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Inflammatory processes in cardiovascular disease: a route to targeted therapies

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Abstract

Inflammatory processes are firmly established as central to the development and complications of cardiovascular diseases. Elevated levels of inflammatory markers have been shown to be predictive of future cardiovascular events. The specific targeting of these processes in experimental models has been shown to attenuate myocardial and arterial injury, reduce disease progression, and promote healing. However, the translation of these observations and the demonstration of clear efficacy in clinical practice have been disappointing. A major limitation might be that tools currently used to measure ‘inflammation’ are insufficiently precise and do not provide information about disease site, activity, or discriminate between functionally important activation pathways. The challenge, therefore, is to make measures of inflammation that are more meaningful, and which can guide specific targeted therapies. In this Review, we consider the roles of inflammatory processes in the related pathologies of atherosclerosis and acute myocardial infarction (AMI), by providing an evaluation of the known and emerging inflammatory pathways. We highlight contemporary techniques to characterize and quantify inflammation, and consider how they might be used to guide specific treatments. Finally, we discuss emerging opportunities in the field, including current limitations and challenges that are the focus of ongoing study.

Inflammation and its failure to resolve are firmly established as central to the development and complications of several cardiovascular diseases[1–3]. Elevated levels of markers of inflammation, such as C-reactive protein (CRP) and serum amyloid A (SAA), have been shown to be predictive of future cardiovascular events across a range of clinical settings[4–6]. The role of the innate immune system in the pathogenesis of cardiovascular diseases has been an area of particular focus, with the appreciation that targeting innate immune function

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in experimental models can variously attenuate disease progression and injury, and promote healing[7–10].

Processes of inflammation contribute to a broad range of cardiovascular diseases; however, in this Review, we consider the roles of inflammatory processes in the related pathologies of atherosclerosis and acute myocardial infarction (AMI), by providing an evaluation of known and emerging inflammatory pathways that are thought to be central to their pathogenesis, and the consequences of their activation. We highlight contemporary techniques to characterize and quantify inflammation, and consider how these might be used to guide specific treatments. Finally, we discuss emerging opportunities in the field, including current limitations and challenges that are the focus of ongoing study.

As interest in this field has expanded, one of the main challenges has been the use of the term ‘inflammation’ to cover a wide variety of processes and observations, some of which are very poorly defined. For instance, ‘inflammation’ can include observation of nonspecific changes in acute-phase proteins (such as CRP and SAA) that are produced in the liver, remotely from the site of pathology, but also encompasses a nuanced understanding of local molecular and cellular processes in the heart or vessel wall that are intimately involved in disease causation. Therefore, our approach is to try to identify and elaborate on mechanistically important inflammatory processes, to link these to clinically relevant biomarkers, and to focus on pathways that are therapeutically tractable.

A major limitation has been that the biomarkers that are currently used to gauge inflammation are crude and nonspecific (for example, CRP). They reflect the downstream consequences of inflammatory activity, but do not provide information relating to the site(s) of activation and cannot be used to discriminate functionally important activation pathways. The challenge, therefore, is to make measures of inflammation that are meaningful to the pathology of interest and which can guide specific, targeted therapies.

Inflammation and cardiovascular disease

Following the first observations of the role of inflammatory processes in cardiovascular disease[3], our understanding of these complex processes has greatly expanded. Their complexity and central roles in the pathogenesis of cardiovascular disease are now at least partially appreciated. Indeed, the term ‘inflammation’ is now used to describe a broad range of processes including those at the site of disease, in the blood, at remote sites, and also as downstream sequelae of disease — with each representing very different biology. Importantly for clinical purposes, techniques have not evolved to enable the reliable, specific, and accurate characterization of these processes.

Acute myocardial infarction

Pathogenesis

AMI most commonly results from the acute rupture of a coronary atherosclerotic plaque resulting in the rapid formation of thrombus in the infarct-related epicardial artery and loss of blood flow distal to the site of occlusion[11]. Other causes include plaque erosions and

calcific nodules[12]. Myocardial ischaemia results in the activation of a well-orchestrated immune response, both locally at the site of injury, but also in the circulating blood and at remote sites (FIG. 1). These processes are important as both mediators of injury and subsequently in repair and recovery[2]. Elevated plasma levels of nonspecific indicators of inflammation (such as high-sensitivity CRP and IL-6) have indicated that their continued upregulation is associated with worse outcomes[13,14].

Inflammatory pathways locally in the myocardium—Myocardial ischaemia leads to cardiomyocyte injury and, if prolonged, death, with release of their intracellular contents and changes to the extracellular matrix. This process results in endogenous signals that trigger a cytokine 'burst', activation of platelets that mediate leukocyte activation and their tissue infiltration[15], and the rapid recruitment of neutrophils to the myocardium, which following degranulation cause direct injury to endothelial cells and production of reactive oxidation species (ROS), proteases, and cytokines[16]. In response, endothelial cells increase expression of leukocyte and platelet adhesion molecules and compromise of intercellular tight junctions facilitates the binding and transmigration of circulating monocytes[17] that coincides with a reduction of *in-situ* neutrophils secondary to a combination of local cell death and egress from the site[18,19].

These endogenous signals are often referred to as 'danger associated molecular patterns' (DAMPs) and include heat-shock proteins[20], high-mobility group protein B[21], low-molecular hyaluronic acid[22], and fibronectin fragments[23], which can all activate innate immune pathways, including Toll-like receptors (TLRs)[24], nucleotide-binding domain leucine-rich repeat containing receptors (NLRs), the complement cascade, and reactive oxygen species (ROS) production.

Of the TLRs, cardiomyocyte expression of TLR4, also a major feature of activated macrophages, is substantially increased in response to DAMPs[25]. TLR4 deficiency is associated with decreased infarct size, a reduction in systemic inflammation[26], and improved left ventricular remodelling[27] in experimental models. In patients after AMI, TLR4 activation in monocytes (the precursors of macrophages) is associated with the development of heart failure[28]. Conversely, TLR2 deficiency is associated with reduced myocardial fibrosis after AMI and improved left ventricular remodelling[29]. TLR4, therefore, seems to be an important component in the initial inflammatory response, and TLR2 central to repair and left ventricular remodelling[30].

Among the various NLRs, the NLRP3 (NACT, LRR and PYD domains-containing protein 3 inflammasome complex has been identified as an important mediator of injury after myocardial ischaemia and includes NLRP3, apoptosis-associated speck-like protein containing a CARD, and the cysteine protease caspase 1[31]. The activated NLRP3 inflammasome binds and activates caspase 1 that converts interleukin-1β (IL-1β) to its active form, and is central to the activation of other cytokines[32]. The complement system consists of cell membrane (and plasma) proteins, many of which circulate as pro-enzymes. Complement activation has an important role in mediating neutrophil and monocyte recruitment acutely to the injured myocardium by the downstream complement effectors C3a and C5a, and the release of monocyte chemotactic protein 1 (also known as C-C motif

chemokine 2; CCL2 by vascular smooth muscle cells, microvascular endothelial cells, and macrophages[33–36].

ROS are also released immediately by ischaemic cardiomyocytes after AMI, and induce leukocyte chemokine upregulation[37], resulting in an increased capacity of endothelial intercellular adhesion molecule 1 (ICAM1) ligands to bind to neutrophils[38], complement activation[39], and the recruitment of additional leukocyte subpopulations that subsequently become activated. Cytokines also contribute to the pathophysiology of AMI, with tumour necrosis factor (TNF), IL-1β, and IL-6 all having central roles. For example, TNF can promote the release of other proinflammatory chemokines and adhesion molecule synthesis in the infarcted myocardium, augmenting further leukocyte infiltration[40]. TNF has interestingly also been shown to be protective of apoptosis, though its net actions are thought to be deleterious in AMI[41]. IL-1β similarly mediates injurious processes and leukocyte infiltration[42]. IL-6 is also upregulated after myocardial ischaemia and modulates its actions through activation of the JAK/STAT (Janus tyrosine kinase/signal transducer and activator of transcription) cascade[43,44].

Activation of remote inflammatory pathways and processes—Initially, ‘classical’ inflammatory monocyte subsets are mobilized and recruited from the blood, but in experimental models, there follows a rapid and massive mobilization of monocytes from the spleen and bone marrow[45], which is also likely to occur in humans[46]. The precise stimuli to effect remote monocyte mobilization are incompletely understood, but include β_3-adrenoceptor activation in the bone marrow[47] and local action of CCL2 via C-C chemokine receptor type 2 (CCR2)[17].

After recruitment to the injured myocardium, blood-derived macrophages in synergy with resident macrophages initially function to scavenge necrotic debris and are active in local proteolysis and phagocytosis[48]. This stage is followed by a second phase consisting of the accumulation of nonclassical macrophages promoting reparative processes, such as angiogenesis and extracellular matrix deposition[17,49]. The precise roles of the various cardiac macrophage subsets either in the steady state or after AMI remain largely unknown. Additionally, the macrophages also communicate with other cell types (such as B cells, neutrophils, and mast cells) to exert further local and remote actions in both tissue injury and repair[50,51]. Leukocyte numbers then return to baseline within 2 weeks in both the heart and blood.

Role of adaptive immunity—In contrast to the innate immune system (consisting primarily of monocyte-derived cells), which provides immediate defence against tissue injury by responding to general factors without pre-exposure, the adaptive immune system (that is, lymphocytes) provides the capacity to react to specific antigens after exposure, and to maintain memories of them.

In AMI, lymphocytes constitute a very small proportion of the infiltrating cell population[52]. However, the higher systemic frequency of activated T lymphocytes in patients with AMI compared with those with stable angina[53,54] suggests that the adaptive immune system is also activated. In particular, $CD4^+CD28^{null}$ T-effector cells are increased in the peripheral

blood of patients after AMI[55], and release proinflammatory cytokines (such as interferon-γ), activating monocytes and tissue macrophages, with the level of increase positively correlated with the risk of future acute coronary events[56]. Conversely, $CD4^+CD25^+$ regulatory T cells (T_{REG}) release anti-inflammatory cytokines, such as IL-10. After AMI, there seems to be an imbalance in T-cell subsets, with a reduction in T_{REG} numbers[57]. This imbalance might indicate that, in these individuals, the counter-regulatory response to the activation of effector T cells is impaired. T lymphocytes have also been shown to have a role in recovery after AMI in experimental models, with the activation of $CD4^+$ T cells required for optimal wound healing and remodelling of the left ventricle[52].

Evidence of inflammation at remote sites—The activation of the immune system after AMI also results in general systemic inflammation, as evidenced by increased plasma levels of inflammatory cytokines, which are positively correlated with adverse outcomes[5]. Observational human studies have also demonstrated that plasma levels of TNF are strongly associated with the occurrence of reperfusion injury after recanalization of the infarct-related artery[58], and with an increased risk of death[59]. Levels of TNF, IL-1, and IL-6 are significantly elevated in patients with AMI complicated by cardiogenic shock when compared with patients with uncomplicated AMI[60]. The extent to which systemic levels of cytokines give meaningful insight into the state of local inflammatory pathways is uncertain, but the complexities of the local processes of inflammation in injury and repair seem to argue against a predictive approach dependent on a single circulating reporter biomarker.

Targeting of biological processes

The ability to target therapeutically these inflammatory central biological processes could potentially attenuate myocardial injury, augment repair and recovery, and reduce the risk of future events. For example, in experimental myocardial infarction, the targeting of inflammatory ‘classical’ monocytes to reduce the total number of monocytes recruited to infarcted, inflamed myocardium by splenectomy or by the administration of angiotensin-converting-enzyme inhibitors has been shown to result in a reduction in infarct size and improved left ventricular function[61]. Similarly, targeting their recruitment with the administration of CCR2-siRNA delivered by nanoparticles also resulted in a reduction in the number of recruited monocytes, with a consequent reduction in infarction size and improved left ventricular function[8,62]. On the basis of these (and other) promising experimental data, a number of agents have been or are currently being investigated in clinical trials (TABLE 1).

To date, however, clinical efficacy has not been demonstrated with any agent. The reasons for this lack of success are likely to be multifactorial. One possible reason might be that the experimental model that has been used in initial therapeutic evaluation is not representative of human disease. Secondly, a common theme to each of these studies is that, in general, enrolment has been on the basis of the crude diagnosis of AMI, without attempts at stratification or ‘mechanistic staging’. As discussed above, the underlying pathogenesis of AMI is a complex interaction between multiple biological processes. In combination with the historical ineffectiveness of general anti-inflammatory drugs in this setting, a ‘one size fits all’ approach might not be appropriate to determine efficacy, and a more-targeted approach to identify patients who would benefit from specific interventions is required.

Molecular techniques—The quantification of a number of different blood components has commonly been used to measure ‘inflammatory’ processes and has included soluble adhesion molecules (such as ICAM1, P-selectin, and vascular cell adhesion molecule 1 [VCAM1]), cytokines (such as IL-1β, IL-6, and TNF)[63], SAA[64], CRP[65], and peripheral white cells[66,67]. With greater understanding of the underlying biology, it is increasingly apparent that these markers actually represent very different biological processes, can be ‘upstream’ or ‘downstream’ of the pathological process of interest, might not be specific, and provide little indication of activity, site, or status. Levels of high-sensitivity CRP, for example, have been observed to be acutely elevated after ischaemic events[68] and, when chronically elevated, have been shown to be associated with an increased risk of future vascular events[69,70]. Mendelian randomization studies, however, have indicated that no causative relationship exists between single nucleotide polymorphisms associated with elevated CRP levels and coronary heart disease[71], and the specific targeting of the molecule was not associated with benefit in preclinical studies[72].

One approach to improve characterization of circulating immune cells after AMI has been to use flow cytometry-based techniques. This powerful approach has enabled more-precise phenotyping of leukocyte populations (such as identification of monocyte subsets) and considerable heterogeneity in both phenotype and function has been observed. Using monocytes as an example, flow cytometry can be used to phenotype the total cell population into subsets on the basis of the cell-surface expression of CD14 and CD16[73], with $CD14^{++}CD16^{-}$ monocytes that express CCR2 thought to be ‘inflammatory’. However, even with these more sophisticated phenotyping techniques, measuring circulating levels of individual soluble biomarkers or the identification of changes in crude counts of leukocyte subtypes is unlikely to provide sufficiently granular information to inform targeted drug selection.

To this end, transcriptomic analysis of circulating monocytes after AMI has identified patterns of gene expression that are conserved between mice and humans[30]. Although not yet formally tested, defined patterns of gene expression in downstream effector cells might plausibly give a more-precise insight into the activity of relevant pathways than measuring soluble biomarkers of uncertain origin and or site of action.

In AMI, circulating monocytes display patterns of gene expression in common with ‘inflammation’ and ‘mitosis’, with CD14, IL-1, and TLR2 identified as highly relevant components of the response using gene-set-enrichment techniques[30,74]. Analogous approaches have been used to validate preclinical models of disease, identify new therapeutic targets[30] and, clinically, gene sets in circulating leukocytes have been used to diagnose cardiac transplantation rejection[75]. Such approaches also borrow from the field of oncology, where they have enabled the reclassification of many malignancies on the basis of molecular signatures that have different prognostic and therapeutic responses[76]. In breast cancer, microarray analysis of frozen breast tissue has been used to develop a 70-gene panel called Mammaprint, which can provide a prognostic measure of the likelihood of distant metastasis within 5 years of completing treatment[77]. Technological advances have enabled single-cell analysis to provide information about the heterogeneity of the human bone marrow cell population against which diseased states and response to drug treatment could

be compared as a basis of mechanistic studies[78]. Complementing transcriptome profiling, proteomic studies have also been used to investigate changes in serum protein levels and, using this approach, haptoglobin has been identified a possible prognostic biomarker after AMI[79]. Similarly, metabolomics[80] and lipidomics[81] methods, although in their infancy, can be also used to identify novel therapeutic targets, and possibly monitor disease activity. Future approaches might enable incorporation of complex, integrated datasets spanning multiple -omics domains and with much greater sampling frequency to allow higher resolution of temporal changes than is currently possible.

Extracellular vesicles have also gained interest as both a diagnostic tool and a potential therapeutic target. They have been shown to be important in cell-to-cell signalling, including in the context of inflammation. Extracellular vesicles carry a variety of cell-derived molecules including proteins[82], mRNA, and microRNA[83], and include exosomes (30–100 nm), multivesicular bodies (also called ectosomes or microparticles: 100–1,000 nm)[84] and apoptotic bodies (1–5 μm)[85,86]. Extracellular vesicles are continually produced by all cell types and, in the setting of AMI, those from endothelial cells and platelets have been shown to have roles in homeostasis of the cardiovascular system including coagulation[87] and tissue repair[88]. After AMI, levels of multivesicular bodies increase[89] and are likely to exert their actions through mediating cell-to-cell signalling via transport of bioactive proteins between cells[90] resulting in direct activation of target cells[91,92]. Several miRNAs found in extracellular vesicles might have roles in cardiovascular disease[93], for example miR-150 released from monocytes that enhances cell migration[94]. Extracellular vesicles might, therefore, be a tool to predict future risk[95], determine disease activity, be a therapeutic target, or provide templates for the engineering of vesicles that consist of a payload with a specific, targeted function.

Imaging techniques—Measuring blood biomarkers, in however sophisticated a way, cannot provide precise information on the distribution of pathology or accurately assess its anatomical extent. In combination with molecular tools, imaging modalities can be employed to determine disease activity and site. MRI is commonly used in the clinical setting and provides information about location and extent of acute myocardial injury, with quantification of myocardial oedema and also of irreversible injury with late gadolinium enhancement indicating myocardial scar.

The administration of ultra-small particle of iron oxide seems to improve specificity with which the location of infiltrating macrophages can be identified[96,97]. In experimental models of AMI, the application of ^{18}fluorodeoxyglucose (^{18}FDG) PET in combination with MRI further improved localization of infiltration of metabolically active leukocytes in experimental models[98], with cellular imaging studies supporting these conclusions[99]. Clinical studies have demonstrated similar findings, which provide the capacity to quantify metabolic activity in the myocardium after infarction[100], monitor continued disease activity, and might also provide a prognostic marker of functional outcome[101].

Further information that we have termed ‘mechanistic staging’ might be obtained from quantitative molecular imaging techniques that target important known processes, ideally on a mechanistic pathway that can be paired to a specific therapeutic agent or intervention. For

example, quantitative molecular imaging has been described for VCAM1 and P-selectin using ‘leukocyte-mimetic’ particles to examine extent and distribution of endothelial activation[102,103], which has also been applied to measure the ‘imprint’ of previous ischaemia[104]. An alternative approach has been to image proteases that are critical in the pathogenesis of disease and has been applied to preclinical models[105] and humans after AMI[100]. Therefore, by utilizing a combination of molecular and imaging techniques, it might be possible not only to identify therapeutic targets, but also to identify patients with ‘active’ disease, localize sites of activity, and monitor response to therapy.

Atherosclerosis

Pathogenesis

Atherosclerosis is a chronic inflammatory process[106]. Immune cells are important mediators from the earliest fatty streaks to late-stage complex plaques. As knowledge of the immune processes in atherogenesis has expanded, opportunities for therapy through their manipulation or modification have become apparent. However, tools to determine disease severity and activity remain fairly crude.

Endothelial cells, lymphocytes, smooth muscle cells, monocytes, and macrophages are all involved in the pathogenesis of atherosclerosis from earliest foam cell formation through to development of advanced plaques (FIG. 2).[107] Initial activation of the endothelium from disruptions to normal shear stress result in an increase in permeability to lipoproteins and upregulation of adhesion receptors[108], and facilitate deposition of lipid from apolipoprotein B (apoB)-containing lipoproteins in the subendothelial space. Endothelial activation also promotes recruitment of circulating monocytes that originate from either the bone marrow[109] or the spleen[45]. Monocyte adhesion depends on the upregulation of a number of cell-adhesion molecules on the luminal surface of the endothelium,[110] notably, ICAM1, P-selectin, and VCAM1; followed by the expression of three major chemokine families, CCR2, CCR5, and CX3C chemokine receptor 1 (CX3CR1), that facilitate the transmigration process[111,112]. After recruited from the spleen and bone marrow[113], monocytes can terminally differentiate into macrophages[114], differentiate and locally proliferate into distinct functional phenotypes[115], or directly influence the phenotype of *in situ* cells (for example, lesional macrophages)[116]. Lipids from retained apoB-containing lipoproteins are taken up by activated macrophages by a number of processes, including phagocytosis of aggregated LDL[117], pinocytosis of LDL[118], uptake of modified apoB-lipoproteins by scavenger receptors, and uptake by CD36. Ongoing lipid accumulation induces their transformation into macrophage-derived foam cells. As the foam cell population grows within lesions in the arterial wall, the rate of accumulation exceeds the rate of clearance[119], and eventually the foam cells coalesce into a lipid-rich necrotic core.

A number of secreted cytokines are also implicated in the pathogenesis of atherogenesis. Cells of both the innate (macrophages) and adaptive (T and B lymphocytes, dendritic cells) immune systems can modulate the local inflammatory milieu. Depending on their local environment, T lymphocytes can be stimulated to secrete proinflammatory T_h1 cytokines such IL-1, IL-6, and TNF; or T_h2 cytokines such as IL-4, IL-10, and IL-13, which promote resolution of inflammation. IL-1 and TNF signalling is primarily mediated by p38 mitogen-

activated protein kinase (MAPK) / nuclear factor (NF)-κB pathways[120]. Conversely, activation of IL-6 signals via signal transducing protein gp130, which activates JAK1 and STAT1 and 3[121] resulting in the activation of endothelial cells and macrophages to produce adhesion molecules and chemokines[122].

The physical properties within the plaque can also have an important role in the propagation of disease. Neovascularization in human plaques originating from the vasa vasorum is thought to contribute to intraplaque haemorrhage, which not only accelerates plaque expansion and inflammation, but also predicts future plaque rupture[123,124]. Angiogenesis is driven by vascular endothelial growth factor, which is a major downstream target of hypoxia inducible factor (HIF) 1α that is induced by a hypoxic environment, especially within the large lipid-rich necrotic core present in advanced atheroma[125,126]. Hypoxia and HIF1α expression has been shown *in vitro* to alter macrophage lipid handling and suppress cholesterol efflux via ABCA1 (the ATP-binding cassette subfamily A member 1) in both mouse[127] and human[128] macrophages. Moreover, *in vitro* and *ex vivo* experiments have implicated hypoxia and HIF1α in driving enhanced glucose uptake, metabolic activity, and polarization of macrophages in human atheroma[129].

In 2003, Rong and colleagues[130] reported that cholesterol-loaded vascular smooth muscle cells lost their differentiation markers and instead expressed macrophage markers. A number of experiments in mice, and notably humans[131–133], have produced *in vivo* data consistent with these phenomena: 30–40% of cells classified as plaque macrophages were of vascular smooth muscle cell origin. It is too early to discern the contribution of these cells to the pathological process during atherogenesis, but at least in cell culture, they do not share the molecular signature or the functional properties of activated macrophages[134].

Targeting of biological processes

The ability to characterize specifically and target the biological processes central to the pathogenesis of atherosclerosis might prevent progression of disease and development of vulnerable plaque. Even more exciting would be the regression of disease, a possibility suggested by a number of animal models and limited clinical data[135]. Although the targeting of various pathways in experimental models of atherosclerosis has been associated with benefit, clear efficacy in clinical studies has not yet been demonstrated for any single agent (TABLE 2). This apparent lack of efficacy might be related to poor representation of human disease by preclinical models used for evaluation therapeutic agent, or a failure to target patients rationally based on mechanistic staging (disease stage, activity, and severity).

Molecular techniques—The relationship between high-sensitivity CRP (which has been used as a surrogate biomarker for ‘inflammation’) and atherosclerosis has been the subject of much interest. A number of studies and meta-analyses have demonstrated an association between elevated CRP levels and an increased risk of cardiovascular disease[65]. Indeed, a reduction in CRP in the context of a statin trial was noted to be an independent predictor for outcome[136]. In the JUPITER study[137] that used high-sensitivity CRP to identify patients at increased risk of vascular events (without elevated LDL-cholesterol levels), patients with high basal high-sensitivity CRP levels were found to be at a significantly increased risk of

future vascular events. Although the association between high-sensitivity CRP and risk is well established, what exactly this biomarker represents is currently unclear.

Similarly, other circulating proteins including VCAM1[138], ICAM1[139] and P-selectin[140] have been measured in the plasma of patients and have been shown to be associated with the level of atherosclerosis. But again, in common with high-sensitivity CRP, what these 'downstream' measures represent in uncertain.

The application of cellular biomarkers has been of interest in atherosclerosis and, by virtue of their central role in pathogenesis, circulating monocytes have been the focus of research[141]. Using flow cytometry, the number of circulating $CD14^{+}CD16^{++}$ monocytes has been shown to be inversely related to plasma HDL levels, and $CD16^{+}$ monocytes are proportional to the severity of atherosclerosis[142], whereas a reduction in the monocyte subpopulation is associated with a reduction in intima–media thickness[143]. Again, these measures do not provide any information about to the underlying pathogenesis of disease. To elucidate the functional characteristics of these 'inflammatory' cells at the level of atherosclerotic plaque, techniques such as laser-capture microdissection (LCM) have been successfully employed to procure intralesional cells in a cell-type-specific and location-specific fashion. In mice, immuno-LCM coupled with downstream gene or protein expression analysis has been used to study lesional macrophages in various stages of atherogenesis, including atherosclerosis regression. The inflammatory state of plaque macrophages was shown to be dynamically regulated as the severity of disease varied[144]. In humans, studies using LCM on surgical plaque explants has also identified genes implicated in lipid metabolism (such as *FABP4* and *LEP*) and activation of the adipokine/peroxisome proliferator-activator receptor (PPAR) signalling pathways[145], as well as a repertoire of inflammatory gene 'signatures'[146], that might be important in mechanistic staging. Early findings utilizing this approach seem to corroborate observations from genome-wide association studies as well as plaque compositional changes in plaque revealed by high-resolution MRI Chai, personal communication. Circulating multivesicular bodies derived from platelets, monocytes, and red blood cells have also been characterized specifically with regard to the miRNA that they contain, and have been shown to have roles in VCAM1 inhibition, activation of plaque macrophages, and in cell-to-cell signalling[147] that are central to atherosclerosis progression[148].

Imaging techniques—To overcome some of the limitations of molecular tools, imaging techniques can provide better characterization of pathogenesis while also providing information relating to the anatomical site of disease by specifically targeting components of the process of interest. Conventional imaging modalities (such as CT and MRI) focus on the presence and extent of disease with limited qualitative information (for example, plaque components), but in combination with molecular contrast agents with specific targets, provide the capacity to determine the presence and site of biological processes of interest. For example, MRI after the administration of recombinant HDL particles containing gadolinium[149] or ultra-small particles of iron oxide[150] can provide information about macrophage infiltration in plaque, or with microparticles of iron oxide targeting VCAM1 can provide information about endothelial activation — a critical step in atherosclerosis pathogenesis[103]. The use of ^{18}FDG-PET in combination with MRI can also improve

characterization of plaque ‘activity’, with uptake indicating macrophage activity[151]; high carotid FDG uptake is an independent predictor of cardiovascular events in asymptomatic patients[152]. The use of other tracers, such as ^{18}F-NaF, have also been used to identify microcalcification in the identification and localization of high-risk coronary plaques[153]. Co-registration of fluorescence molecular tomography and CT with the aid of protease sensors has also been demonstrated to be useful in imaging atherosclerosis in a murine model, and has also been applied to determine response to therapy[154].

Intravascular fluorescence imaging techniques have increasingly been utilized in clinical practice with intravascular ultrasonography and optical coherence tomography (OCT) increasingly used to identify atherosclerotic plaque and diagnose rupture, erosions, calcific nodules, and the presence and distribution of calcification, lipid, and fibrous tissue[12]. Targeted molecular agents have been used to identify lipid-rich, metabolically active plaque in rabbit arteries[155,156]. Dual-modality OCT and near-infrared autofluorescence (NIRAF) using a specialized OCT–NIRAF catheter has been shown to be feasible and safe in humans and, indeed, NIRAF enhances the sensitivity to detect vulnerable thin-cap fibroatheroma[157].

In summary, the combination of molecular tools and imaging techniques provides the capacity to characterize active inflammatory processes, the sites of disease, and also enables longitudinal monitoring of disease progression and response to therapy.

Future implications

In combination with improved understanding, the ability to characterize and mechanistically stage underlying pathological biological processes will enable a more-tailored approach to the management of patients presenting with, or at high risk of, cardiovascular disease. New therapies will be focused on targeting specific pathological processes, especially those related to inflammation, at the appropriate stage of disease and on the basis of current activity. The same tools can also be used to monitor response to treatment[158] (including toxicity[159]). Some preclinical examples include studies in which either IL-13 (which polarizes macrophages to the M2 state) or a ligand of resolving receptors on macrophages were effective in retarding the progression of atherosclerosis[160,161].

New biomarkers and imaging techniques could also be applied when evaluating the efficacy of existing and new therapies and be used as surrogate clinical end points (as opposed to the ‘hard’ clinical end points), resulting in increased statistical power of studies, a reduction in the number of patients that need to be enrolled, reduced cost, and a shorter timescale. Finally, the majority of reports to date using many of these novel tools have demonstrated only ‘proof of concept’. Future studies demonstrating clinical benefits associated with their application in conjunction with technological advances (for example, platform integration and miniaturization) enabling the feasibility of point-of-care testing are required to make these interventions more broadly applicable to patients beyond research centres[162].

Conclusions

A number of biological processes contribute to the pathogenesis of cardiovascular disease. These have historically all been termed ‘inflammatory’, but this oversimplification does not

accurately convey the heterogeneity of the processes involved. Experimental data suggest that the specific targeting of multiple biological processes can result in attenuation of injury and augmentation of reparative processes. However, despite these very promising results, clear efficacy of anti-inflammatory therapeutics has not been demonstrated in clinical practice. Currently available tools to measure and monitor inflammation are often nonselective, represent downstream sequelae of inflammation, and do not provide any information about presence, extent of activity, or location. By using improved informative techniques to characterize inflammation, a more selective, rational targeted therapeutic approach can be employed. Future efforts will focus on these areas, with a goal of personalized medicine to complement existing treatment strategies, and the ultimate aim of improving clinical outcomes.

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Biographies

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Key points

- Inflammation and its failure to resolve are firmly established as central to the development and complications of several cardiovascular diseases
- Targeting of inflammatory processes in experimental models has been demonstrated to be beneficial in attenuating myocardial and arterial injury, reducing disease progression, and promoting healing, but clinical translation has been disappointing
- Current tools to measure ‘inflammation’ are nonspecific and represent downstream sequelae of biological processes, but provide little insight into disease state, site, or activation pathways
- Contemporary molecular techniques (such as proteomics and gene-expression analysis) improve our ability to characterize underlying biological processes, and identify activation pathways as biomarkers and as a basis to develop new therapeutics
- Noninvasive imaging tools enable the identification of activation of specific pathways and their sites, and can be used to monitor response to therapy

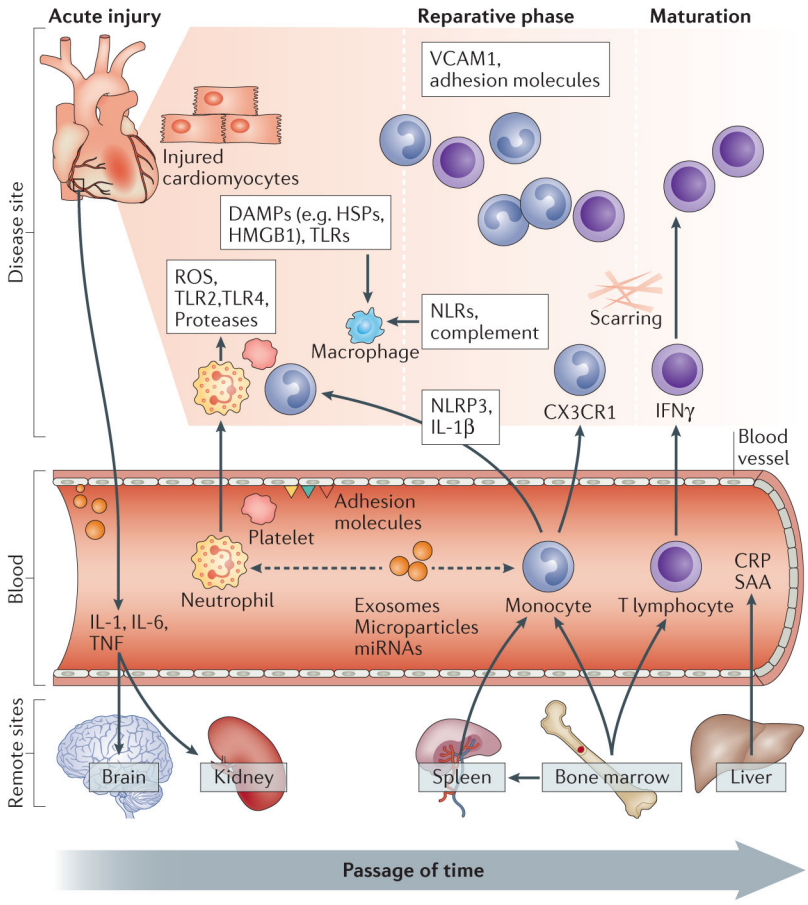

Figure 1.

Biological pathways central to the pathogenesis of acute myocardial infarction (AMI). Immediately following AMI, a number of local processes are activated with release of reactive oxygen species and cytokines with infiltration of circulating neutrophils and monocytes resulting in acute myocardial injury. Simultaneously a number of remote sites are also activated (e.g. spleen, bone marrow) via signalling pathways that result in further activation of the immune system and injury. Following this, a reparative phase ensues predominantly mediated by monocytes and T-lymphocytes resulting in tissue repair and recovery with upregulation of processes involved in angiogenesis and extracellular matrix deposition. Abbreviations: ROS: reactive oxygen species; TLR: toll-like receptors; DAMPS: damage associated molecular patterns; HSP: heat shock proteins; HMGB1: high mobility group box 1 protein; VCAM: vascular cell adhesion molecule; NLR: NOD-like receptor; NLRP3: NOD-like receptor family pyrin domain containing 3; IL: interleukin; TNF: tumour necrosis factor; IFN: interferon; CX3CR1: CX3 chemokine receptor 1; miRNA: micro ribonucleic acid; CRP: C reactive protein; SAA: serum amyloid A.

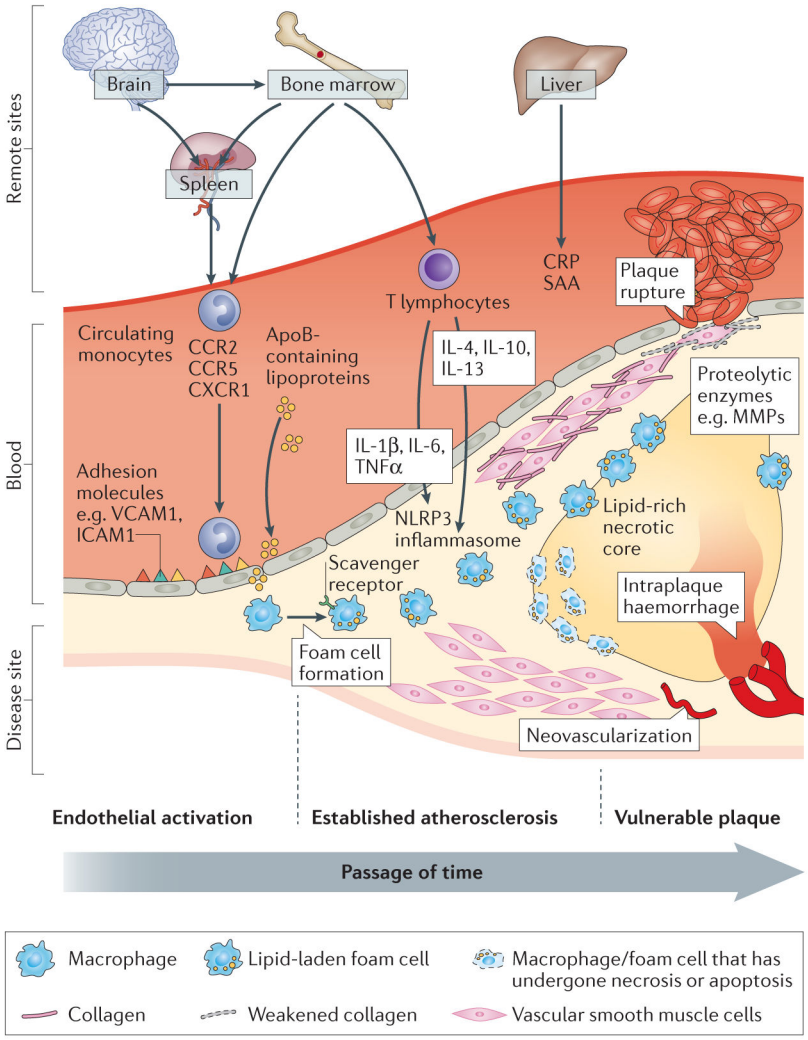

Figure 2.

Biological processes central to the pathogenesis of atherosclerosis. Endothelial cells, lymphocytes, smooth muscle cells, monocytes, and macrophages are all involved in the pathogenesis of atherosclerosis from earliest foam cell formation through to development of advanced plaques. Initial activation of the endothelium from disruptions to normal shear stress result and facilitate deposition of lipid in the subendothelial space. Endothelial activation also promotes recruitment of circulating monocytes where they terminally differentiate into macrophage or differentiate and locally proliferate into distinct functional phenotypes. Activated macrophages take up lipid and results in their transformation into macrophage-derived foam cells. As the foam cell population grows within lesions in the arterial wall, the rate of accumulation exceeds the rate of clearance, and eventually the foam cells coalesce into a lipid-rich necrotic core. Abbreviations: VCAM: vascular cell adhesion molecule; ICAM: intercellular cell adhesion molecule; CCR: chemokine-chemokine receptor; CX3CR1: CX3 chemokine receptor; IL: interleukin; TNF: tumour necrosis factor; ApoB: Apoliporotein B; CRP: C reactive protein; SAA: serum amyloid A; MMP: matrix

metalloproteinase; NLRP3: NOD-like receptor family pyrin domain containing 3; VSMC: vascular smooth muscle cells.

Table 1

Anti-inflammatory agents in acute myocardial infarction

Target Drug	Preclinical studies			Clinical studies		
	Animal model	**Outcomes**	**Result**	**Population**	**Outcomes**	**Result**
IL-6 Tocilizumab	Mice	Improved LV remodelling[163]	✓	117 patients with NSTEMI	Reduction in troponin T level[164]	✓
IL-1β Canakinumab	Mice	Improved LV remodelling and reduction in cardiomyocyte apoptosis after AMI[165] Impaired LV remodelling and suppression of pro-collagen gene expression[166]	–	NA	NA	NA
IL-1R Anakinra	Mice and rats	Improved LV remodelling and reduction in cardiomyocyte apoptosis after AMI[167]	✓	182 patients with NSTEMI 20 patients with STEMI	Reduction in hsCRP at 14 days, same event rate at 30 days, significant excess in MACE in IL-1Ra group at 1 year[168] No difference in clinical outcomes, reduction in incidence of heart failure with treatment[169]	-
Tumour necrosis factor Etanercept	Rats	Improved LV ejection fraction after AMI, significant reduction in infarct size[170]	✓	26 patients with AMI	Reduced neutrophil count and IL-6 level, increased monocyte–platelet aggregates[171]	–
MMP-2 PG-116800	Mice	Reduction in leukocyte infiltration and cardiac rupture, and improved survival rate[172]	✓	203 patients with STEMI and low LV ejection fraction	No improvement in LV ejection fraction at 6 months[173]	–
MMP-2, MMP-9 Doxycycline	Mice	Reduction in LV dilatation after AMI[174]	✓	110 patients with STEMI	Reduction in ventricular dilatation at 6 months[175]	✓
C-reactive protein	Pigs	Removal of C-reactive protein reduced infarct size[176]	✓	NA	NA	NA
P-selectin Inclacumab	Dogs	Significant reduction in infarct size and neutrophil infiltration[177]	✓	544 patients after NSTEMI	Reduction in biomarkers after PCI[178]	✓
α_1-Antitrypsin	Mice	Reduction in caspase 1 activity, preservation of viable myocardium, and improved LV remodelling[179]	✓	10 patients with STEMI	Reduction of hsCRP level[180]	✓
C3/C5 (complement) Pexelizumab	Rats	Reduction in myocardial infarction size by 44%[181]	✓	5,745 patients with STEMI	No difference in outcomes with treatment[182]	–
Integrin / CD18	Dog Primate	Nearly 50% reduction in infarction size[183] Reduction in infarct size[184]	✓	420 patients with STEMI 394 patients with STEMI	No difference in infarct size[185] No difference in coronary blood flow, infarct size, or rate of ECG normalization[186]	–
Immunoglobulin	Rats	No improvement in survival or LV function[187]	–	62 patients with STEMI	No effect on LV remodelling at 6 months[188]	–
P38 MAPK Losmapimod	NA	NA	NA	3,503 patients with AMI	No difference in outcomes[189]	–

AMI, acute myocardial infarction; ECG, electrocardiogram; hsCRP, high-sensitivity C-reactive protein; LV, left ventricular; MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; NA, not available; NSTEMI, non-ST-segment elevation myocardial infarction; PCI, percutaneous coronary intervention; STEMI, ST-segment elevation myocardial infarction; ✓, positive study; –, neutral study; X, negative study.

Table 2

Anti-inflammatory agents in atherosclerosis

Target Drug	Preclinical studies			Clinical studies		
	Animal model	**Outcomes**	**Result**	**Population**	**Outcomes**	**Result**
IL-1β Canakinumab	Rats	Reduction of carotid neointima formation after injury[190]	✓	17,200 patients with MI 189 patients with type 2 diabetes mellitus and pre-existing vascular disease	First occurrence of MACE, change in carotid plaque burden (ongoing)[163] No effect on vascular structure and function, but trend towards retarded progression of atherosclerosis by MRI[164]	–
Recombinant IL-1ra	*Apoe*$^{-/-}$ mice	Reduction in fatty lesion formation[191]	✓	NA	NA	NA
Tumour necrosis factor Etanercept, infliximab	*Apoe*$^{-/-}$ *Tnf*$^{+/-}$ mice	Reduction in atherosclerosis plaque progression[192]	✓	2,126 patients with rheumatoid arthritis (meta-analysis)	Trend towards reduction in all cardiovascular events[193]	✓
IL-12 subunit p40 Ustekinumab	*Apoe*$^{-/-}$ *Il12*$^{-/-}$ mice	Reduction in atherosclerosis[194]	✓	3,117 patients with psoriasis (pooled analysis)	Overall cardiovascular events were similar between treatment and placebo groups[195]	–
IL-17A Secukinumab	*Apoe*$^{-/-}$ mice	Reduction in atherosclerosis lesion area[196]	✓	3,993 patients with psoriasis (pooled analysis)	Overall cardiovascular events were similar between treatment and placebo groups[197]	–
p38 MAPK (Losmapimod)	*Ldlr*$^{-/-}$ mice	Reduction in foam cell formation and expression of CCL2 and VCAM1[198]	✓	99 patients with atherosclerosis receiving stable statin therapy	Reduction in vascular inflammation in most inflamed areas and reduction in inflammatory biomarkers[199]	✓
CC-chemokines 35K protein to inactivate CC-chemokines	*Apoe*$^{-/-}$ mice	Reduction in atherosclerosis, macrophage content, and lipid content in plaque[200]	✓	NA	NA	NA
P-selectin Inclacumab	Rabbits	Reduced intimal hyperplasia after injury[201]	✓	380 patients with saphenous vein graft disease	No effect on saphenous vein graft disease progression[202]	–
VCAM1	*Apoe*$^{-/-}$ mice	Reduction in plaque formation and reduced infiltration of CD45^{+} cells[203]	✓	NA	NA	NA
sPLA$_2$ Varespladib	*Apoe*$^{-/-}$ mice	Reduction in atherosclerosis[204]	✓	5,145 patients after ACS	Significant increase in the risk of AMI, no reduction in risk of recurrent events[205]	X
LpPLA$_2$ Darapladib	Pigs	Reduction in atherosclerosis[206]	✓	15,828 patients with stable CAD	No reduction in the primary end point of cardiovascular death, myocardial infarction, or stroke[207]	–
Serine protease inhibition	Rabbits	Reduction in plaque growth after injury[208]	✓	NA	NA	NA

Target Drug	Preclinical studies			Clinical studies		
	Animal model	**Outcomes**	**Result**	**Population**	**Outcomes**	**Result**
Colchicine	Rabbits	Reduction in lipid deposition in aortic plaque[209]	✓	532 patients with stable coronary disease	Significant reduction in major cardiac events[210]	✓
Methotrexate	Rabbits	Reduction in neoatheroma formation and intimal thickening[211]	✓	7,000 patients with stable CAD and diabetes mellitus or metabolic syndrome	Effects on nonfatal MI, nonfatal stroke, and death (ongoing)[212]	–

ACS, acute coronary syndrome; AMI, acute myocardial infarction; CAD, coronary artery disease; CCL2, C-C chemokine 2 (also known as monocyte chemoattractant protein 1); $LpPLA_2$, lipoprotein-associated phospholipase A_2; MACE, major adverse cardiovascular event; MAPK, mitogen-activated protein kinase; MI, myocardial infarction, $sPLA_2$, secretory phospholipase A_2; VCAM1, vascular cell adhesion molecule 1.