inflammation, and these suggestions are currently being tested in larger trials that, additionally, aim to assess any effects on coronary heart disease.[30] Long-term use of statins (β-hydroxy-β-methylglutaryl coenzyme A [HMG-CoA] reductase inhibitors) appear to produce rapid and sustained reductions in plasma concentrations of C-reactive protein that are apparently unrelated to the degree of cholesterol lowering achieved.[31] Preliminary reports that cardioprotection with pravastatin or lovastatin may increase with increasing evidence of baseline inflammation, however, require testing in larger samples,[19,32] as does a report, based on a subgroup analysis involving only 36 cases and a nonsignificant interaction test, which has claimed that lovastatin is not cardioprotective in individuals with lower than average plasma concentrations of low-density lipoprotein cholesterol and of C-reactive protein.[32]

CONCLUSIONS

In retrospect, insufficient attention has generally been given to methodological issues that limit the interpretation of evidence from published observational studies of various "inflammatory" factors and coronary heart disease, and this may have created a sense of premature certainty about the existence of real epidemiological associations. Larger and more rigorous studies are needed to confirm or refute the existence of any such associations, and, ideally, these studies should involve serial measurements of "inflammatory" markers (and of potential confounding factors) to reduce residual biases. Moreover, as has recently been the case for plasma fibrinogen, larger-scale observational studies are needed that can include complementary information on the genetic determinants of various "inflammatory" markers, thereby providing unconfounded tests of causality based on "Mendelian randomization."[21,22] As yet, data about the possible determinants of low-grade systemic inflammation (whether vascular or extravascular) are preliminary and inconclusive, but some novel hypotheses, such as those related to subclinical renal disease, have not yet been thoroughly examined. Similarly, a few randomized intervention studies, based on fairly small numbers of cases and on subgroup analyses of marginal statistical significance, have generated intriguing hypotheses that require testing in larger samples. As "inflammatory" hypotheses of coronary heart disease are scientifically attractive and potentially important, the results of such studies will be awaited with great interest.

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Surfing the Heart

The Scientist – EuroSCORE – Heart Art

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The Scientist

http://www.the-scientist.com

The Scientist is an international news magazine published in print and on the Web. Its aim is to provide "a unifying forum for discussion of the following topics—news, research, profession, and technology—that drive scientific progress."
The web magazine is interactive, ie, visitors can write and express opinions, and the contents are most of the times provocative and stimulating. Useful resources are available too. Advertising provides the economic support so that registration is free for on-line articles.

EuroSCORE

http://www.euroscore.org

EuroSCORE stands for European System for Cardiac Operative Risk Evaluation. The site provides an on-line version of a validated algorithm for calculating predicted operative mortality in patients undergoing cardiac surgery. In the site it is explained how the algorithm was implemented: nearly 20 000 consecutive patients from 128 hospitals in eight European countries were studied and information was collected on 97 risk factors in all of the patients. A scoring system was prepared from the database, then tested and validated: this scoring system is EuroSCORE. References relative to the scientific bases of the scoring system are provided in detail and related full-text papers are accessible.
The visitors can play around with the EuroSCORE calculator and even discover their own surgical risk!

Heart Art

http://www.heartart.nl/

This is a highly original web site approaching the heart from a quite different perspective, and which is guaranteed to provide a welcome break for the cardiologist!
It defines itself as a site with "the heart as a theme, offering modern classics for offices or reception rooms, supplements for your private art collection, and special gifts for special occasions."
This site presents a wide range of contemporary artists, who, each in their own way, have been inspired by the heart. HeartArt features a Prints section, two Galleries (with paintings, etc), Gift Ideas (ranging from books, candles, and postcards to ties and jewelry, all in connection with the heart), as well as an Exhibition Agenda.
And if you think this has nothing to do with cardiology, well, think again! the Exhibition Agenda features, among others, events organized by the European Society of Cardiology, who, incidentally, is one of the web site's major sponsors!

All sites accessed June 1st, 2003

Dialogues Cardiovasc Med. 2003;8:97

Icons of Cardiology

Henry Pickering Bowditch and the founding of American physiology

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Although physiological research has been carried out since the work of Galen in the 2nd century AD, the field languished until the 17th and 18th centuries when a new era of discovery was stimulated by investigators like William Harvey and Stephen Hales in England, Luigi Galvani and Alessandro Volta in Italy, and Antoine de Lavoisier in France. Modern experimental physiology emerged in the second quarter of the 19th century when laboratories were established by François Magendie and Claude Bernard in France, and Johannes Müller in Germany.[1] These and other physiology laboratories developed within the continental university system and so became integrated with European medical education. In America during the latter half of the 19th century, however, medicine was usually taught by clinical practitioners who devoted no more than a few hours each week to their students. Because few medical schools at that time were associated with universities,[2] experimental physiology had little influence on American medicine. This began to change at the end the 19th century, when research laboratories were established at Johns Hopkins, where the laboratory was founded by Henry Newell Martin, who had trained in England; at Yale, where the laboratory began under Russel H. Chittenden, who had studied under one of Bernard's students; and at Harvard. The latter, which became "the center of a widespread influence in physiology and scientific medicine in [the United States]," [1] was established by Henry Pickering Bowditch, who had studied in both France and Germany.

Henry Pickering Bowditch *(1840-1911).*

Reproduced from reference 1: *Howell WH. The American Physiological Society during its first 25 years. In:* History of the American Physiological Society Semicentennial 1887-1937. *Baltimore, Md: American Physiological Society; 1938:1-89. Copyright © 1938, American Physiological Society.*

BIOGRAPHY

Bowditch was born in Boston in 1840 to one of New England's leading families. After receiving his BA from Harvard, he entered the Lawrence Scientific School in Cambridge, Massachusetts, intending to study chemistry, comparative anatomy, and natural history.[3,4] In 1861, after the outbreak of the Civil War, Bowditch enlisted as a cavalry officer in the Union Army and fought in several battles. He was discharged after being wounded, but following his recovery he immediately reenlisted and saw further action. Shortly after Lee's surrender at Appomattox, Virginia, he resigned from the army to resume his studies at the Lawrence School. He subsequently entered Harvard Medical School where he received his MD in 1868. While a medical student he met Charles E. Brown-Séquard, a French experimental physiologist who had come to Harvard to establish a Physiological and Pathological Institute.[2] Although this ambitious plan was not realized, Bowditch was drawn to Paris to study with Brown-Séquard. Because of poor health the latter became unable to accept students so that Bowditch went instead to work with Claude Bernard, whose laboratory turned out to be small and not well equipped. This led Bowditch to seek the advice of Willy Kühne, a German physiologist who was then working with Claude Bernard. Kühne suggested a two-year course of study with four of Germany's leading scientists: Max Schultze, Karl Ludwig, Rudolf Virchow, and Hermann von Helmholtz.[3] This long period of training was possible because of the financial support and encouragement that Bowditch received from his family.[2] In 1869, after studying histology with Schultze,

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Bowditch went to Leipzig to work with Ludwig. This collaboration proved to be so successful that Bowditch abandoned his plan to study with Virchow and Helmholtz; instead he spent two years with Ludwig with whom he published two papers, one of which described the all-or-none law of muscle contraction and the staircase phenomenon in cardiac muscle (5, see below).

In 1869, Bowditch had been asked by Charles W. Eliot, then president of Harvard, to teach a graduate course at Harvard Medical School, but chose instead to remain with Ludwig to avoid "sacrificing a large part of the benefit to be derived from my studies in the German universities" (cited by 1). In 1871, after Eliot had increased the offer to assistant professorship of physiology, Bowditch returned to Boston "filled with the spirit of Bernard and Ludwig."[6] He took full charge of instruction in physiology "with elaborate finished lectures and careful detailed demonstrations" that were characteristic of the Leipzig school[4]; at the same time he set up equipment he had brought from Leipzig in a renovated attic and, with generous support from his father, became one of the first full-time American physiologists. Bowditch's laboratory trained undergraduate medical students and more advanced scholars who, as was the practice at that time, paid a fee for training under his guidance. The educational value of this laboratory experience was noted by Eliot who, in seeking to raise funds for Harvard Medical School in 1874, wrote:

> The medical faculty of the university is leading in a reform in medical education, which is of the utmost consequence, not only to the community, but to the country… In order to enlarge the range of medical knowledge it is absolutely essential that there should be investigators in medical science.[2]

Bowditch became professor of physiology at Harvard in 1876 and, between 1883 and 1893 served as dean of the medical faculty where he became a leading advocate for a rigorous 4-year course of study to earn a medical degree, including time spent in the experimental laboratory. After stepping down as dean, Bowditch devoted much of his time to planning and fund-raising for Harvard Medical School. He resigned his professorship because of failing health in 1906, and died 5 years later.

THE ALL-OR-NONE LAW AND THE STAIRCASE PHENOMENON

Bowditch published his major contributions to cardiovascular physiology when, as a student of Ludwig, he noted that the pressure developed by the frog heart depends on the "time which passes between it and a preceding contraction."[5] In describing the changing contractile response that follows a period during which "the heart had been in perfect rest over several minutes" *(Figure 1),* Bowditch stated:

> The first twitch… is the smallest, and each following one is increasing in size in such a way that with the rising number of twitches, the amount of increase is getting smaller and smaller until it completely vanishes.[5]

Bowditch called this step-like increase in pressure a "Treppe" (German for staircase). Bowditch also examined the pressure developed in pairs of stimuli and found that the second response was of greater magnitude, a manifestation of the staircase phenomenon now called "postextrasystolic potentiation." He noted that prolonging the interval between stimuli decreased the extent to which the second twitch was enhanced. Bowditch also discovered the "all-or-none" law when he observed that the strength of contraction is independent of that of the stimulus:

> The induction current of the smallest strength which can induce a cardiac twitch does not cause the weakest of all twitches, and also the extent of the latter does not grow to an unsurpassable maximum if the intensity of the stimulating current increases. In our subjects *the induction current either induces a twitch or not* [italics added]; and if it does so, it also provokes the most extensive twitch which the induction current at a given time can produce at all.[5]

Bowditch concluded that changes in the intensity of the contractile response caused by altering stimulation frequency "lies in the variable properties of the muscle fiber itself," stating: "it seems to be hardly necessary to stress the immense practical implication of this sentence."[5] In this latter regard, however, he was wrong; it was not until the 1950s, more than 80 years later, that the "variable properties" described by Bowditch came to be understood as changes in myocardial contractility.[7]

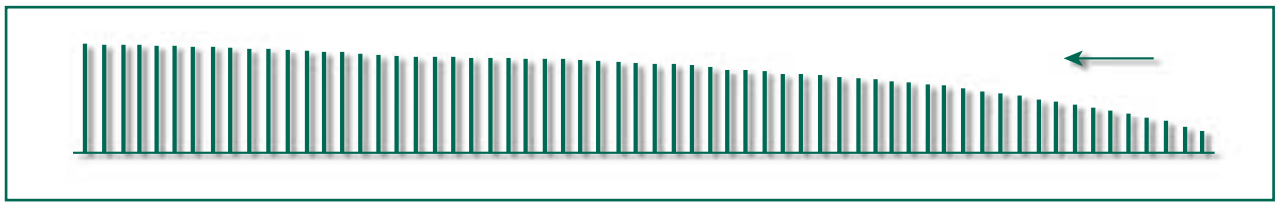

Figure 1. *The staircase phenomenon (Treppe) as shown in a woodcut from reference 5. The ordinate is the pressure developed by a frog heart; time is on the abscissa. This figure, which lacks scales showing absolute values for pressure and time, should be read from right to left.*

Adapted from reference 5: *Bowditch HP. Über die Eigenthümlichkeiten der Reizbarkeit, welche die Muskelfasern des Herzens zeigen [On the Properties of the Excitability Evidenced by the Muscle Fibers of the Heart].* Berichte der Kön.-Sächs Gesellschaft der Wissenschaften Mathematisch-Physische Klasse. *1871;23:652-689.*

CONCLUSION

By transplanting the intellectual approach and methodology of experimental physiology from Europe to the United States, Bowditch made a major contribution to American medicine. His positions as dean of Harvard Medical School and as a founder of the American Physiological Society (of which he was second president) enhanced his ability to integrate experimental physiology into the medical curriculum. Walter Cannon noted:

> The traditions of the [American Physiological] Society, particularly its character as an association to encourage research, are largely the result of [Bowditch's] initiative. His example and his genuine appreciation of new work as it was reported at meetings of the Society was a wholesome stimulus to young men beginning physiological investigation.[6]

Howell, who was both a colleague and a good friend, attributed Bowditch's success to his personality, stating:

> He was a man of commanding and distinguished appearance. His beard and heavy mustache and his general carriage were suggestive of his military experience during the war, and his fine face when in repose had a certain severe dignity, which helped to make him an impressive presiding officer. Indeed, the writer can recall no one in his experience who could conduct a scientific meeting with the same attractive combination of authority and courtesy. But business aside and in personal intercourse he was cordial and friendly and, at times, even jovial with his great hearty laugh and his almost boyish enjoyment of fun… Needless to say, he was loved and respected by all of his fellow members of the [American Physiological] Society.[1]

Summarizing Bowditch's impact on American medicine, Osler wrote: "Men of his type raise a community. Not many are needed in a country; a teaspoonful of such yeast raises a mighty big lump of dough."[8]

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Regional accumulations of T cells, macrophages, and smooth muscle cells in the human atherosclerotic plaque

L. Jonasson, J. Holm, O. Skalli, G. Bondjers, G. K. Hansson

Arteriosclerosis. 1986;6:131-138

The human atherosclerotic plaque is a heterogeneous structure, particularly in its advanced state, consisting of a variety of cell types. From morphological studies, the presence of myofibrils in many cells suggested a smooth muscle cell origin, and the resemblance of some cells to monocytes argued that the inflammatory cell component of plaques contained macrophages. However, prior to this paper by Jonasson et al, there had been little characterization of the cellular components within plaques, their lineage and degree of differentiation, or any indication of their functional state.

Jonasson et al examined human carotid arterial plaques from surgical specimens, and a panel of monoclonal antibodies were used to identify T lymphocytes, macrophages, and smooth muscle cells. In addition, T lymphocytes were removed from lesions for functional studies of activation including rosetting and the presence of HLA-DR. Macrophages were found throughout the plaque, particularly around the lipid core region, with T cells comprising a significant portion of the fibrous cap. Of particular interest, desmin-positive smooth muscle cells, a marker of a more undifferentiated phenotype, were frequently seen in the plaque, but not in the normal vessel.

This study provided definitive proof, based on the lineage-specific staining of cell-surface antigens that appear on the surface of only one cell type, of the composition of human plaques. Importantly, the study identified the predominant cell type in different regions of the plaque, macrophages in the "necrotic" core, smooth muscle cells in the fibrous cap, and, particularly, the presence of desmin-positive smooth muscle cells, a phenotype previously identified in the chronic intima after arterial injury. Macrophages were also seen, particularly in the shoulder region and infiltrating the fibrous cap, and also noteworthy was the high proportion of T cells in the fibrous cap, many of which demonstrated markers of activation.

Not only did this study outline the characteristics and location of different cell types in different regions of the plaques, it also laid the foundation for the now widely held hypothesis of plaque rupture, and the repair reaction that underlies acute plaque progression. In particular, it has become apparent that plaques frequently rupture through thin fibrous caps, associated with low smooth muscle cell contents in caps infiltrated by activated macrophages and lymphocytes. The close approximation of all three cell types seen in this study has fostered the idea that cell:cell interactions regulate plaque behavior. Thus, macrophages/T cells and smooth muscle cells have opposing effects on matrix structure, macrophages may be activated by cytokines produced from T cells, and T cells may be activated by macrophage-presented antigens. In addition, macrophage and T-cell cytokines may promote the smooth muscle cell phenotypic change seen as an accumulation of desmin-positive cells within the plaque. The predictions made in this study, that critical cell:cell interactions, both direct and induced through cytokines, regulate plaque behavior, has also provided the basis for current therapies aimed at suppressing both inflammatory cell accumulation and activation.

1986

US painter Georgia O'Keeffe dies, aged 98; the European Space Agency's Giotto probe obtains the first closeup pictures of the nucleus of Halley's comet; and Jacques Chirac is named Prime Minister of France

The interleukin-1 family: 10 years of discovery

C. A. Dinarello

FASEB J. 1994;8:1314-1325

Cytokines are small, highly active molecules that are produced by a variety of cells in response particularly to injury or infection. Interleukins (IL), the subject of this historical perspective and review, are one of the founding members of the family of cytokines. IL-1 was simultaneously studied by many groups in different areas, characterizing a huge range of biological properties before cloning of mouse IL-1α and IL-1β in 1984 demonstrated that the same molecules were responsible for the promiscuous properties.

Since these initial breakthroughs, a further member has been isolated, interleukin-1 receptor antagonist (IL-1ra), blocking both binding and biological activity of IL-1α and IL-1β. While IL-1α and IL-1β are potent agonists, inducing biological responses in the picomolar or femtomolar range, IL-1ra is an endogenous competitive inhibitor; the host response to external or internal stimuli thus depends upon the net occupancy of the IL-1 receptors by these three molecules. In most cells, the IL-1α and IL-1β responses are similar, although some cell-specific responses are apparent; such specificities are underscored by the finding of distinct receptor binding sites for IL-1α and IL-1β. IL-1 signaling follows the same sorts of pathways as seen in the toll-like receptors (TLRs), activating nuclear factor kappa B (NF-κB), and triggering transcription of a number of proinflammatory genes and cytokines, including itself. The potency of these molecules is reflected in the multiple and complex regulatory systems that prevent their production and activity under normal circumstances. Thus, IL-1ra is one of very few high-affinity natural inhibitors. IL-1α has no signal peptide, and thus is mostly cytoplasmic. In contrast, IL-1β secretion is controlled at multiple levels. Most IL-1β mRNA is not translated and requires a second signal for translation. This generates pro–IL-1β, which requires cleavage for activity. The cleavage enzyme, IL-1β converting enzyme (ICE), is a caspase enzyme, which must also be activated from a proform, and a number or endogenous and virus products inhibit ICE. Even if IL-1β does get secreted, IL-1ra and IL-1 receptors are also secreted in excess and can sequester active cytokine.

So where does IL-1β fit into atherosclerosis? IL-1 receptors are found on a variety of vascular cells, including smooth muscle cells, and both macrophages and T lymphocytes that participate in atherosclerosis. Macrophages in particular are potent sources of IL-1 after activation as foam cells, which may be mediated by scavenger-receptor binding. Endothelial cells and smooth muscle cells also synthesize IL-1, which may be triggered by activation of ICE via ligands such as CD40. IL-1 promotes vascular smooth muscle cell proliferation, augmenting effects of growth factors, and proliferation is blocked by IL-1ra. Growth factors also increase IL-1ra expression, creating a scenario where overexpression of both cytokine and receptor is present in atherosclerosis, generating a positive feedback loop leading to progressive inflammation. IL-1 is also one of the major stimuli to the systemic inflammatory state seen in atherosclerosis, mediated in part by IL-1–mediated release of IL-6, and circulating levels of IL-1 may be increased in unstable coronary syndromes. A number of protective mechanisms may also maintain vessel integrity by inhibiting IL-1. For example, apoptosis of endothelial cells is blocked in part by agents such as nitric oxide (NO), which inhibit ICE.

1994

The Swedish men's tennis team defeats Russia in Moscow to win their 5th Davis Cup; Kim Il Sung, President of North Korea (1945-1994) dies, aged 82; and the Nobel Peace Prize is awarded to Yitzhak Rabin, Shimon Peres, and Yasser Arafat

Adhesion molecules—Part 1

P. S. Frenette, D. D. Wagner

N Engl J Med. 1996;334:1526-1529

Adhesion molecules and adhesion receptors regulate a wide variety of physiological situations and pathologies, such as embryogenesis, wound repair, cell growth, and differentiation. This review summarizes the classes of adhesion receptors and indicates where adhesion molecule expression has been implicated in disease.

Adhesion receptors consist of four families, the integrins, members of the immunoglobulin superfamily (cell adhesion molecules or CAMs), cadherins, and selectins. Integrins are membrane glycoproteins that have bidirectional signaling, modifying the affinity of the integrin for its ligand in addition to transmission of ligand-receptor signaling into the cell. The CAMs contain immunoglobulin-like structures and are frequently involved in homophilic interactions. The cadherins form molecular links between adjacent cells, for example, at adhering junctions, again forming homophilic interactions. In contrast to other adhesion molecules that bind to proteins, the selectins interact with carbohydrate ligands on endothelial cells and leukocytes.

Adhesion receptors associate with a variety of cytoplasmic proteins and cytoskeletal components. Links to the latter stabilize cell:cell junctions, whereas interactions with cytoplasmic proteins are responsible for signal transduction from integrins. The signals transduced by adhesion molecules depend upon the specific ligand and receptor, but, in general, result in adaptation to the local cell environment. Thus, interactions between different extracellular matrices result in cell differentiation or proliferation/activation via adhesion molecule signaling, and lack of signaling may promote apoptosis. Defective adhesion molecule signaling may also result in loss of cell anchorage or homophilic interactions, a prerequisite for both local invasion and metastasis in cancer. In contrast, an increase in adhesion molecule expression with heterophilic interactions can promote cell migration and invasiveness. These properties are due in part to the cytoskeletal rearrangement that occurs following adhesion molecule signaling. Evidence is accumulating for the role of adhesion molecules in atherosclerosis. Thus, sites predisposed to atherosclerosis show increased expression of vascular cell adhesion molecule (VCAM), which binds to leukocytes found in early plaques, in particular to monocyte/macrophages and T lymphocytes. In humans, platelet–endothelial cell adhesion molecule–1 (PECAM 1) is normally expressed by endothelial cells and some lipid-filled macrophages, but VCAM-1 is absent. Intercellular adhesion molecule–1 (ICAM-1) and E-selectin are present focally in endothelial segments in some normal vessels. In contrast, plaques express high levels of ICAM-1 in both endothelial cells and macrophages, and both E-selectin and VCAM-1 show increased expression in the endothelium over plaques. VCAM-1, ICAM-1, and E-selectin are increased in the adventitial vessels in atherosclerosis, and also in intimal neovessels, where they may regulate monocyte migration to deep regions of the plaque. Interestingly, circulating adhesion molecules such as sICAM-1, sVCAM-1, (s, for soluble), and P-selectin are associated in some studies with acute manifestations of atherosclerosis such as acute coronary syndromes and a worse clinical outcome. It is not clear whether this is a direct proadhesion effect, or reflects endothelial damage and/or inflammatory cell release of these molecules.

Although these studies implicate adhesion molecule expression in atherogenesis, direct evidence proving their importance is lacking. Although VCAM-1 knockout mice develop significantly less atherosclerosis than control animals, the results for ICAM-1 knockout are controversial. However, β-hydroxy-β-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor treatments reduce both local expression of adhesion molecules and circulating soluble forms, indicating that prevention of leukocyte trafficking into plaque may be one mechanism of their action.

1996

Aung San Suu Kyi's National League for Democracy is banned in Burma; legendary jazz vocalist Ella Fitzgerald dies, aged 79; and Mount Ruapehu erupts in New Zealand

The tumor necrosis factor ligand and receptor families

F. Bazzoni, B. Beutler

N Engl J Med. 1996;334:1717-1725

Tumor necrosis factor (TNF) is a cytokine with a multitude of actions. Originally characterized as an agent able to kill tumor cells, it also is a catabolic hormone (cachectin) and a proinflammatory molecule, acting as a mediator of toxic shock. TNF is synthesized particularly by inflammatory cells of the monocyte/macrophage lineage, but binds to receptors that are widespread. TNF receptors come in two types, the type I receptor (p55) and the type II receptor (p75), and are part of a larger family that includes CD95/Fas, tumor necrosis factor–related apoptosis-inducing ligand (TRAIL) receptors I and II, CD40, nerve growth factor receptors, CD27, CD30, and OX40. This review summarizes the ligands, signaling, and response of TNF receptors.

In almost all cases, the signaling follows a stereotypic pattern. Each receptor is a transmembrane protein consisting of two subunits, which may be identical. Binding of trimeric ligand to specific receptor causes receptor aggregation and (proximity) activation. Receptor activation permits binding of an adapter molecule to a cytoplasmic domain of the receptor (for example TRADD [TNF-receptor–associated death domain] to TNF-R1, FADD [Fas-associated death domain] to Fas), which binds further signaling molecules through protein:protein interactions. In receptors signaling apoptosis (TNF-R1, Fas, TRAIL R I and II), the intracellular domain contains a protein motif known as a death domain, required for recruiting upstream caspases in apoptosis signaling. TNF-receptor family members also bind TRAF proteins (TNF-receptor-associated factors), which signal via a series of kinase enzymes, resulting in activation of nuclear factor kappa B (NF-κB). TRAFs can also induce apoptosis even though some receptors lack death domains, in part by autologous expression of other TNF-like ligands. Oddly, NF-κB activation may inhibit apoptosis by directly inducing proteins of the BCl-2 family; the balance between death and survival therefore depends upon the intracellular levels of inhibitors of apoptosis.

In addition to inducing (or inhibiting) apoptosis, receptor activation promotes inflammation and cell proliferation. These signals are particularly important for the deletion of autoreactive T lymphocytes. Thus, a phenotype resembling systemic lupus erythematosus is seen in mice with inactivating mutations in Fas or Fas-L, and human disease is characterized by splenomegaly, lymphadenopathy, and autoimmune disease. CD40 ligand mutations cause X-linked immunodeficiency, caused by clonal expansion of antigen-responsive B cells.

TNF-like ligands and receptors appear to play a number of significant roles in vascular disease. TNF receptors are widespread within the vessel wall, being expressed by endothelial cells, vascular smooth muscle cells, and macrophages. Activation of TNF receptors on endothelial cells and smooth muscle cells rarely induces apoptosis, however, as receptors are mostly internal, and high levels of inhibitors of apoptosis are present. In contrast, the major action appears to be proinflammatory signaling mediated through NF-κB. Thus, TNF-R1 activation causes expression of adhesion molecules on endothelial cells and activates ICE, thus increasing interleukin IL-1β release, and can augment the production of growth factors. CD40 ligation also has profound effects on coagulation, in part by inducing tissue factor expression. The profound proatherogenic effect of signaling through these molecules is demonstrated by mice that lack either CD40 or CD40 ligand and that develop significantly less atherosclerosis than control animals. This finding has emphasized the prospect of therapies aimed at inhibiting signaling through these molecules to either slow progression of atherosclerosis or promote plaque stability, in part by interrupting the cycle of chronic inflammation.

1996

Death of Pol Pot, leader of the Khmer Rouge;
Desmond Tutu retires as archbishop of Cape Town;
and US sprinter Michael Johnson breaks
the world 200-m record at the Altanta Olympics

Scavenger receptor family proteins: roles for atherosclerosis, host defence and disorders of the central nervous system

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The first scavenger receptor (SR) was discovered in 1979, as a mechanism by which oxidized low-density lipoprotein (LDL) is preferentially taken up by macrophages. Since this discovery, a family of receptors has emerged, expressed by many cells, including macrophages, smooth muscle cells, and endothelial cells. The scavenger receptor family of proteins can be divided into class A (type I and II macrophage scavenger receptors with collagenous structure, MARCO), class B (CD36, scavenger receptor class B1), mucin-like (CD68/macrosialin, dSR-CI), and endothelial (LOX-1, lectin-like oxidized LDL) receptors. These receptors have a number of structural and functional homologies, which in part led to their discovery.

The range of these receptors also reflects their heterogeneity of function. Thus, type I and type II receptors can mediate uptake of oxidized low-density lipoprotein (LDL), although these are by no means the only receptors that do this. Type I and II receptors also mediate phagocytosis and clearance of apoptotic cells, although again, other receptors can perform this function and in addition clear oxidized red cells. Type I and II receptors are also required for macrophage adhesion and ingestion of intracellular bacteria as part of the innate defense reaction. CD36 also binds and internalizes oxidized LDL and is important in apoptotic clearance, but, in addition, is partly responsible for the binding adhesion and aggregation of platelets. Finally, scavenger receptor BI (SRBI) can mediate binding of a variety of lipids, including oxidized and acetyl LDL, normal LDL, and high-density lipoprotein (HDL). As SRBI is predominantly expressed in the liver, it is seen as having a major role in HDL-derived cholesterol transport.

Yamada et al summarize the structure and function of scavenger receptors and also their role in disease. In particular, scavenger receptors are implicated in both the initiation of atherosclerosis and its subsequent progression. Scavenger receptors were originally implicated in atherosclerosis by virtue of receptor-mediated uptake of modified LDL, with resultant pathological deposition of cholesterol in the vessel wall. However, uptake of modified LDL also triggers endocytosis, phagocytosis, adhesion, and signal transduction, and thus their role in atherosclerosis may be more prominent.

Type I and II scavenger receptors are expressed in macrophages and smooth muscle cells in atherosclerotic plaques and may promote foam cell formation and lipid retention in the vessel. Indeed, in knockout mice experiments, both type I and II knockouts are associated with reduced plaque formation in both apolipoprotein E (ApoE) and LDL receptor knockout backgrounds. Interestingly, these effects are observed in the absence of reductions in circulating LDL, arguing strongly for a role of these receptors in local retention of LDL in the vessel wall. Both CD36 and SRBI are also expressed in human atherosclerotic plaques, and may mediate the residual LDL uptake seen in type I and II receptor knockout mice. In addition to the role in mediating oxidized LDL uptake, increasing evidence suggests that scavenger receptors, in particular CD36, mediate phagocytosis of apoptotic cells in atherosclerotic plaques. The cross-reaction of these receptors with acetyl LDL argues also that lipid blockade of these receptors reduces apoptotic clearance in atherosclerosis, which may account for the accumulation of dead cell debris seen in the "necrotic cores" of these lesions.

1998

France beats Brazil 3-0
in the World Cup final in Paris;
UN Secretary General Kofi Annan signs
a treaty establishing the International Criminal
Court at the Hague; and the Tour de France
is rocked by allegations of drug use resulting
in the suspension of the Festina team

Activation of receptor for advanced glycation end products: a mechanism for chronic vascular dysfunction in diabetic vasculopathy and atherosclerosis

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Diabetics get atherosclerosis. More than this, diabetics get earlier, more diffuse, and aggressive atherosclerosis, and have a worse outcome of their vascular disease compared with nondiabetics. While these clinical facts are well established, the mechanism of diabetic vascular disease has been more difficult to dissect. Specifically, it has been difficult to identify differences in cell activation or signaling of diabetic vascular disease that underlie the different biological and clinical behaviors of this disease. Recently, an increasing role has been elucidated for receptors for advanced glycation end products (RAGEs), the subject of this review and study.

RAGEs are a member of the immunoglobulin family superfamily of cell surface receptors whose ligands play a significant pathogenetic role in many disease states. In atherosclerosis, one particular class of ligands, known as glycoxidation products or advanced glycation end products (AGEs), is particularly noteworthy. AGEs occur in diabetics at sites of oxidant stress and are posttranslational modifications of cell-surface and intracellular proteins. Both hyperglycemia (provided by the diabetic state) and oxidation (provided by the local environment in atherosclerosis) promote their formation. Ligand binding to receptor has a number of downstream signaling consequences. First, receptor binding enhances receptor expression (possibly by nuclear factor kappa B [NF-κB] activation), generating a positive feedback loop. Second, RAGE ligation triggers cellular activation. Although the signaling pathways involved are not fully characterized, they include activation of $p21^{ras}$, mitogen-activated protein kinase (MAPK), and NF-κB, resulting in transcription of target genes. Smooth muscle cells, endothelial cells, and macrophages all express RAGEs. The biological response to RAGE ligation is therefore dependent upon the cell in question. Ligation results in smooth muscle cell proliferation and matrix synthesis, and, in endothelium, results in reduced barrier function, reduced nitric oxide (NO) production, increased adhesion molecule formation, and altered coagulation properties. In almost all cases, activation of vessel wall cells would be predicted to promote atherosclerosis,

In atherosclerosis, as in many other chronic inflammatory diseases, RAGE expression increases and is sustained over years. In diabetic vascular lesions, RAGE expression also correlates with sites of AGE production, and this leads to the hypothesis that RAGE/AGE interactions generate a chronic inflammatory state, which, superimposed upon a further stimulus, such as lipoprotein accumulation in atherosclerosis, promotes the formation of an aggressive and unstable atherosclerotic plaque. Proof of principle studies in apolipoprotein E (ApoE) knockout mice that have also been rendered diabetic using streptozotocin have demonstrated the potency of RAGE/AGE interactions in promoting atherosclerosis. Diabetic ApoE knockout mice developed faster, more extensive atherosclerosis than their nondiabetic controls, associated with RAGE lesions and AGE deposition at sites of lesion formation. Blockade of RAGEs using a soluble form of the receptor to sequester ligand dose-dependently reduced both the extent and complexity of atherosclerosis, as well as AGE deposition.

Importantly, the identification of RAGE/AGE interactions provides the basis for novel therapies designed to inhibit atherosclerosis and interrupt the spiral of increasing chronic inflammation that promotes plaque instability. These agents appear to work independently of cholesterol lowering and may represent a potent mechanism for slowing and stabilizing atherosclerosis in diabetics.

1999

Shakespeare in Love wins seven Oscars, including Best Film; the fossil of *Anomocephalus africanus*, the earliest ancestor of the mammals, is discovered in South Africa; and Bertrand Piccard and Brain Jones become the first people to fly around the world in a balloon

NF-κB: pivotal mediator or innocent bystander in atherogenesis?

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Few molecules can signal as many different functions, some of which are opposing, as nuclear factor kappa B (NF-κB). NF-κB is a transcription factor of the Rel family, characterized by a homologous region that mediates dimerization, nuclear translocation, DNA binding, and transcription. NF-κB is normally kept inhibited by a family of inhibitors, the IκBs, which mask the nuclear localization signal and thus promote retention in the cytoplasm. Diverse stimuli activate NF-κB through phosphorylation of the IκB kinase (IKK) complex, promoting proteosomal degradation, with resultant release of NF-κB to translocate to the nucleus. NF-κB activates a huge variety of genes that are relevant to atherosclerosis, including cytokines, chemokines, adhesion molecules, and genes that regulate cell proliferation and survival. NF-κB also activates part of the IKK complex, increasing its own inhibitor. In this way, NF-κB signaling is self-limiting and transient.

So what is the evidence that implicates NF-κB as a critical regulator of atherosclerosis, the subject of this review? Activated NF-κB is found in atherosclerotic plaques and after arterial injury in smooth muscle cells, endothelial cells, and macrophages, but not in normal vessels, ie, guilt by presence. Many targets of NF-κB are both present in atherosclerotic plaques and would be predicted to initiate atherogenesis and promote lesion formation. These include the leukocyte adhesion molecules (LAM), vascular cell adhesion molecule–1 (VCAM-1), intercellular adhesion molecule–1 (ICAM-1), and E-selectin, the chemokines monocyte chemotactic protein–1 (MCP-1) and interleukin 8 (IL-8), tissue factor (which regulates coagulation), and cyclin D1 (which promotes cell proliferation). In many cases, there is experimental evidence that inhibition of these effector molecules or their receptors (eg, MCP-1, CR2) dramatically inhibits atherosclerosis in animal models. The promoters of these genes contain NF-κB binding sites, and cytokine induction of these genes requires NF-κB.

Second, NF-κB is activated by a large number of stimuli associated either with the onset or progression of atherosclerosis. Thus, angiotensin II, a series of cytokines, oxidized lipids, and advanced glycosylation end-products (AGEs) bind to their cognate receptors and, through a series of adapter proteins and intermediate kinases, trigger NF-κB activation. These stimuli provide a link through NF-κB to many of the common risk factors for atherosclerosis, namely hypertension, diabetes, and hypercholesterolemia, Other, less well-established risk factors may also result in activation of NF-κB, including infectious agents and homocysteinemia. This discussion argues strongly that NF-κB activation is all bad, and inhibiting NF-κB should be a major therapeutic aim in atherosclerosis. Predictably for such a multifunctional regulator as NF-κB, this is all too simplistic. In addition to signaling inflammation, NF-κB is a potent suppressor of cell death that may be generated by that inflammation. Cell death of endothelial, smooth muscle, and inflammatory cells has been implicated in both plaque progression and plaque instability. NF-κB activates a variety of inhibitors of apoptosis, blocking apoptosis at a variety of steps. NF-κB also induces genes that protect against oxidant stress, such as HO-1 and superoxide dismutase, which inhibit inflammation in the vessel wall.

At present, the precise status of NF-κB in inducing/progressing atherosclerosis or protection is unclear. A remarkable series of correlations strongly suggest that NF-κB plays a key role in atherosclerosis, including the presence of activated NF-κB and NF-κB–induced genes and activation by known risk factors. We await direct evidence via tissue specific overexpression or knockout of NF-κB components before formal proof of this concept.

2001

French painter Balthus dies at the age of 92; British yachtswoman Ellen MacArthur becomes the first woman to complete the Vendée Globe solo race around the world, taking second place; and Tom Cruise and Nicole Kidman file for divorce

New therapeutic targets revealed through investigations of innate immunity

R. J. Ulevitch

Crit Care Med. 2001;29(7 suppl):S8-S12

The innate immune system comprises a mechanism by which cells induce genes for potent antimicrobial peptides upon exposure to pathogens, without formation of antibodies. This is a rapid response, without a previous exposure to generate acquired (antigen-specific) immune responses. The signaling pathways responsible for this innate immunity have recently been identified following pioneering work in a number of laboratories, including the author's, and are summarized in this review.

In general, receptors for innate immune responses are either scavenger receptors or the recently characterized toll-like receptors (TLRs). (The original toll receptors were described in *Drosophila* as a mediator of innate immunity.) For example, bacterial lipopolysaccharide (LPS)–binding protein/C14, which regulates the response to Gram-negative bacteria, activates TLRs. The receptor complex is multiprotein, consisting, for example, of CD14, TLR4, and a secreted protein MD-2 that promotes CD14-dependent binding of LPS to TLR4. Analysis of other TLRs indicates that they bind components of outer membranes of all bacteria and have unique tissue and cellular distributions.

TLR signaling to the nucleus involves a series of kinase steps and activation of NF-κB. TLR activation triggers events similar to those activated by interleukin receptors. For example, TLR4 activation signals through myeloid differentiation Factor 88 MyD88, interleukin 1 (IL-1)-receptor–activated kinase (IRAK), and TRAF6. Beyond this point, the signaling becomes murkier. A number of protein kinases, including the rho/rac/cdc42 system of GTPases, have been implicated, although none definitively proven. Somehow, these pathways trigger gene transcription for antimicrobial peptides. TLRs are also integral points for a number of responses that comprise innate immunity. TLR activation, for instance, can trigger apoptosis and both synthesis and secretion of proforms and release of proinflammatory cytokines such as IL-1β. The balance between cell activation and cell death is determined by the relative expression of multiple species of protein kinases that regulate these processes, including p38, JNK, IKK-β, and PKB/Akt.

So what has this signaling system, characterized as the response in septic shock/multiorgan failure, got to do with atherosclerosis? The answers lie with both the stimuli and the receptors. Both LPS and lipoproteins activate TLRs, including those on macrophages, via CD14/TLR2, for example. Increasing evidence implicates chronic macrophage activation as a major stimulus for both atherogenesis and plaque rupture. Furthermore, infectious organisms have been implicated as causing atherosclerosis, evidenced by increased serum levels of reactive antibodies and the detection of organisms within macrophages in plaques. TLR signaling in macrophages may be responsible for increased NF-κB signaling and macrophage activation, with release of proinflammatory cytokines within lesions. TLR signaling may also directly promote apoptosis, a feature of advanced atherosclerotic plaques.

Thus, the TLR signaling system may represent a further mechanism by which macrophage activation is both triggered and maintained in atherosclerosis. Clearly, both identification of TLR ligands and blocking receptors may lead to new therapeutics in this area, in addition to therapies aimed at preventing or limiting the response in septic shock.

2001

The International Olympic Committee awards Beijing the right to host the 2008 Olympic Games; violence erupts between antiglobalization protesters and police at the Genoa G8 summit; and an American patient receives the world's first self-contained mechanical heart transplant at the Jewish Hospital in Louisville

Chemokines and disease

C. Gerard, B. J. Rollins

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Over 50 chemokine ligands and over 20 G-protein–coupled chemokine receptors have been identified to date. Within the vasculature, chemokine receptors are expressed by endothelial cells and vascular smooth muscle cells, and, in particular, by inflammatory cells invading and taking up residence within the vessel wall. Chemokines can be divided into inducible agents, whose expression is elicited by many stimuli that alter tissue homeostasis and traffic leukocytes to the site of tissue injury, and constitutive chemokines, responsible for basal leukocyte trafficking into lymphoid organs. Although these agents clearly evolved as host response to injury, it seems that their inducibility and high expression can result in a prolonged activation of leukocytes with concurrent tissue damage.

This review focuses on the role of chemokines in specific diseases, eg, multiple sclerosis, cancer, human immunodeficiency virus (HIV) infection, allergic inflammatory disease (asthma), and vascular disease (atherosclerosis and transplant vasculopathy). A number of common themes emerge. First, in all of the diseases discussed, excessive chemokine and chemokine expression is seen, associated with leukocyte infiltration into the target cells/organ. Second, there are few instances of disease-specific chemokine receptor or chemokines; rather there is a huge overlap in ligand-receptor specificity, suggesting that chemokine redundancy occurs. In addition, most diseases are associated with a temporal and spatial pattern of specific chemokine and receptor expression, arguing for a multicomponent pathological response, with cooperative activity of different cell deaths in the pathogenesis of the disease process. Such a multicomponent chemokine response is seen in atherosclerosis. Thus, activated endothelial and smooth muscle cells release chemokines that promote leukocyte emigration (eg, monocyte chemotactic protein–1 [MCP-1]), and smooth muscle cell migration/proliferation. Other chemokines, such as IL-8, stromal-derived factor (SDF-1), interferon-gamma–inducible protein (IP-10), I309, and the chemokine receptor CXCR2 are also seen in animal models of atherosclerosis, and may also have a role in leukocyte trafficking (eg, IP-10 promotes $CXCR3^+$ T lymphocyte migration). Given the multitude of chemokines seen in atherosclerosis, it is truly remarkable that loss of either MCP-1 or its receptor CCR2 can result in a large reduction in atherosclerosis, arguing that at best only partial redundancy occurs in chemokine signaling in atherosclerosis. MCP-1 also potently promotes tissue factor expression, inducing a local procoagulant state. In humans, MCP-1, RANTES (regulated on activation, normal T-cell expressed, and presumably secreted), LD78, eotaxin, and fractalkine are present in atherosclerotic plaques, although we lack evidence of any chemokine-specific activity in human disease. Finally, chemokines are seen as important links between known risk factors for atherosclerosis and the vessel response. Thus, altered shear stress in hypertension or disordered blood flow promotes endothelial expression of chemokines, oxidized lipids promote macrophage expression of chemokines and chemokine receptors, and putative infectious agents in atherosclerosis such as cytomegalovirus can induce chemokine receptors.

Taken together, these studies suggest that chemokine/chemokine receptor antagonism may be beneficial in human atherosclerosis. Indeed, some currently available drugs of proven benefit, such as β-hydroxy-β-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, also inhibit MCP-1 expression. In addition, small molecule inhibitors of specific chemokines can prevent leukocyte migration in animal vessels. We await human studies to verify both the principle of this approach and its clinical utility in atherosclerosis.

2001

Sir Donald Bradman, the greatest cricketeer of the 20th century, dies in Adelaide, aged 92; Libyan secret service agent Abdel-Baset-al-Megrahi is found guilty of the bombing of Pan Am flight 103 over Lockerbie; and Ariel Sharon, leader of the Likud party, wins the Israeli election

Inflammation and atherosclerosis

P. Libby, P. M. Ridker, A. Maseri

Circulation. 2002;105:1135-1143

Atherosclerosis is an inflammatory disease—a widely held and almost uniformly accepted principle. This simple truism belies a checkered history in hypotheses accounting for the generation of atherosclerosis. Indeed, we have traveled a long way from the concept of atherosclerosis being a simple lipid storage disease, through a smooth muscle cell-driven "neoplastic" response to injury, to the recognition that inflammation is fundamental to both atherogenesis and the clinical consequences of atherosclerosis. The authors of this review have contributed in no small part to this change in thinking.

The evidence for inflammation in atherosclerosis is legion. The earliest lesions of atherosclerosis consist of lipid-laden foam cells generated by emigration of inflammatory cells into the vessel, mediated by expression of adhesion molecules on the endothelium. Inflammatory cells are present in all stages of atherosclerosis and demonstrate multiple features of activation, such as cell-surface markers and expression/secretion of active biological molecules. Such molecules promote the vast majority of other features of atherosclerosis, such as smooth muscle cell migration/proliferation and formation of a locally procoagulant plaque. Importantly, the clinically significant sequelae of atherosclerosis, plaque rupture with subsequent vessel occlusion, are greatly increased in plaques with high inflammatory cell components. Thus, inflammation initiates lesion formation, promotes plaque progression, and drives plaque rupture. The recognition of the importance and ubiquity of inflammation in atherosclerosis has resulted in the predominant role that inflammation occupies in all current models of atherogenesis. Inflammation in atherosclerosis is not just a local phenomenon; advanced atherosclerosis is also a systemic inflammatory state. Thus, multiple markers of inflammation are increased in atherosclerosis (eg, C-reactive protein [CRP], serum amyloid A [SAA], interleukin 6 [IL-6], soluble adhesion molecules) and predict a high-risk subgroup after acute coronary syndromes. Indeed, such markers may have as great a positive value as conventional risk factors for the development of disease, and inflammation provides an important link between such risk factors and atherosclerosis. Thus, oxidized lipids directly promote inflammatory cell migration and activation, and both hypertension and diabetes can induce proinflammatory cytokines, via angio-tensin II and advanced glycosylation end-products (AGEs), respectively. In addition, circulating markers of inflammation have been implicated in direct pathogenesis of athero-sclerosis. CRP, for example, activates complement, induces expression of adhesion molecules, promotes tissue factor expression, and enhances expression of monocyte chemotactic protein–1 (MCP-1).

Accumulating evidence also demonstrates that inhibiting inflammation suppresses both the generation and the clinical consequences of atherosclerosis, and, conversely, inhibiting atherosclerosis reduces atherosclerosis. Thus, β-hydroxy-β-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors markedly reduce the proportion of plaques comprised of inflammatory cells and their activation, and also reduce serum markers of inflammation. In addition, inhibition of single inflammatory molecules or their receptors can markedly inhibit the development of atherosclerosis in animal models. Libby et al present an overview of inflammation in atherosclerosis as a local and systemic process that mediates and governs every stage of plaque development and biological behavior. This sets the stage for clinical testing of a wide range of targeted anti-inflammatory therapies in atherosclerosis. Finally, inflammatory markers may be used for risk stratification and both for targeted therapy and monitoring the effect of therapy.

2002

HRH Queen Elizabeth, the Queen Mother, dies at the age of 101; UNITA and Angola's armed forces agree a cease-fire, ending 27 years of civil war; and the world's oldest man, Yukichi Chaganji, turns 113 on Kyushu island, Japan

Inflammation & Coronary Artery Disease

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selected by **Peter Libby, MD**

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