Do we know enough about the immune pathogenesis of acute coronary syndromes to improve clinical practice?

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Summary

Morbidity related to atherosclerosis, such as acute coronary syndromes (ACS) including unstable angina and myocardial infarction, remain leading causes of mortality. Unstable plaques are inflamed and infiltrated with macrophages and T lymphocytes. Activated dendritic cells interact with T cells, yielding predominantly Th1 responses involving interferon-gamma (IFN-γ) and tumour necrosis factor-alpha (TNF-α), while the role of interleukin 17 (IL-17) is questionable. The expansion of CD8nullCD4 or CD8 T cells as well as pattern recognition receptors activation (especially Toll-like receptors; TLR2 and TLR4) is characteristic for unstable plaque. Inflammation modifies platelet and fibrin clot characteristics, which are critical for ACS. Understanding of the inflammatory mechanisms of atherothrombosis, bridging inflammation, oxidative stress and immune regulation, will allow for the detection of subjects at risk, through the use of novel biomarkers and imaging techniques including intravascular ultrasound, molecular targeting, magnetic resonance imaging (MRI) and 18F-fluorodeoxyglucose (18F-FDG) positron emission tomography (PET). Moreover, understanding the specific inflammatory pathways of plaque rupture and atherothrombosis may allow for immunomodulation of ACS. Statins and anti-platelet drugs are anti-inflammatory, but importance of immune events in ACS warrants the introduction of novel, specific treatments directed either on cytokines, TLRs or inflammasomes. While the prime time for the introduction of immunologically inspired diagnostic tests and treatments for atherosclerosis has not come yet, we are closer than ever before to finally being able to benefit from this vast body of experimental and clinical evidence. This paper provides a comprehensive review of the role of the immune system and inflammation in ACS.

Keywords

Acute myocardial infarction, atherothrombosis, inflammation, atherosclerosis, immunity

Introduction

The role of the immune system and inflammation in the pathogenesis of atherosclerosis and coronary artery disease has been well established during the past two decades (1, 2). In spite of this, morbidities related to atherosclerosis remain the leading cause of mortality worldwide. In particular, acute coronary syndromes (ACSs) represent life-threatening conditions during the natural history of coronary atherosclerosis including unstable angina (UA) and acute myocardial infarction (AMI). Unraveling the immune mechanisms of atherosclerotic plaques has led to attempts to treat atherosclerotic plaque progression using immunomodulation. Immune mechanisms are also involved in the pathogenesis of risk factors such as hypertension or hypercholesterolaemia (3, 4). Much less is known about the immune mechanisms of acute events, as the majority of studies have focused on their thrombotic nature (5). Thus, most of the therapies for ACS modulate coagulation and platelet function (6). These therapies have greatly improved clinical outcomes in ACS. While it is very important to develop effective new therapies to limit the consequences of acute coronary events, it is much more important to prevent them. However, we are still unable to effectively predict and prevent ACS occurrence. The term ‘vulnerable plaque’ has been introduced indicating the high risk of plaque rupture or erosion, which subsequently leads to ACS. The vulnerable plaque is, however, difficult to detect, and no biomarkers have been identified so far to allow for early detection of the disease. Recent advances in the understanding of the role of inflammation in ACS give promise for more forthcoming progress in this field.

This review will focus on the immune pathogenesis of ACSs and will address possible clinical (either diagnostic or therapeutic) applications of this knowledge.

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Immune events in ACS

While thrombus formation is the most critical step in ACS, it has been known for over a decade now that inflammation is exacerbated during both UA and MI (7). Gene arrays performed on unstable plaques have very often pointed to various immune associated genes alongside matrix metalloproteinases and tissue factor (TF) (8, 9). This inflammatory response has been initially thought to be induced by tissue damage in ACS. While there is no doubt that immunological response to damage is critical in modulating long term consequences of ACS such as heart failure (10–12), recent studies point to the fact that specific immune mechanisms may underlie plaque instability, and may even modulate platelet function and thrombus formation (13, 14). Immunopathogenetic studies of atherosclerotic plaques from patients with ACS as well as studies in animal models show activated macrophages, T cells and dendritic cells at sites prone to plaque rupture or erosion (15–17). Studies of platelet transcriptome, which give insight into events directly preceding acute coronary events in humans, also revealed several changes in immune related genes, such as CD69 and myeloid-related protein-14 (MRP14) (18). Thus, inflammatory responses in ACS include systemic immune activation, local inflammation in the vulnerable plaque and immune reactions associated with the thrombotic event itself.

Systemic inflammation in ACS

High C-reactive protein (CRP) levels, as indicators of systemic inflammation are accepted risk factors for acute coronary events (19). Moreover, ACS is associated with increased counts of total leukocytes, and in particular with an increased proportion of T lymphocytes (20–22). Increased CD4+CD28null T cells [23] and decreased CD4+CD25+Foxp3+ regulatory T (Treg) cells producing anti-inflammatory interleukin (IL)-10 and transforming growth factor (TGF)-β were identified in the peripheral blood of ACS patients (24, 25). At the same time, increased Th1 [interferon [IFN]-γ, IL-1beta, IL-12 p70 and regulated upon activation, normal T-cell expressed, and secreted (RANTES)] and decreased Th2 cytokines and expression of C-C chemokine receptor type 3 (CCR3) were identified (26). Th17 cells (CD4+ T-cells producing IL-17) are increased in ACS (27). However, there is no consensus regarding changes of lymphocyte subpopulations in the peripheral blood in ACS (27).

Increased levels of CD14+ monocytes were found in ST-segment elevation myocardial infarction (STEMI) and non-ST-segment elevation myocardial infarction (NSTEMI) patients, when compared with unstable angina (28). Monocyte subpopulations in ACSs appear to be altered as well. Peak CD14+CD16+ monocyte levels are increased in AMI patients (29). Moreover, circulating and localised at culprit site CD14+CD16+ monocytes were identified to show largest expression of TLR4 and suggested to be involved in AMI pathogenesis (30).

Systemic inflammation is associated with altered cytokine profiles in plasma in acute coronary events. While smaller studies demonstrate increased IFN-γ or IL-17 levels, and decreased inhibitory TGF-β1 (31), many larger studies attempting to link specific cytokine or chemokine levels to ACS have been inconclusive (32). For instance, while RANTES is increased in vulnerable and unstable plaques, the plasma levels do not seem to adequately reflect the risk (32), although the jury is still out on this particular issue (33). One explanation might be that inflammatory responses occur locally in the plaques, which may not be transferred to the peripheral blood level measurements sufficiently. Thus, we must focus on local immune events within the unstable plaque or coronary thrombus.

A vulnerable plaque is an inflamed plaque

Sites of atherothrombosis leading to ACS are associated with plaque rupture (60%), plaque erosion (30%), calcified nodules (2–7%) or very rarely with isolated intra-plaque haemorrhage (34, 35). Plaque rupture has been initially considered predominantly mechanical, as it occurs at sites of turbulent blood flow (5). Very soon several distinct biological features of rupturing plaques became apparent. They are characterised by the presence of a thin fibrous cap, overlying a necrotic core (thin-cap fibroatheromas; TCFA) which is heavily infiltrated by macrophages (density up to 26%) and T lymphocytes, pointing the attention to the critical role of the immune system in plaque vulnerability (Fig. 1) (5, 36, 37).

Recent studies also point to the important role of perivascular adipose tissue in this process (38, 39). These inflammatory characteristics of plaque are related to the immune nature of atherosclerosis itself (which has been expertly reviewed elsewhere [2, 40]). However, culprit lesions in ACS show particularly strong immune activation, characterised by, for example, a high level of human leukocyte antigen (HLA)-DR (17) or Toll-like receptor (TLR1, 2 and 4) expression (41) (Table 1). Interestingly, both M1 and M2 type macrophages were identified in vulnerable plaque regions, although it is now disputed how the functional balance between them relates to plaque instability (42).

Matrix metalloproteinases (MMP-7, MMP-8, MMP-12, MMP-13, MMP-14 and particularly MMP-9) have been implicated in plaque rupture (43), as they decrease collagen content by degradation and suppression of its production by apoptotic smooth muscle cells (44) leading to a thinning of the fibrous cap and a generation of necrotic core. MMPs are released from activated macrophages and foam cells at the most vulnerable, shoulder regions of plaques (13, 43). Activated macrophages and neutrophils in vulnerable plaque also produce reactive oxygen species (ROS), which inhibit tissue derived inhibitors of metalloproteinases (TIMPs), further activate MMPs (43, 45) and exacerbate endothelial dysfunction. This may link up with recent discoveries of predictive role of myeloperoxidase in ACS to inflammation (46). This link between matrix metalloproteinases activation and inflammation is the critical step in atherothrombosis (36).
Alongside macrophages, CD4+ and to a lesser extent CD8+ T cells are abundantly present in unstable regions of atherosclerotic plaques. In particular, Th1 lymphocytes, producing IFN-γ and tumour necrosis factor (TNF)-α, have been abundant in unstable plaques (36, 47), while anti-inflammatory, T regulatory cells secreting IL-10 and TGF-β attenuate atherosclerosis and stabilise plaque morphology (36, 48). Some studies confirm lower numbers of forkhead box P3 (FoxP3)+ T regulatory cells in unstable plaques (49), but others show their abundance (50). However, it is not the number of T regulatory cells, but their function that matters. Autoimmune diseases are often associated with increased numbers of dysfunctional T regulatory cells, which fail to provide inhibitory effects.

Interestingly, while IL-17 has been found to play a pro-inflammatory role in numerous autoimmune diseases, a recent study has demonstrated that a lack of IL-17 is associated with an increased formation of unstable, vulnerable plaques (51). Moreover clinical studies show that activation of Th17/Th1 and Th1, but not of Th17 cells, is associated with ACS (52).

CD28null CD4 and CD8 T cells are very important players in the immunopathogenesis of plaque instability. Clonally expanded CD4+CD28null T cells with strong pro-inflammatory properties were found in unstable coronary plaques, and may amplify and sustain inflammatory processes (23). The CD4+CD28null T cells in ACS are characterised by an overexpression of alternative co-stimulatory receptors (OX40 – inducible co-stimulator and 4–1BB – cytotoxic T lymphocyte associated antigen-4, programmed death-1) (53), indicating mechanisms of their activation in ACS.

Recent studies point particular attention to the role of dendritic cells (DCs) in cardiovascular disease (54) as well as unstable plaques. Their interactions with plaque residing T cells is the primary feature of rupture-prone plaque, even in the absence of thin-cap fibroatheromas (55). These DCs form clusters at the rupture-prone plaque regions with frequent DC-T cell contacts (55). They show markers of DC activation such as HLA-DR and CD83, and produce chemokines like CC chemokine ligands (CCL) 19 and CCL21 (56). Moreover, plasmacytoid dendritic cells in unstable regions produce INF-α, which further stimulates inflammatory processes.

**Figure 1: Role of immune system in atherosclerotic plaque rupture.**
Infiltration with macrophages, T cells, B cells and neutrophils occurs at all vessel wall layers (including perivascular adipose tissue; PVAT) and thrombus itself and is especially strong at the site of plaque rupture. Activated leukocytes produce matrix metalloproteinases (MMPs), which degrade collagen leading to fibrous cap thinning and plaque rupture or erosion. Immune cells are also present and modulate thrombus formation. A white, platelet-rich, thrombus forms at the rupture site, while a red, erythrocyte-rich, thrombus propagates both proximally and distally and may lead to total vessel occlusion. Based on [20, 23, 125]. TLR – Toll-like receptor; PVAT – perivascular adipose tissue; IL – interleukin; IFN – interferon; MIF – macrophage migration inhibitory factor; MMP – matrix metalloproteinase; CCL – CC chemokine ligand; IP-10 – interferon-gamma-inducible protein-10; GM-CSF – granulocyte-macrophage colony-stimulating factor; HLA-DR – human leukocyte antigen-DR.
reaction in the plaque (57, 58). IFN-α also induces TNF-related apoptosis-inducing ligand (TRAIL) on CD4+ T lymphocytes. TRAIL binds to its receptors, TRAIL-R1 and TRAIL-R2 which can be expressed on vascular smooth muscle cells (VSMCs) and leads to the activation of Fas-associated death domain (FADD), caspase-8 and subsequently caspase-3 activation, which results in VSMC death (57, 58). Apoptosis of VSMCs, immune cells and endothelium can be induced also by oxidative stress, oxidized lipoprotein (oxLDL), hypoxia or IFN-γ (59) and is a very important factor in plaque destabilisation (59). Various proinflammatory stimuli may activate inflammasomes (molecular platforms, which assemble multiple factors to provide caspase-1 activation) (60).

To summarise, activated DC in shoulder regions and instability-prone areas of plaques produce chemokines, and thus regulate immune cell traffic into these regions of plaques. They may also augment T-cell stimulation and provide optimal conditions for T lymphocyte activation, resembling the microenvironment in organised lymphoid tissues in the vulnerable regions of atherosclerotic plaques (56).

Mast cells have also been identified in atherosclerotic plaques, especially at sites of plaque rupture, erosion or haemorrhage (61). Pro-inflammatory cytokines, histamine, tryptase, leukotriens, thromboxane and chymase, secreted by mast cells can induce MMPs while proteases such as tryptase and chymase can cleave extracellular matrix, causing plaque destabilisation and/or intraplaque haemorrhage (61, 62). Mast cells can also contribute in macrophage apoptosis and leukocyte migration (61).

Finally, much less is known about the role of activated B cells and neutrophils in plaque instability, although this is now a very active area of research which will likely bring many valuable new findings in the near future. Cells involved in immune regulation of ACS have been summarised in Table 2.

While vulnerable, “hot” plaque is a major risk factor for the causation of an acute coronary event, it must be remembered that plaque rupture is not always associated with ACS. Some ruptured plaques can even be clinically silent, indicating that other, most likely pro-thrombotic mechanisms must coincide with plaque rupture.

Inflammation modifies thrombus formation

Thrombosis is also the most critical mechanism for ACS including STEMI (36). The local microenvironment, including inflammatory milieu, is critical for thrombus formation. Intracoronary ACS levels of cytokines [IL-1α, IL-6, IL-8, IL-12, IFN-α, macrophage migration inhibitory factor (MIF), macrophage inflammatory protein (MIP)-1α, monocyte chemotactant protein (MCP)-1, tissue plasminogen activator inhibitor (tPAI)-1, interferon-gamma-inducible protein (IP)-10, eotaxin and granulocyte-macrophage colony-stimulating factor (GM-CSF)] are increased when compared with aortic blood (20–22). While this may be a marker of unstable plaque inflammation, it will certainly affect platelet function as well as fibrin generation. Coronary thrombi differ, both in architecture and composition (20–22) from other circulatory thrombi (20, 63). Fibrin accounts for 55 ± 18%, platelets for 17 ± 18%, red blood cells for 11 ± 9%, crystals of cholesterol for 5 ± 8%, while leukocytes for 1.3 ± 2.0% of ACS intracoronary thrombi (21). While leukocyte content is not predominant, they may play an important modulatory role. Leukocytes include an increased content of CD14+ monocytes and a decreased content of CD66b+ granulocytes, B cells and T cells, compared to aortic blood (20). The Toll-like receptors, TLR2 and TLR4, were over-expressed on monocytes and granulocytes (TLR2) in the intracoronary thrombi and play a functional role (20). Leukocytes are activated in the thrombus as is indicated by the elevated number of proinflammatory cytokines presence (64).

An increasing body of evidence suggests that inflammation in the thrombus is not just a bystander, but actively modifies the thrombosis. Activated inflammatory cells, such as macrophages in the plaque or thrombus, release pro-thrombotic TF and plasminogen activator inhibitor (PAI)-1 (65, 66). Cytokines such as TNF-α or IL-1 as well as CD154 (CD40L), a ligand binding to CD40 on the leukocytes, induce TF expression (66, 67). All of the following, activated CD4+ T lymphocytes, neutrophils and macrophages as well as non-immune platelets, endothelial cells and smooth muscle cells, produce CD40L (66, 68). Cytokines produced by leukocytes including IFN-γ and IL-4 are associated with residual platelet reactivity in patients with ACS on dual antiplatelet therapy (14). Importantly, TLR1 and TLR6 are expressed on platelets although their role in ACS has not been well established yet (69).

The interaction between leukocytes and platelets is, however, two-directional, as platelets produce inflammatory modulators which may differentially modulate and traffic monocytes or T cells into the thrombus. These include RANTES, TGF-β, platelet-derived growth factor, platelet factor 4, trombospordin or nitric oxide (66). RANTES may be especially important as it triggers monocyte or neutrophil accumulation on inflamed or atherosclerotic endothelium, initiating acute coronary events (70). Circulating neutrophil-platelet and monocyte-platelet aggregates in ACS have also been reported (71–73).

Finally, inflammation may also alter fibrin structure, generating abnormally dense fibrin networks that resist fibrinolysis (74). This is particularly important as fibrin contributes to the majority of intracoronary thrombi.

Taking into account the above-mentioned examples of leukocyte-platelet interface it is reasonable to assume with some degree of confidence that thrombosis activates inflammation and that inflammation can amplify thrombosis. However the exact mechanisms of these interactions and possible therapeutic implications are still unclear.

Immune system in myocardial injury

Myocardial injury and heart failure are the main consequences of ACS (75). However, injured myocardium by inducing severe immune responses may also play modulating role in the natural his-
tory of the disease. Ischaemia-reperfusion injury or necrosis to the myocardium leads to the generation of *alarmins* or danger-associated molecular patterns (DAMPs) from the cells or the extracellular matrix (61, 76) (Fig. 2). They elicit innate immune reactions similar to immune activation induced via pathogen-associated molecular patterns (PAMPs), through TLRs. TLRs include at least 13 molecules, expressed by various cell types including leukocytes as well as vascular cells. These molecules can be either expressed on the cell membrane (TLR1–2, TLR4–6 and TLR11) or in the internal cellular compartments (TLR3, TLR7–9 and murine TLR13) (61). TLR2 and TLR4 have been the most intensely studied in the context of atherosclerosis and ACS and are summarised in Table 1. A role of other TLRs in cardiovascular diseases, such as the contribution of TLR3 and TLR7/8 in viral cardiomyopathy and/or myocarditis was reported (77). On the other hand TLR4, TLR5 and TLR9 are involved in adverse myocardial remodelling and/or contractile dysfunction (77). A more detailed description of TLRs function and role in cardiovascular diseases has been reviewed recently elsewhere (61, 77–79).

The importance of infiltrating neutrophils, monocytes and macrophages in ischaemia-reperfusion injury is very well defined (Fig. 2) (80). Several chemoattractants including IL-8 (chemo-kine (C-X-C motif) ligand (CXCL)-8), CXCL-1, MCP-1 or fractalkine and adhesion molecules (L- and P-selectin; intercellular adhesion molecule (ICAM)-1) are implicated in leukocyte recruitment into the damaged myocardium during ACS (81). Neutrophils and monocytes release multiple inflammatory mediators such as TNF-α, proteases or ROS which contribute to myocardial injury. Myocardial injury characterised by impaired ejection fraction recovery was recently linked to increased CD14+CD16* monocyte subset during ACS (29). Moreover, monocytes/macrophages activated within myocardial infarct can efflux to the periphery (10%/24 hours) to blood, liver, and spleen and abrogation of this was deleterious for infarct healing, and accelerated heart failure (82). Modulating the immune mechanisms of myocardial damage may be a promising drug target for its prevention during ACS.

**Are ACS driven by acute and chronic infections?**

Taking into account the profound involvement of immune activation in ACS, infections or other systemic inflammatory reactions could be associated with increased ACS risk. Indeed, up to 30% of MIs occur after upper respiratory tract infection (83), and the risk of ACS is greatly increased following respiratory, but not urinary, infections (84). The risk of MI or stroke is at its highest for the few days after infection (85) and remains elevated for over 21 days (86). Several other acute infections have been linked to ACS. These include gastroenteritis, *N. meningitidis, C. canimorsus* and *S. aureus* infections (85).

Chronic infectious agents such as *Ch. pneumoniae* or *P. gingivalis*, initially linked to atherosclerosis, may increase risk of acute coronary events (87–89). However, their direct role in ACS is controversial and has not been supported by strong evidence.

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**Figure 2: Myocardial infiltration by immune cells during the course of myocardial infarction.** Neutrophils and monocytes migrate into myocardial tissue attracted by IL-8, MCP-1 and other chemokines. Augmented release of inflammatory mediators, proteases and reactive oxygen species contributes to enhanced myocardial damage. Immune cells also contribute to proper healing and remodelling after myocardial infarction. Activated monocytes/macrophages also migrate from infarcted myocardium and may elicit systemic inflammatory response (82). DC – dendritic cell; TLRs – Toll-like receptors; DAMPs – danger-associated molecular patterns; Hsp – heat shock proteins; HMGB-1 – high-mobility group box 1; IL-8 – interleukin-8; CXCL-1 – chemokine (C-X-C motif) ligand 1; MCP-1 – monocyte chemoattractant protein-1; monocytes/macrophages and T cells are presented as in Figure 1.
Infections may contribute to atherosclerosis and ACS development through diverse mechanisms, including direct effects on cells in the vascular wall, circulating cytokines and inflammatory mediators, TLRs activation as well as initiation of autoimmune reactions (90). DC numbers, as well as perivascular macrophage and T cell content, increased in the coronary artery intima and media of patients with systemic infections (85, 91) who died as a result of ACS (91). Infections enhance hypercoagulable states (92) and promote endothelial dysfunction (85, 93) also through the innate immunity, by TLRs’ activation.

Thus we must remember that patients with severe coronary disease, admitted to hospital or in ambulatory practice with upper respiratory tract infection should be considered at high risk of ACS. This knowledge is, however, underestimated in current clinical management, possibly due to the lack of effectiveness of antimicrobial therapies in preventing ACS. The latter results most likely from the presence of some specific pathogen-related characteristic, host susceptibility to infection or concomitant viral infections (89, 90) and should not overshadow epidemiological evidence linking infections to ACS. Annual influenza vaccination, with inactivated vaccine decreases ACS risk (94) for coronary artery disease (CAD) patients and seems more cost-effective than primary and secondary statin prevention and beta-blocker use after MI (83). Moreover, pneumococcal vaccination also decreases MI rate (95).

It may therefore, be worth considering if the vaccinations for CAD patients should be as widely used as they are for subjects with chronic obstructive pulmonary disease?

### Increased ACS risk in autoimmune diseases

Several systemic autoimmune disorders such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), antiphospholipid syndrome (APS) or primary Sjögren syndrome (pSS), are associated with enhanced atherosclerosis development and with an increased cardiovascular risk of ACS (96). The underlying mechanisms, which involve both unspecific immune activation and molecular mimicry, are still under intense investigation. Particular interest has focused on APS, which is characterised by recurrent thrombosis and pregnancy loss with antiphospholipid antibodies (aPL) like anticardiolipin (aCL), anti-beta2-glycoprotein-I (anti-beta2GPI), or lupus anticoagulant presence. Atherosclerotic events in APS can be mediated by the procoagulant and proinflammatory effects of aPL on endothelium or via autoantibody-mediated thrombosis. Systemic lupus erythematosus (SLE) is associated with a four- to eight-times increase in the risk of CAD and MI, independently of traditional risk factors, and which is also attributed to the presence of auto-antibodies as novel risk factors for ACS (96). Similarly, RA is associated with increased CAD, and the occurrence of ischaemic events of atypical presentation. Moreover, mortality and recurrence of ACS is greater in RA patients (96). Interestingly, in this group of patients a particularly high expansion of CD4+CD28null T cells was found (97). Th17/Treg imbalance, type 1 IFN and TRAIL are among important potential mechanisms linking autoimmune diseases and ACS (97). Moreover, autoimmune mechanisms appear to play a role in ACS even in the absence of autoimmune disease. For example, aCL profile (high IgG and low IgM) is associated with a three-fold increase of the risk of the recurrence of an acute coronary event even in the absence of SLE (98). Further understanding of the mechanisms linking auto-

### Table 1: Toll-like receptors 2 and 4 (TLRs) and their ligands involvement in atherosclerosis and ACS (61, 77–79).

<table>
<thead>
<tr>
<th>TLR</th>
<th>Pathogen-associated molecular patterns</th>
<th>Danger-associated molecular patterns</th>
<th>Atherosclerosis</th>
<th>Acute coronary syndromes</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Peptidoglycans</td>
<td>HMGB-1</td>
<td>TLR2 found in human coronary arteries and primary adventitial fibroblasts, TLR2 ligands enhance neointima formation and increases atherosclerosis in ApoE/- mice [126], TLR2 mRNA, its ligands (EDA and hs60), and regulators like IRAK-M are increased in advanced plaques in ApoE/-</td>
<td>Overexpressed on monocytes and granulocytes in coronary thrombi [20], TLR2/- mice or anti-TLR2 treatment [127] reduces myocardial ischemia/reperfusion injury, TLR2/- mice – improved remodelling after MI [128]</td>
</tr>
<tr>
<td>4</td>
<td>Cell surface lipopoly-saccharides [129]</td>
<td>Hsp60, HMGB-1, Hyaluronic acid, Fibronectin-EDA</td>
<td>TLR4 found in human coronary artery plaques and adventitia, Periadventitial application of lipopolysaccharide augmented neointima formation; TLR4/-protected [129] TLR4 mRNA, its endogenous ligands (EDA and hs60) as well as regulating mediators, (IRAK-M) are increased in advanced plaques in ApoE/-</td>
<td>Overexpressed on monocytes in coronary thrombi [20], TLR4 +/- reduced the LV remodeling and preserved systolic function [130], Smaller myocardial infarctions in TLR4/-, Attenuated IR-induced myocardial apoptosis and cytokine expression in TLR4/- mice [120]</td>
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immune disorders and ACS will provide vital proof for the role of immune mechanisms of ACS. We need to better understand if the immune reactions increasing risk of ACS are of a more unspecific nature or whether they are associated with specific antigen stimulations related to these infections or autoimmune reactions.

**Immune system markers in the diagnosis and prediction of ACS**

**Immune-based biomarkers of vulnerable plaque**

The primary diagnostic aim in ACS is to identify patients at risk, prior to the event. This is the only way to successfully limit the number of adverse outcomes, but is currently not possible. The taking into account of high CRP levels in addition to traditional risk factors can improve cardiovascular risk prediction (99). However, even the use of an extended biomarker panel, including the use of inflammatory markers such as CRP and fibrinogen, only modestly altered the final results of the risk prediction for the first cardiovascular event in the large Framingham Heart Study population (100).

Assessment of specific components of the immune system, such as selected cellular markers, may aid in ACS risk stratification in future. This may include T cell repertoire, characterised by CD4+CD28null T lymphocyte expansion (101) or soluble CD40 ligand determination in patients at risk. CD40L has been used to identify patients for antiplatelet treatment with abciximab to reduce cardiovascular event rate (102). Similarly, pro-inflammatory lipoprotein-associated phospholipase A2 (103) or myeloperoxidase have been shown to predict imminent ACS (104, 105).

Finally, microRNA markers as well as metabolomic approaches, partially related to the immunopathogenesis, may in future increase the sensitivity and specificity of prediction of ACS. However, at the moment we do not seem to have a ready prospect for the quick and affordable detection of vulnerable plaque.

**Molecular imaging of inflamed plaques**

Much greater progress has been made in the possibilities of invasive and non-invasive imaging of “hot”, unstable spots within atherosclerotic plaques. The majority of these techniques are based on the detection of plaque inflammation in conjunction with assessment of fibrous cap thickness and necrotic core determination. So far the most widely clinically assessed – optical coherence tomography (OCT) and virtual histology – intravascular ultrasound (VH-IVUS) or hybrid imaging using computed tomography coronary angiography (CTCA) allow for visualisation of thin fibrous cap and necrotic core. New modalities will base more on visualising specific inflammation such as 18F-fluorodeoxyglucose (18F-FDG) positron emission tomography (PET), or magnetic resonance imaging (MRI) and myocardial perfusion imaging techniques, like single photon emission computed tomography (SPECT) (36, 106).

Particular interest has been raised by 18F-FDG positron emission tomography. It is a gold standard for the detection of cancer metastasis, but has been successfully used to image metabolically active atherosclerotic plaques. 18F-FDG accumulation is directly correlated with plaque macrophage content (107). The anti-inflammatory effect of treatments such as statins can also be visualised (108). Increased coronary uptake of 18F-sodium fluoride (18F-NaF) and 18F-FDG by PET is associated with plaque inflammation, prior cardiovascular events (109). Authors of a recent review even posed the question – are we ready for the prime time? (108). While this approach is very promising its costs and usefulness need to be verified by large clinical trials before we will be able to pronounce it a success.

More recently Gaemperli et al. proposed the use of 11C-labelled PK11195 (the selective ligand for the translocator protein characteristic for macrophages), which may in turn, prove itself to be more specific for imaging plaque inflammation than FDG by PET/CTA (110).

High-resolution MRI allows us to discriminate plaque composition, including lipid core, intraplaque haemorrhage, fibrosis, calcifications or arterial thrombus age (111). Moreover, MRI using magnetic contrast agents targeted for macrophages or other inflammatory cells and molecules, neovascularisation, thrombotic material, high-density lipoprotein (HDL) or fibrin have all been tested at the pre-clinical level (111). Moreover, MRI detection of areas with high MMP activity has been proposed (112). Ultra-small superparamagnetic iron oxides (USPIOs) targeted for adhesion molecules such as vascular cell adhesion molecule (VCAM)-1 or for molecules involved in apoptosis have been effectively used in ApoE-/- mice for vulnerable plaque identification (113).

Electric impedance spectroscopy (EIS) imaging shows that impedance values differ depending on the cellular composition of plaque, in particular in relation to the presence of CD31 (neovascularisation), CD36 (scavenger cells) and MMP-3 (114). This may create new opportunities for vulnerable plaque imaging in the clinical setting.

Further development of these approaches, will be enabled by a better understanding of the role individual cells of the immune system play in the pathogenesis of plaque instability, as this will identify the molecular targets we need to image to identify atherosclerosis-prone sites.

**Immune system targeting in ACS**

**Animal models**

Animal models of atherosclerosis and plaque rupture, such as ApoE-/- mice, have been extensively used to investigate therapeutic approaches to limit plaque instability (40). However, there are no perfect animal models for ACS. Firstly, immunosuppressive
### Table 2: Cellular involvement in ACS in humans.

<table>
<thead>
<tr>
<th>Cell</th>
<th>Acute coronary syndrome</th>
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<tr>
<td><strong>Lymphocytes</strong></td>
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| CD3+ | ● HLA-DR expression on both CD4+ and CD8+ T cells in UA patients [131]  
● Increased CD3+CD4+HLA-DR+ T cells presence in AMI and SA patients [132] |
| CD4+CD28null | ● Expanded in patients with UA, clonally multiplied found in culprit, but not non-culprit lesions [23]  
● Associated with ACS recurrence [101]  
● Increased in DM patients and correlate with first cardiovascular event, as well as with worse ACS outcome [133] |
| Th17 T cells | ● Increased in ACS patients [24]  
● Increased in ACS (Th17/Th1 subset) but no difference between AMI, UA, SA [52]  
● High IL-17 transcoronary gradient is associated with higher troponin I [134] |
| T regulatory (Treg) | CD4+CD25+ high/Foxp3+ | ● Decreased in ACS patients as compared to SA and controls [24]  
● Compromised functional properties in ACS subjects [25]  
● Lower Treg cells in NSTACS, than in STEMI, SA or in controls, highest levels in STEMI patients [135] |
| Effector memory T cells (TEM: CD3+CD4+CD45RA-CD45RO+CCR7-) | ● Increased in AMI and SA [125]  
● HLA-DR+TEM and CXCR3+TEM increased in AMI and SA [125] |
| T-cell receptor zeta-chaindim CD4+ T cells | | ● Increased in ACS patients,  
● Enhanced transendothelial migratory capacity [136] |
| Cytokines producing | | ● Increased IFNγCD4+ and CD8+ T cells in UA than in SA or controls [11]  
● Increased IFNγ producing CD4+ (Th1) T cells in ACS, than in SA [137]  
● 1–2 weeks after UA increase in CD4+ T cell IL-2 and IL-4 with decrease in CD4+ and CD8+ IFNγ production, rebound to the original levels at three months [11]  
● Reduced TGF-β1 producing cells (Th3) in ACS [31] |
| Natural killer cells (NK) | | ● Lower prevalence in AMI patients, than in control subjects [138]  
● Reduced (CD56(dim)) in ACS and SA patients as compared to controls, concomitant loss of function  
● Reduced invariant NK (iNK) T cells in AMI patients, iNK T cells as restenosis predictors [138] |
| **Dendritic cells (DC)** | |
| Myeloid DC (mDC) | | ● Reduction of circulating precursors of mDC in SA, UA and AMI [139]  
● Inverse correlation between mDC and hsCRP or IL-6 [139]  
● Increased mDC in vulnerable carotid plaques [139] |
| Plasmacytoid DC (pDC) | | ● No change in circulating precursors of pDC presence in SA, UA, AMI [139]  
● IFN-alpha production by pDC in vulnerable plaque [57, 58] |
| **Monocytes** | |
| CD14+ | | ● Increased in STEMI and NSTEMI when compared with unstable angina [28]  
● Higher CD11b/CD18 on monocytes in coronary than in aortic blood in UA [140]  
● Increased monocyte LFA-1 (CD11a/CD18), Mac-1, VLA-4, ICAM-1 expression in myocardial infarction patients as compared to controls [141] |
| CD14+CD16- | Peak levels after AMI (on day 3) negatively associated with myocardial salvage and ejection fraction recovery [29]  
No significant changes during three days after UA event [29] |
| CD14+CD16+ | Decreased in AMI patients on admission as compared to UA and SA subjects [29]  
Peak values after AMI (on day 5) higher than in SA patients [29]  
Show largest expression of TLR4 [30] |
| **Other leukocytes** | |
| Neutrophils | | ● Reduced myeloperoxidase intracellular index in ACS patients [142]  
● Higher CD11b/CD18 (Mac-1) on Neu in coronary than in aortic blood UA [140]  
● Increased Mac-1 expression in MI [141] |
| Mast cells | | ● Increased activity (histamine; tryptase; leukotriens; thromboxane) in ACS [62]  
● Higher ACS risk of atopic individuals [62]  
● Links between allergic reactions, mast cells and ACSs occurrence |
| Eosinophils | | ● Hypereosinophilia presenting as ACS with normal coronaries  
● Cases of eosinophilia and ACSSs recurrence, lower rate of ACSSs on prednisone treatment [143] |

(NSTE) ACS – (non–ST segment elevation) acute coronary syndrome; STEMI – ST segment elevation myocardial infarction; AMI – acute myocardial infarction; UA – unstable angina; SA – stable angina; NCA – normal coronary artery; TLR – toll-like receptor; TGF-β1 – transforming growth factor beta-1; IFNγ – interferon γ; IL – interleukin; NK – natural killer; CRP – C-reactive protein; IFN – interferon; LFA-1 – lymphocyte function-associated antigen-1 (CD11a/CD18); VLA-4 – very late activation antigen-4; ICAM-1 – intercellular adhesion molecule-1.
Clinical immunology of ACS

Treatment using low-dose (lacking systemic immunosuppressive complications) FK506 decreased inflammatory cell density and reduced necrotic core, while increasing collagen content, indicating a more stable plaque morphology. 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) and anti-platelet drugs have pleiotropic effects reducing plaque inflammation. Simvastatin stabilises plaques across a variety of species including humans, and is associated with an increase in T regulatory lymphocytes in atherosclerotic plaques (115). Non-selective cyclooxygenase (COX)-1 and 2 inhibition, using low dose aspirin in LDL-receptor deficient mice on a high fat diet, decreases circulating ICAM-1, MCP-1, TNF-α and stabilises atherosclerotic plaques (116). The similar effects of a selective COX-2 blockade, stabilising plaques, have been described in a pig model of atherosclerosis (117).

More specific approaches include the use of immunomodulating cytokines such as TGF-β1, which limits plaque instability and reduces other complications of atherosclerosis (118). As Toll-like receptors have been shown to play a very important role in plaque inflammation (Table 1), the combined inactivation of TLR2 and TLR4 attenuated inflammation and increased plaque stability in an ApoE−/− mouse model (119). Finally the blocking of certain immunogenic epitopes may also prove useful. For example, the use of the anti-Hsp60 antibody attenuated ischaemia-reperfusion-induced myocardial apoptosis and cytokine expression in mice (120).

The use by subjects of experiments of various non-pharmacological approaches, such as being subjected to regimens of constant exercise, have been shown to strongly inhibit the presence of both oxidative stress and inflammation in the plaque (121).

The aspects described above are selected from a large body of evidence. We need to be careful when translating these findings directly to human disease and treatment. The use of larger animals such as pigs would seem the appropriate choice in a selection of clinically relevant approaches using antithrombotic agents e.g. aspirin, lipid lowering drugs e.g. statins or blood pressure lowering treatments e.g. ACE-I with known anti-inflammatory properties are not listed.

### Table 3: Selected interventional clinical studies or meta-analyses (if present, described preferentially) of interventions targeted at immune system in ACS

<table>
<thead>
<tr>
<th>Clinical study</th>
<th>Intervention</th>
<th>Population</th>
<th>Effect</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Giugliano et al. (meta-analysis)</td>
<td>Corticosteroids</td>
<td>AMI patients</td>
<td>Associated with 26% decrease in mortality (11 trials). However analysis limited to studies &gt; 100 patients and RCTs shows no benefit. No harm and possible beneficial effects on mortality.</td>
<td>[144]</td>
</tr>
<tr>
<td>MUNA trial</td>
<td>Methyl-prednisolone (48 h course)</td>
<td>Unstable angina patients</td>
<td>Reduced CRP, but trend toward worse event-free survival noticed. No improvement of short-term (30-day) outcome.</td>
<td>[145]</td>
</tr>
<tr>
<td>DAVIT II study</td>
<td>NSAIDs (randomisation in the 2nd week after AMI).</td>
<td>AMI patients</td>
<td>Trend to lower mortality, lower reinfarction and major cardiac events.</td>
<td>[146]</td>
</tr>
<tr>
<td>NUT-2</td>
<td>Addition of meloxicam (COX-2 inhibitor) to aspirin and heparin for 30 days.</td>
<td>Non-ST-segment elevation acute coronary syndrome patients</td>
<td>Lower rate of recurrent angina, myocardial infarction or death as well as secondary composite variable (coronary revascularisation, MI and death).</td>
<td>[147]</td>
</tr>
<tr>
<td>COMPLY</td>
<td>Pexelizumab (an anti-CS complement mAb) 2 mg/kg bolus &lt; 6 h after onset and 0.05 mg/kg/h for 20 h</td>
<td>STEMI patients undergoing fibrinolysis</td>
<td>Did not reduce infarct size (CK-MB assessment) or adverse clinical outcomes.</td>
<td>[148]</td>
</tr>
<tr>
<td>COMMA</td>
<td>Pexelizumab 2 mg/kg bolus ± 0.05 mg/kg per h for 20 h infusion</td>
<td>STEMI patients undergoing primary PCI</td>
<td>Did not reduce infarct size (determined by CK-MB assessment), but lowered 90-day mortality rate.</td>
<td>[149]</td>
</tr>
<tr>
<td>Armstrong et al.</td>
<td>Pexelizumab 2 mg/kg bolus &lt; 6 h after onset and 0.05 mg/kg/h for 20 h</td>
<td>STEMI patients undergoing primary PCI</td>
<td>Did not affect mortality rate.</td>
<td>[150]</td>
</tr>
<tr>
<td>LIMIT AMI</td>
<td>Recombinant, humanised anti-CD18 mAb (subunit of beta2 integrin adhesion receptor) – intravenous bolus 0.5 or 2 mg/kg, added to recombinant tissue plasminogen activator</td>
<td>STEMI patients &lt;12 h from onset</td>
<td>Induced transient leukocytosis, but did not affect coronary blood flow, infarct size or ST-segment elevation resolution. Possible interference of heparin.</td>
<td>[151]</td>
</tr>
<tr>
<td>HALT-MI study</td>
<td>Antibody against CD11/CD18 (HuZ3F2G, 0.3 or 1 mg/kg) within 6 hours of chest pain onset, before angioplasty</td>
<td>STEMI patients</td>
<td>No infarct size decrease. No differences according in corrected TIMI frame and clinical events. High doses increased in minor infections.</td>
<td>[152]</td>
</tr>
<tr>
<td>PSALM</td>
<td>Recombinant P-selectin glycoprotein ligand-immunoglobulin (75 or 150 mg rPSGL-Li i.v., P-selectin antagonist), within 6 h of onset</td>
<td>Alteplase treated STEMI patients</td>
<td>No benefits in coronary vessel patency, myocardial tissue reperfusion or function recovery. Prematurely stopped after 88 patients, due to no efficacy in larger trial (RAPSODY).</td>
<td>[153]</td>
</tr>
</tbody>
</table>
Clinical studies

It is much more difficult to assess the effects of anti-inflammatory treatments on plaque stability in humans. Imaging modalities such as intravascular ultrasound or FDG-PET have been used in small to moderate-size studies. Both systemic anti-inflammatory treatments as well as the local delivery of drugs, particularly through drug-eluting stents (DES), have been used to stabilise plaques. The major studies are summarised in Table 3. In particular, statin use has been recommended for plaque stabilisation. Moreover several studies which are testing the use of anti-cytokine and immunomodulating treatments in humans are currently under way.

Local delivery of anti-proliferative and anti-inflammatory molecules on DES is valuable, as DES are clinically used. DES have been associated with superior features of plaque stabilisation, although the possible anti-endothelial effects of some of the drugs available on stents must be considered (122). Anti-inflammatory drugs such as sirolimus (rapamycin), a calcineurin inhibitor,
which inhibits T cell activation by interfering with IL-2 actions, shows superiority over paclitaxel – a pure antimitotic agent, which stabilises intracellular polymers of microtubules. Use of other, newer immunosuppressant agents on stents such as zotarolimus or everolimus appears very promising in clinical trials (123, 124).

Local delivery of anti-inflammatory medications may be also provided by direct, ‘one shot’ drug delivery to the area of high risk plaque during angiography or PCI. Among other anti-inflammatory approaches photodynamic therapy, cooling, heating and so-notherapy of vulnerable plaques have been proposed (35).

In summary, several methods, including statins, aspirin and other antiplatelet treatments have anti-inflammatory effects and induce plaque stabilisation. However, we are now close to introducing specific treatments targeting the various individual immune mechanisms of ACS.

Conclusions

Immune mechanisms are critical for the development of plaque rupture and atherothrombosis. The understanding of these specific mechanisms brings us closer to the ability to successfully detect unstable atherosclerotic plaques and identify patients at high risk of ACS. Moreover, attempts are made to employ immunomodulating therapies in the treatment of atherothrombosis and prevention of ACS.

However, before we can achieve that we need to further uncover the very specific mechanisms of immune involvement in ACS, so that novel therapies are free of strong immunosuppressant activities, which are associated with significant adverse clinical outcomes.

While the prime time for the clinical use of the results of many years of research into the immune mechanisms of atherosclerosis is not there yet, we are closer than ever before to being in a state of finally benefitting from this vast body of experimental and clinical evidence.

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Conflicts of interest

None declared.

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