Pathophysiological role of neutrophils in acute myocardial infarction

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Summary

The pathogenesis of acute myocardial infarction is known to be mediated by systemic, intraplaque and myocardial inflammatory processes. Among different immune cell subsets, compelling evidence now indicates a pivotal role for neutrophils in acute coronary syndromes. Neutrophils infiltrate coronary plaques and the infarcted myocardium and mediate tissue damage by releasing matrix-degrading enzymes and reactive oxygen species. In addition, neutrophils are also involved in post-infarction adverse cardiac remodelling and neointima formation after angioplasty. The promising results obtained in preclinical models with pharmacological approaches interfering with neutrophil recruitment or function have confirmed the pathophysiological relevance of these immune cells in acute coronary syndromes and prompted further studies of these therapeutic interventions. This narrative review will provide an update on the role of neutrophils in acute myocardial infarction and on the pharmacological means that were devised to prevent neutrophil-mediated tissue damage and to reduce post-ischaemic outcomes.

Keywords

Atherosclerosis, neutrophils, inflammation, acute myocardial infarction

Introduction

Atherosclerotic plaque rupture promotes atherothrombosis, vessel occlusion and consequent ischaemic organ damage. This pathophysiological sequence is the primary cause of acute cardiovascular events (such as myocardial infarction [MI] and stroke). Both systemic and intraplaque atherosclerotic inflammation represent main risk factors, closely related to each other in determining the plaque composition and related vulnerability (1). In fact, culprit lesions are typically not flow-limiting stenoses, but rather inflamed lipid-loaded lesions (2). Although the clinical assessment of coronary atherosclerosis severity is based on vascular lumen occlusion degree determination (3), retrospective analyses as well as prospective observations suggested that in 60–70% of the patients with acute coronary syndromes (ACS), the culprit site exhibits a diameter narrowing below 70% and frequently below 50% (2). Macrophages are the most abundant cell subtype within atherosclerotic plaques. However, other leukocytes are also well represented and have important roles in determining local inflammation and in promoting plaque rupture (4). Among these, polymorphonuclear neutrophils (PMNs) are now under the spotlight for their role in acute cardiovascular events and, in particular, during the very early and late stages of atherogenesis. Previous lack of research attention to neutrophils was potentially dependent on their rare detection, short life span and high tissue turnover (5). As of now, most of the knowledge on the role of PMNs within atherosclerotic plaques has derived from studies on experimental mouse models, human atherosclerotic plaque biopsies and epidemiological data. The aim of this narrative review is to highlight the step of coronary atherogenesis and post-infarction cardiac remodelling which PMNs partake in. In addition, we will also focus on pharmacological intervention strategies targeting PMNs in acute MI as potential sources of future therapeutic perspectives.

Neutrophils in atherogenesis

In the last two decades, a large number of studies documented a direct correlation between a high number of circulating white blood cells (WBC) and an increased cardiovascular risk. The pathophysiological hypotheses (6) underlying this observation have been interestingly reviewed by Coller in 2005 (7). In subsequent analyses, a high neutrophil count and neutrophils/lymphocytes...
(N/L) ratio have been proved as useful prognostic predictors in patients with ACS (8) (▶Table 1).

In addition, individual studies as well as multivariate analyses showed a direct correlation between neutrophil count and cardiovascular risk factors, such as hypertension (34), metabolic syndrome (21), type 2 diabetes mellitus (35), smoking (36), hyperlipidaemia (37), obstructive sleep apnea (38) and end-stage renal disease (39). Finally, PMN-related biomarkers (such as systemic levels of MPO and elastase) were also shown associated with atherosclerosis (▶Table 2).

Low amounts of PMNs were initially found within human atherosclerotic plaques and this might explain why PMNs have been classically neglected in the pathophysiology of atherosclerosis. Mehta et al. firstly showed in 1989 an increased neutrophil count in patients with ACS (8) compared to controls, as well as a positive correlation between neutrophil count and coronary stenosis complexity (r=0.28; p=0.002) and is independent stenosis complexity predictor (OR: 4.05; CI 1.9–10.4; p=0.038).

### Table 1: Neutrophil count and cardiovascular risk.

<table>
<thead>
<tr>
<th>Author (reference)</th>
<th>Year</th>
<th>Study design (n)</th>
<th>Target</th>
<th>Outcome and Prediction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dinerman J. L. et al. [9]</td>
<td>1990</td>
<td>Case-control (93 patients; 17 AMI, 29 UA, 25 SA and 22 healthy controls)</td>
<td>Neutrophil count</td>
<td>Positively correlates with CAD presence. AMI/UA vs. SA/control group (p&lt;0.01)</td>
</tr>
<tr>
<td>Bell D. et al. [10]</td>
<td>1990</td>
<td>Case-control (97 patients; 32 AMI, 30 IHD and 35 healthy controls)</td>
<td>Neutrophil count</td>
<td>Positively correlates with CAD presence. AMI (p&lt;0.0001) and IHD (p&lt;0.004) vs healthy controls</td>
</tr>
<tr>
<td>Mazzone A. et al. [11]</td>
<td>1993</td>
<td>Case-control (29 patients; 15 UA and 14 SA)</td>
<td>Neutrophil count (CD11b)</td>
<td>Positively correlates with coronary inflammation in coronary ACS. UA vs. SA group (p&lt;0.01)</td>
</tr>
<tr>
<td>Takeshita S. et al. [12]</td>
<td>1997</td>
<td>Case-control (166 patients; 68 ACS, 83 CAD and 15 SA)</td>
<td>Neutrophil count</td>
<td>Positively correlate with CAD severity (p&lt;0.05)</td>
</tr>
<tr>
<td>Leckie M. J. et al. [13]</td>
<td>2004</td>
<td>Case-control (53 patients; 9 UA, 18 SA and 26 healthy controls)</td>
<td>Neutrophil count</td>
<td>Positively correlates with CAD presence. UA (p=0.007) and SA (p&lt;0.001) group vs. healthy controls</td>
</tr>
<tr>
<td>Avanzas P. et al. [14]</td>
<td>2004</td>
<td>Prospective cohort study (55 AMI NSTEMI)</td>
<td>Neutrophil count</td>
<td>Positively correlates with coronary stenoses complexity (r=0.36; p=0.007)</td>
</tr>
<tr>
<td>Avanzas P. et al. [15]</td>
<td>2004</td>
<td>Case-control (150 patients; 121 CAD and 29 healthy controls)</td>
<td>Neutrophil count</td>
<td>Positively correlates with coronary stenoses complexity (r=0.28; p=0.002) and is independent stenosis complexity predictor (OR: 4.05; CI 1.9–10.4; p=0.038)</td>
</tr>
<tr>
<td>Haumer M. et al. [16]</td>
<td>2004</td>
<td>Prospective observational (398 patients with peripheral artery disease)</td>
<td>Neutrophil count</td>
<td>Positive predictor of major adverse cardiovascular events (multivariate analysis: HR 1.83; CI 1.29–3.00; p&lt;0.017)</td>
</tr>
<tr>
<td>Grau A. J. et al. (CAPRIE study) [17]</td>
<td>2004</td>
<td>Prospective observational (18558 patients with previous cardiovascular disease)</td>
<td>Neutrophil count</td>
<td>Positive predictor of recurrent ischaemic events (multivariate analysis: RR 1.09; CI 1.06–1.13; p&lt;0.001)</td>
</tr>
<tr>
<td>Gillum R. F. et al. (NHANES I study) [18]</td>
<td>2005</td>
<td>Prospective observational (5027 healthy controls)</td>
<td>Neutrophil count</td>
<td>Independent death risk factor from all causes (RR 1.29; CI 1.14–1.47; p&lt;0.01) and CVD death (RR 1.39; CI 1.15–1.67; p&lt;0.001)</td>
</tr>
<tr>
<td>Horne B. D. et al. [19]</td>
<td>2005</td>
<td>Prospective observational (3227 patients angiographically enrolled)</td>
<td>N/L ratio</td>
<td>Positive predictor of death/AMI (multivariate analysis: HR 0.83; CI 0.68–1.03; p&lt;0.001)</td>
</tr>
<tr>
<td>Nijm J. et al. [20]</td>
<td>2005</td>
<td>Case-control (105 patients; 20 UA, 45 SA and 45 healthy controls)</td>
<td>Neutrophil count</td>
<td>Positively correlates with CAD severity. Increasing neutrophil count in UA (p&lt;0.05) and SA (p&lt;0.05) vs control group and in UA vs SA group (p&lt;0.01)</td>
</tr>
<tr>
<td>Tsai J. C. et al. [21]</td>
<td>2007</td>
<td>Prospective observational (1872 T2DM patients)</td>
<td>Neutrophil count; N/L ratio</td>
<td>Correlates with systolic BP (r=0.002, p=0.002) and low HDL-cholesterol (r=−0.003, p=0.004). N/L ratio positive correlates with CAD risk (multiple linear regression: OR 237.458; CI 50.242–1147.02; p=0.001)</td>
</tr>
<tr>
<td>Zazula A. D. et al. [22]</td>
<td>2008</td>
<td>Prospective observational (178 patients with chest pain)</td>
<td>N/L ratio (N/L) ratio</td>
<td>Is predictor of ACS diagnosis in chest pain (p&lt;0.0001)</td>
</tr>
</tbody>
</table>
phil elastase activity in patients with ACS (40). Dinerman (9) and Bell (10) in 1990 and others later confirmed these results by flow cytometry. This difficulty in detecting PMNs in human and mouse atherosclerotic plaques can be attributed to their biological features, but also to the methods that are commonly used to detect them. Firstly, within inflamed tissues, PMNs rapidly undergo apoptosis and are cleared by phagocytes (57). On the other hand, activated human PMNs also might exhibit marked phenotypic changes by acquiring antigen-presenting cell features or shedding their surface membrane receptors (58). These plastic and dynamic properties might modify the expression of neutrophil specific markers. In addition, for many years, sensitive and specific detection methods for PMNs also lacked. Anti-mouse Ly6G antibodies allowed to identify PMNs in atherosclerosis mouse models (from early stages to rupture-prone lesions) (59, 60). Similar results were obtained by combining the staining for MPO and elastase (61) or formyl peptide receptor (FPR2) and p22phox (62). Apoe−/−Lysm<sup>cre/cre</sup> mice allow tracking PMNs in late atherosclerotic plaques. Using this model, Rotzius et al. noticed a PMN-endothelium interaction and PMN accumulation in shoulder region of mouse atherosclerotic plaques, thus suggesting a significant PMN recruitment from the vessel lumen to the arterial wall (63). These results from human and mouse studies suggest that neovessels, which have a more fragile in lipid core, might be a preferred target for PMN homing.

Table 1: Continued

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</thead>
<tbody>
<tr>
<td>Papa A. et al. [23]</td>
<td>2008</td>
<td>Prospective observational (422 CAD patients)</td>
<td>N/L ratio</td>
<td>Positive predictor of cardiac death (multivariate analysis: HR 8.13; CI 1.42–46.57; p&lt;0.02)</td>
</tr>
<tr>
<td>Karabinos I. et al. [24]</td>
<td>2009</td>
<td>Prospective observational (160 NSTEMI patients)</td>
<td>Neutrophil count</td>
<td>Positively correlate with major in-hospital events (p=0.02), independently predict major in-hospital events (OR: 7.74; CI: 2.79–21.47; p&lt;0.001) and is an independent in-hospital prognostic factor (OR: 6.52; CI: 1.56–27.22; p=0.01)</td>
</tr>
<tr>
<td>Poludasu S. et al. [25]</td>
<td>2009</td>
<td>Prospective observational (327 lower N/L ratio, 45 higher N/L ratio)</td>
<td>N/L ratio</td>
<td>Positively correlates with mortality rate (p&lt;0.001) and is independent long-term mortality predictor (covariates analysis: HR 2.1; CI 1.1–4; p=0.02</td>
</tr>
<tr>
<td>Setianto B. Y. et al. [26]</td>
<td>2010</td>
<td>Case-control (79 patients; 54 AMI, 25 UA)</td>
<td>neutrophil count</td>
<td>Positively correlates with CAD severity. AMI vs UA group (p&lt;0.001).</td>
</tr>
<tr>
<td>Muhmmed Suliman M. A. et al. [27]</td>
<td>2010</td>
<td>Prospective observational (300 ACS patients)</td>
<td>N/L ratio</td>
<td>Positively correlates with all-cause in-hospital mortality (t test: p&lt;0.003)</td>
</tr>
<tr>
<td>Husser O. et al. [28]</td>
<td>2010</td>
<td>Prospective observational (267 reperfused STEMI patients)</td>
<td>Neutrophil count</td>
<td>Positively correlate with larger infarction (p=0.0001), independently predict larger infarction (OR 1.14; CI 1.04–1.26; p=0.008) and major adverse cardiac events (HR 1.2; CI 1.1–1.4; p=0.003).</td>
</tr>
<tr>
<td>Park B.-J. et al. [29]</td>
<td>2011</td>
<td>Cross-sectional (849 healthy controls)</td>
<td>N/L ratio</td>
<td>Positively correlates in multivariate analysis with arterial stiffness (OR 2.12; CI 1.18–3.83; p=0.012) and coronary calcium score (OR 2.19; CI 1.02–4.70; p=0.045)</td>
</tr>
<tr>
<td>O’ Hartaigh B. et al. [30]</td>
<td>2012</td>
<td>Prospective (3316 patient undergoing coronary angiography)</td>
<td>Neutrophil count</td>
<td>Positive predictor of CVD mortality (multivariate analysis: HR 1.93; CI 1.39–2.67; p&lt;0.001).</td>
</tr>
<tr>
<td>Arbel A. et al. [31]</td>
<td>2012</td>
<td>Prospective observational (3005 patient undergoing coronary angiography)</td>
<td>N/L ratio</td>
<td>Positive predictor of CAD presence and severity (OR 2.45; CI 1.76–3.42; p&lt;0.001). Also positively correlated. N/L ratio correlate with CVD events both in crude HR 2.3 (CI 1.72–3.16; p&lt;0.001) than after regression analysis: HR 1.55 (CI 1.09–2.2; p=0.01</td>
</tr>
<tr>
<td>Kaya N. et al. [32]</td>
<td>2012</td>
<td>Case-control (394 repeating angiography; 196 progressive disease and 198 non-progressive disease)</td>
<td>N/L ratio</td>
<td>Positively correlates with progressive disease (multivariate analysis: RR 2.267; CI 1.068–4.815; p=0.003</td>
</tr>
<tr>
<td>Park J. J. et al. [33]</td>
<td>2012</td>
<td>Prospective observational (325 patients with STEMI treated with PTCA)</td>
<td>N/L ratio</td>
<td>Positive predictor of mortality (HR 3.12; CI 1.14–8.55; p&lt;0.05)</td>
</tr>
</tbody>
</table>
Neutrophils in the pathophysiology of plaque rupture

A potential relationship between the risk of plaque rupture and its composition and structure has been recently reported (64). Specific histological features characterise the so-called “vulnerable plaque” (65). The feature that is most highly predictive of plaque rupture is the necrotic lipid core, a mixture of necrotic cellular debris, macrophages, foam cells, growth factors, cytokines and extracellular lipid pools composed of free cholesterol, cholesterol crystals, and cholesterol esters. A large, eccentric lipid core unbalances circumferential plaque stress to the shoulder lesion, where nearly 60% of plaque ruptures tend to occur (66). A necrotic lipid core also contributes to inflammation, thrombosis, proteolytic plaque breakdown, and physical stress at the fibrous cap level (67). The fibrous cap is a connective tissue layer which covers the necrotic lipid core and separates it from the arterial lumen. If necrotic lipid core miss there is no fibrous cap and the plaque cannot rupture. Structural components of the fibrous cap components include matrix molecules such as collagen, elastin, and proteoglycans that are

Table 2: Neutrophil products and cardiovascular risk.

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Mehta J. et al. [40]</td>
<td>1989</td>
<td>Case-control (57 patients; 17 AMI or UA, 20 SA and 20 healthy controls)</td>
<td>HNE</td>
<td>Plasma levels (B peptide) positively correlate with CAD severity. AMI/UA group vs SA (p&lt;0.01) and healthy controls (p&lt;0.001)</td>
</tr>
<tr>
<td>Dinerman J. L. et al. [9]</td>
<td>1990</td>
<td>Case-control (93 patients; 17 AMI, 29 UA, 25 SA and 22 healthy controls)</td>
<td>HNE</td>
<td>Plasma levels (B peptide) positively correlate with CAD severity. AMI (p&lt;0.02) and UA (p&lt;0.006) vs healthy controls</td>
</tr>
<tr>
<td>Bell D. et al. [10]</td>
<td>1990</td>
<td>Case-control (97 patients; 32 AMI, 30 IHD and 35 healthy controls)</td>
<td>HNE</td>
<td>Plasma levels (B peptide) positively correlate with CAD severity. AMI (p&lt;0.0001) and IHD (p&lt;0.004) vs control group</td>
</tr>
<tr>
<td>De Servi S. et al. [41]</td>
<td>1995</td>
<td>Case-control (60 patients; 38UA and 22 SA)</td>
<td>Neutrophil adhesion molecule CD11/CD18b</td>
<td>Plasma levels positively correlate with CAD severity. UA vs SA (p&lt;0.0001)</td>
</tr>
<tr>
<td>Amaro A. et al. [42]</td>
<td>1995</td>
<td>Case-control (42 patients; 19 CAD and 23 not CAD)</td>
<td>HNE</td>
<td>Plasma levels positively correlate with CAD presence (p&lt;0.05)</td>
</tr>
<tr>
<td>Biasucci L. M. et al. [43]</td>
<td>1996</td>
<td>Case-control (86 patients; 16 AMI, 30 UA, 40SA)</td>
<td>MPO</td>
<td>Intracellular MPO was reduced in AMI and UA vs SA (p&lt;0.01)</td>
</tr>
<tr>
<td>Koşar F. et al. [44]</td>
<td>1998</td>
<td>Case-control (185 patients; 135 CAD, 50 not-CAD)</td>
<td>HNE</td>
<td>Plasma levels positively correlate with CAD presence (p&lt;0.001) and plaque complexity (p&lt;0.001)</td>
</tr>
<tr>
<td>Smith F. B. et al. [45]</td>
<td>2000</td>
<td>Prospective observational (207 patients with SA)</td>
<td>HNE</td>
<td>Plasma levels positively correlate with cardiovascular events risk during follow-up (RR: 1.74; CI 1.04–2.95; p&lt;0.005)</td>
</tr>
<tr>
<td>Zhang R. et al. [46]</td>
<td>2001</td>
<td>Case-control (333 patients; 158 CAD, 175 CAD)</td>
<td>MPO</td>
<td>Plasma levels positively correlate with CAD presence (p&lt;0.001). MPO is an independent factors CAD (multivariate analysis: OR 20.4; CI 8.9–47.2, p&lt;0.001)</td>
</tr>
<tr>
<td>Garlíchs C. D. et al. [47]</td>
<td>2003</td>
<td>Case-control (60 patients; 15 AMI, 15 UA, 15 SA and 15 healthy controls)</td>
<td>PMNs apoptosis</td>
<td>Serum of ACS patients inhibits PMNs. AMI/UA vs SA/healthy controls (p&lt;0.001)</td>
</tr>
<tr>
<td>Buffon A. et al. [48]</td>
<td>2002</td>
<td>Case-control (52 patients; 33UA, 13 SA and 6 healthy controls)</td>
<td>MPO</td>
<td>Intracellular MPO inversely correlate with CAD severity. UA vs SA and healthy (p&lt;0.05 for all comparisons)</td>
</tr>
<tr>
<td>Sainchez de Miguel L. et al. [49]</td>
<td>2002</td>
<td>Case-control (69 patients; 31 AMI, 18 UA and 20 healthy controls)</td>
<td>eNOS iNOS</td>
<td>In circulating PMN higher iNOS (p&lt;0.05) and lower eNOS (p&lt;0.05) levels in AMI/UA vs healthy controls</td>
</tr>
<tr>
<td>Smith C. et al. [50]</td>
<td>2006</td>
<td>Case-control (100 patients; 40 UA, 40 SA, 20 healthy controls)</td>
<td>NAP-2</td>
<td>Plasma levels positively correlate with CAD severity. UA vs SA (p&lt;0.05) and healthy controls (p&lt;0.001).</td>
</tr>
</tbody>
</table>
derived from vascular smooth muscle cells (VSMCs). Fibrous cap thickness is one of the most well-known markers of plaque vulnerability. Plaque rupture only occurs when the fibrous cap is extremely thin (68). Additional distinctive features of the vulnerable plaque are represented by: outward remodelling, inflammatory cell infiltration within the fibrous cap and increased plaque neovascularisation (69). Through their activation, adhesion and infiltration, neutrophils might influence all of these parameters of atherosclerotic plaque vulnerability and their multiple roles are actually under investigation in both humans and animal models (5).

The presence of neutrophils was clearly documented in human vulnerable atherosclerotic plaques by Naruko et al. (70) and by Ionita et al. (71). In addition, Leclercq et al. also showed a positive correlation between PMN markers and shoulder haemorrhages as well as in the necrotic core in human atherosclerotic plaque (72).

On the other hand, the study by Zernecke et al. recently showed that neutrophil intraplaque infiltration might be critical also during early atherogenesis (59). In particular, the chronic blockade of neutrophil gelatinases (i.e. MMP-1, -8, and -13), which have been shown to cleave several immune modulatory molecules, such as growth factors (stromal cell-derived factor), cytokines (transforming growth factor [TGF]-β, tumour necrosis factor [TNF]-α, interleukin [IL]-1β), chemokines (IL-8), and endothelin (ET)-1. Another important property is represented by the gelatinase ability to cleave and control their own and other MMP activities (78). PMN granules have also been shown to contain and release some collagenases (i.e. MMP-1, -8, and -13), which were recently described to increase atherogenesis and extracellular matrix degradation in atherosclerotic mice (79). Consistent with this study, MMP-8 has been shown to potentially increase plaque vulnerability, cleaving type I and III collagen and activating angiogenesis (AT)-I conversion to AT-II. Moreover, MMP8 knockout mice exhibited reduced endothelial cell activation as compared to controls.

Neutrophil-derived components in plaque vulnerability

PMNs are activated by micro environmental inflammatory signals, inducing mediators that are contained in their own intracellular granules and vesicles (Figure 1) (73). Neutrophil gelatinases have been shown to play a critical role in mouse atherogenesis and atherothrombosis, as deduced from experiments in knockout animals (74-76).

Gelatinases mainly degrade type IV and denatured collagens (gelatins) but also many matrix substrates: collagen types (I, V, VII, X, and XI), elastin, fibronectin, laminin, aggrecan, vitronectin, brevican, neurocan and decorin (77). In addition, gelatinases have been shown to cleave several immune modulatory molecules, such as growth factors (stromal cell-derived factor), cytokines (transforming growth factor [TGF]-β, tumour necrosis factor [TNF]-α, interleukin [IL]-1β), chemokines (IL-8), and endothelin (ET)-1. Another important property is represented by the gelatinase ability to cleave and control their own and other MMP activities (78). PMN granules have also been shown to contain and release some collagenases (i.e. MMP-1, -8, and -13), which were recently described to increase atherogenesis and extracellular matrix degradation in atherosclerotic mice (79). Consistent with this study, MMP-8 has been shown to potentially increase plaque vulnerability, cleaving type I and III collagen and activating angiogenesis (AT)-I conversion to AT-II. Moreover, MMP8 knockout mice exhibited reduced endothelial cell activation as compared to controls.

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<tbody>
<tr>
<td>Mocatta T. J. et al. [51]</td>
<td>2007</td>
<td>Prospective case-control study (668 patients; 512 AMI, 156 healthy controls)</td>
<td>MPO</td>
<td>Plasma levels positively correlate with ACS (p&lt;0.001) and mortality (OR 1.8; CI 1–3; p=0.034)</td>
</tr>
<tr>
<td>Roman R. M. et al. [52]</td>
<td>2010</td>
<td>Prospective case-control study (308 patients; 130 ACS, 178 SA)</td>
<td>MPO</td>
<td>Plasma levels positively correlate with CRP (r=0.39; p&lt;0.001) and CAD severity (p&lt;0.05) in ACS (not in SA), MPO have prognostic value (OR 3.8; CI 1.2–12; p&lt;0.05)</td>
</tr>
<tr>
<td>Naruko T. et al. [53]</td>
<td>2010</td>
<td>Case-control (467 patients; 236 UA, 146 SA, 85 healthy controls)</td>
<td>MPO</td>
<td>Plasma levels positively correlate with CAD complexity (p&lt;0.0001) in UA (not in SA). MPO is an independent risk factor for complex lesions (multivariate analysis: OR 12.49; CI 3.24–48.17, p=0.0002)</td>
</tr>
<tr>
<td>Cojocaru I. M. et al. [54]</td>
<td>2010</td>
<td>Case-control (150 patients; 78 IS; 72 healthy controls)</td>
<td>MPO</td>
<td>Plasma levels positively correlate with IS (RR 8.188; CI 4.038–16.600; p&lt;0.0001)</td>
</tr>
<tr>
<td>Setianto B. Y. et al. [26]</td>
<td>2010</td>
<td>Case-control (79 patients; 54 AMI, 25 UA)</td>
<td>sCD40L level</td>
<td>Plasma levels positively correlate with neutrophil count in UA (r=0.607, p&lt;0.002)</td>
</tr>
<tr>
<td>Kafkas N. et al. [55]</td>
<td>2012</td>
<td>Case-control (140 patients; 25 STEMI, 40 NSTEMI, 35 UA, 40 SA and 20 healthy controls)</td>
<td>NGAL</td>
<td>Plasma levels positively correlate with CRP (r=0.685; p&lt;0.0001) and neutrophil count (r=0.511, p&lt;0.0001)</td>
</tr>
<tr>
<td>Salonen I. et al. [56]</td>
<td>2012</td>
<td>Prospective (53 patients with ischaemic heart disease)</td>
<td>MPO</td>
<td>Plasma levels positively correlate with PMNs count (p=0–003) and CRP (p=0.02). MPO in an independent risk factor for ATS (no correlation with other CV risk factor).</td>
</tr>
</tbody>
</table>
Elastase is stored at high concentrations PMN azurophilic granules and is primarily detected in the shoulder region of atherosclerotic plaques (81). Elastase substrates include the majority of the components of the extracellular matrix. In particular, elastase-mediated digestion of elastin, collagen, fibronectin and proteoglycans has been shown to expose recognition sites that bind cellular integrin and tyrosine receptors, thus promoting leukocyte chemotaxis (82). More recently, neutrophil elastase has also been demonstrated to cleave the macrophage receptor for hemooglobin-haptoglobin complexes (CD163). Considering that hemooglobin intraplaque accumulation after intraplaque hemorrhages promotes plaque vulnerability, neutrophil elastase may contribute to plaque vulnerability also through this mechanism (83). Azurophilic granules also release a human serine proteinase: proteinase 3 (PR-3) that may promote fibrous cap thinning by its ability to degrade elastin and other macromolecules of the extracellular matrix (such as fibronectin, laminin, vitronectin, and collagen types I, III, and IV) (84). Moreover, PR-3 may activate gelatinases (MMP-2 and MMP-9), thus further contributing to extracellular matrix degradation (85) and to PMN transendothelial recruitment (86). Activated PMNs in atherosclerotic plaque exhibit an abrupt increase in oxygen consumption, known as phagocyte respiratory burst (62) that allows for reactive oxygen species (ROS) generation. NADPH oxidase, the key enzyme in ROS production in PMNs, is a multicomponent complex made of proteins (gp91phox and Gp22phox) inserted in the plasma membrane and of polypeptides located in the cytoplasm (the Gp40phox/Gp47phox/Gp67phox complex). Among the different ROS that are produced as a consequence of NADPH activity, hydrogen peroxide ($\text{H}_2\text{O}_2$) might also act as second messenger, which changes pH and ion flux, triggers proteolytic enzyme release and acts as a myeloperoxidase (MPO) substrate (87). MPO is the major protein in PMN primary granules and catalyses the hydrogen peroxide-mediated oxidation of halide ions to hypochlorous acid (HOCl), chloramines, aldehydes, hydroxyl radicals, singlet oxygen and ozone (88). The first demonstration of MPO within atherosclerotic plaque was in 1994 (89). More recently, Malle et al. immunohistochemically demonstrated a colocalisation of myeloperoxidase and hypochlorite-modified...
proteins in atherosclerotic plaques (90). Subsequent studies have shown that intraplaque MPO expression is an independent predictor of both presence and severity of coronary artery disease (91). MPO-generated ROS might actively promote atherosclerotic plaque vulnerability through different mechanisms. In the first place, MPO-derived oxidants generate peroxided lipids (92), which are recognised by macrophage scavenger receptors (93) and promote the development of the necrotic lipid core. In addition, MPO-generated ROS contribute to extracellular matrix degradation by activating MMP-7, MMP-8 and MMP-9 (94), by increasing PMN survival (95) and by promoting smooth muscle cell switch to a fibroblastic phenotype (96).

Interaction between neutrophils and other atherogenic cells

PMNs were shown to influence the atherosclerotic inflammatory response also through their interaction with both circulating immune cells and vascular cells (Figure 2). Such interactions are mediated through a variety of membrane-bound receptors for endothelial adhesion molecules, extracellular matrix proteins, cytokines and chemokines. Activated PMNs also synthesise and release cytokines and chemokines (such as TNF-α, CXCL-8, interferon [IFN]-γ, CCL3, CCL4, and IL-17), thus recruiting other circulating inflammatory cells or promoting intraplaque cell differentiation (97). For instance, IL-17 producing T-lymphocytes, named Th17, show a marked pro-inflammatory activity (98), which ultimately leads to atherosclerotic lesion progression (99, 100), and also might favor PMN recruitment and activation (101). In turn PMNs exhibit a remarkable chemotactic activity on Th17 (102). Despite a still unclear role in atherogenesis (103), IL-17 might represent a soluble mediator linking T lymphocytes with neutrophils. TNF-α has been shown increase PMN survival and PMN antigen presenting cell properties within inflamed tissues (104, 105). Additionally, the neutrophil product MPO is able to bind to the mannan receptors (MMRs) on resident macrophages, leading to ROS

Figure 2: Interactions between neutrophils and immune and vascular cells in atherogenesis. MPO: myeloperoxidase; ROS: reactive oxygen species; TNF-α: tumour necrosis factor-α; IL-1β: interleukin-1β; IFN-γ: interferon-γ; MIP: macrophage inflammatory protein; GM-CSF: granulocyte-macrophage colony-stimulatory factor; APC: antigen presenting cells; G-CSF: granulocyte colony-stimulatory factor; EC: endothelial cell; VSMC: vascular smooth muscle cell; ECM: extracellular matrix.
Role of neutrophils in atherothrombosis and coronary neo-intima formation after percutaneous transluminal coronary angioplasty (PTCA)

Recently, atherothrombosis emerged as a highly complex event, in which not only activated platelets (PLTs), but also PMNs have emerged as critical players within the coronary thrombus (122). PLTs were shown to promote PMN adhesion and activation enhancing chemokine release (CXCL4, CXCL7 and CCL5) and expression of inflammatory receptors, co-stimulatory molecules and scavenger receptors in vitro and in a mouse model of atherosclerosis (123, 124). The CD40-CD40L co-stimulatory pathway has also been suggested to be involved in the interaction between PLTs and PMNs. Through soluble CD40L release, PLTs stimulate PMNs to release ROS, which, in turn, activate PLTs (125). Specifically, this mechanism was proposed to contribute to neointima formation after arterial injury (126). PMNs might also activate PLTs by a novel mechanism only recently described as a part of the innate immune response: the formation of neutrophil extracellular traps (NETs), a meshwork of extracellular DNA fibers comprising histones and antimicrobial proteins. Fuchs et al. have demonstrated the role of NETs in PLT activation, adhesion and aggregation in vitro (127), further supporting a direct role for PMNs in atherothrombosis.

A potential protective role for PMNs in neointima formation after both balloon angioplasty and stenting has recently been proposed. PTCA is a clinically effective method for improving blood flow through stenosed or occluded arteries (128). However, restenosis, resulting from neointimal hyperplasia, negative remodelling, and elastic recoil limit the long-term success of this procedure (129). PTCA frequently induces a severe injury that would expose to the blood flow the neointima composed of a mixture of smooth muscle cells and inflammatory cells (130). Although their role is still under debate, an early inflammatory response to arterial injury is characterised by PMN influx. Similarly to early atherogenesis, the pathophysiological recruitment of PMNs within neointima might be related to the dysregulation of the CXCL12/CXCR4 axis that might influence neutrophil-mediated pro-atherosclerotic activities (59). Studies on β2 integrins (131, 132) and P-selectin (133) defective mice, showing a reduction in neointima thickness, suggested that PMN adhesion promotes restenosis. However, subsequent studies demonstrated that both β2-integrins and P-selectin are poorly specific for PMNs (134). Likewise, the blockade of the chemokine receptor CXCR2 (critical for neutrophil chemotaxis towards CXCL1) was found to delay, rather than promoting, endothelial recovery after arterial injury (135, 136). Recently, Xing et al. assessed the role of PMNs in neointima formation by administering IL8RA- and/or IL8RB-transduced endothelial cells (ECs) to a rat of vascular injury. An accelerated re-endothelialisation of the injured area in association with downregulated expression of inflammatory mediators, PMN infiltration, and neointima formation were observed (137). Potential direct interactions between PMNs, ECs and EPCs have been recently demonstrated by Soehnlein et al. PMN-derived cathelicidin (LL-37) promotes re-endothelialisation and thereby limited neointima formation after stent implantation. Drawing from these findings, the authors generated a LL-37-coated stent. LL-37-trigger EPC-mediated function resulting in pro-angiogenic factors release (VEGF and EGF) and enhancing EC migration and proliferation and reducing their apoptosis (138). Overall, a potential protective role for PMNs in PTCA and stenting appears highly likely, with these cells possibly acting as potential promoters of re-endothelialisation. However, these conclusions still await further confirmation.

Post-infarction cardiac remodelling

Following infarction, the myocardium undergoes major changes both in its function and structure (139). Dying cardiomyocytes together with damaged extracellular matrix (ECM), activate a reparative response and an immune reaction, the latter being initially...
characterised by the involvement of innate immunity cells. Multiple innate immune pathways (such as toll-like receptors [140, 141]), receptors for Advanced Glycation End-product (AGEs) [142], complement system [143] and ROS [144, 145] were shown to be active in this phase. Through intracellular signalling cascades, these signals converge to activate nuclear factor (NF)-κB [146], which, in turn, induces the synthesis of inflammatory cytokines, such as TNF-α [147], IL-1β [148], IL-6, and chemokines, such as CCL2, CCL4, CXCL1, CXCL8 [149]. Although strongly recruited at the infarction site, PMNs are unlikely to have an important role in late remodelling processes: from 3 to 7 days after the acute event, PMN infiltration resolves in both mouse and canine models [150]. In addition, no significant long-term effects of neutrophil depletion in animal models of acute MI could be detected [151, 152]. Indeed, PMNs may contribute to the healing response: the release of apoptotic mediators (such as annexin A1 and lactoferrin) was shown to inhibit further PMN recruitment [153] and to promote the release of anti-inflammatory molecules, such as IL-10 and TGF-β [57]. On the other hand, neutrophils have also been shown as pivotal players in post-infarction healing by potentially favouring the recruitment of inflammatory monocytes [118], instead of the reparatory monocytes [154]. In fact, Soehnlein et al. showed that PMN components (i.e. PMN-derived LL-37 and heparin-binding protein [HBP/CAP37/azurocidin]) might directly activate inflammatory monocyte extravasation in mice [118]. Although highly speculative, the early release of these neutrophilic mediators in mice might affect the cardiac recruitment of different monocytes subsets (Ly-6C[high] and Ly-6C[low] mononuclear cells, respectively) in the first week post-infarction [154]. The relevant activation of monocytes in the first phases after an acute ischaemic event (such as stroke of acute MI) was recently confirmed by Dutta et al., showing that a marked increased monocyte intraplaque recruitment might accelerate atherogenesis in ApoE/- mice submitted to an acute MI [155]. Vice versa, the detrimental role of PMN infiltration in the first hours after cardiac ischaemia is well known. The reperfusion of the ischaemic myocardium is necessary to rescue tissue from death [156]. However, at the same time, the restored blood flow halts the ischaemic process by supplying oxygen and nutrients, but triggers a cascade of inflammatory and toxic mediators [157]. Frame et al. [158] and Farb et al. [159] found more important signs of myocardial injury after reperfusion than after prolonged ischaemia, while Rochitte et al. [160] documented a progressive increase in tissue damage and microvascular obstruction during the hours after reperfusion. Over the last two decades, experimental evidence suggested that PMNs may also directly damage cardiomyocytes through the release of toxic products, such as reactive oxygen species and proteolytic enzymes [161]. In particular, compelling evidence for a key role of PMNs in ischaemia-reperfusion-mediated myocardial damage stems from studies of pharmacologic and genetic interventions aimed to prevent PMN recruitment or function. For instance, Liehn et al. investigated the role of CXCL12/CXCR4 axis in an experimental model of acute MI in mice [162]. Using heterozygous (+/−) CXCR4 animals (characterised by a lower expression of CXCR4 on bone marrow-derived mononuclear cells) [163], the authors showed that these mice developed a reduced scar area at four weeks post-MI when compared to wild type littermates [162]. Importantly, this beneficial remodelling was associated with reduced infarct size and leukocyte infiltration (i.e. neutrophils and monocytes) in the early hours after the acute MI [162]. This article confirmed that early interference with PMN recruitment after an acute MI might be a promising therapeutic approach to reduce infarct size and adverse cardiac remodelling after an acute MI [162].

Treatments targeting PMN activities in coronary atherogenesis and post-infarction cardiac remodelling

Statins

Statins are a class of lipid-lowering drugs more effectively through their action on 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA), the rate-limiting enzyme of the mevalonate pathway for cholesterol synthesis (164). The beneficial effects of statins are clearly related to the reduction in cholesterol levels they induce, but also to their complex “pleiotropic” anti-inflammatory activity. Dunzendorfer [165] and Kaneider [166] were the first to document the ability of statins to induce leukocyte apoptosis and inhibit leukocyte transendothelial migration [167-171]. Statins were shown to inhibit PMN migration and activation in vitro. Cerivastatin inhibited adhesion molecule expression (ICAM-1, P-selectin, and E-selectin) on endothelial cells, thus preventing neutrophil adhesion to endothelial cells [172, 173]. Bandoh et al. [174] and Kowalski et al. [175] reported the anti-oxidant effect of fluvastatin and atorvastatin, which reflects the ability of these drugs to scavenge ROS and to inhibit NADPH-dependent ROS generation. The metabolic effects of statins are not limited to cholesterol, but also affect cholesterol intermediate products that are involved in several cell-signalling pathways. Isoprenoids, such as geranylgeranyl pyrophosphate (GGP) and farnesyl pyrophosphate (FPP), promote the activation of GTPases, including RhoA [176]. This protein, together with its effector Rho kinase, is thought to control cytoskeleton rearrangement, cell migration and ROS generation [177] in inflammatory cells. Statins were shown to modulate the levels of these metabolites and, as a result, to dampen PMN migration [178] and platelet-PMN interaction [179]. Finally, Nakamura et al. showed that fluvastatin treatment in atherosclerotic mice suppressed atherosclerotic plaque rupture by reducing MMP-9 expression, endothelial adhesion molecule expression and PMN infiltration thus confirming pleiotropic anti-inflammatory properties for statins. Such effects may reflect, at least in part, a direct anti-inflammatory activity of fluvastatin on PMNs [180].

HDL and HDL apolipoproteins

High-density lipoprotein (HDL)’s beneficial role is complex and likely involves several mechanisms, including anti-inflammatory effects [181]. HDL and apolipoprotein(a)-I regulate PMNs activation, as detected by CD11b expression, by decreasing lipid rafts abundance [182], specialised plasma membrane areas with a role
in oxidative burst, calcium channel trafficking (183) and NADPH oxidase activity (184). In addition, apo lipoproteins inhibit PMN-mediated release of TNF-α, IL-1, IL-8, IL-1 receptor antagonist (IL-1Ra) and degranulation (185, 186). In vivo, infusion of HDL or apoA-I significantly attenuates PMN intima infiltration in rabbits (187, 188), by acting both on endothelial cell activation (188, 189) and circulating PMNs (190-192). This potential protective role of HDL on leukocyte-mediated pro-atherosclerotic activities was partially confirmed by Yvan-Charvet et al., who recently showed that transplantation of bone marrow knockout for adenosine triphosphate-binding cassette (ABC) transporters (Abca1 and Abcg1) into apolipoprotein A-1 transgenic mice (animal with elevated HDL) was associated with a reduced leukocytosis, and accelerated atherogenesis (193). This study indicated that Abca1, Abcg1 in presence of elevated HDL levels might abrogate both conditions. More recently, Murphy et al. using the same knockout mice (characterised by leukocytosis due to the high proliferation of hematopoietic progenitor cells in the bone marrow) identified a specific role for the proteoglycan-bound ApoE on these leukocyte progenitors to promote cholesterol efflux via Abca1/Abcg1, resulting in a final abrogation of leukocytosis and atherosclerosis in mice (194). Finally, Westerterp et al. showed that the increase in HDL levels was inversely associated with hematopoietic progenitor cell mobilisation in the same mouse model (knockout for Abca1, Abcg1 and ApoE) characterised by atherosclerosis and concomitant leukocytosis (195). Taken together, these interesting studies from animal models might support cholesterol efflux pathways as potential therapeutic targets to reduce neutrophil-mediated activities in atherosclerosis.

**Treatments that selectively target PMN chemokines**

Tissue reoxygenation during reperfusion triggers an inflammatory reaction that is characterised by high levels of cyto-chemokines and consequent leukocyte recruitment, potentially causing greater damage to affected organs than ischaemia itself. Chemokine-induced PMN recruitment and activation within ischaemic organs is believed to be a pivotal mechanism of reperfusion injury. CXC chemokines, including CXCL1, 2, 3, 5 (ligands of CXCR2) and CXCL6–8 (ligands of both CXCR1 and CXCR2) were shown to attract neutrophils in both human and mouse atherosclerotic plaques and infarcted tissues (196). On the other hand, CC chemokines have been considered as critical neutrophil chemoattractants in certain inflammatory microenvironments mimicking the atherosclerotic plaque and the ischaemic myocardium (197-199). Thus, selective blockade of CC and CXC chemokines has been evaluated as a therapeutic strategy to improve the outcomes of MI in several animal models. Monoclonal antibody treatments against CXCL8 (200) or CCL5 (201), chemokine-binding proteins (202), as well as CXCR1/2 competitive antagonists (203) were recently investigated both in vivo and in vitro with promising preliminary results. Widdowson et al. firstly illustrated the therapeutic potential of CXCR1/2 blockade using IL-8 inhibiting molecules that were identified from phenolic urea series (203). Maher et al. documented the efficacy of monoclonal antibodies against CXCL8 in reducing inflammation, and therefore the symptoms, in patients with chronic obstructive pulmonary disease (COPD) (200). However, the authors did not focus on cardiovascular outcomes in this study. In 2010, in a mouse model of acute MI/reperfusion injury, we demonstrated that treatment with the CXC chemokine-binding protein Evasin-3 was associated with a reduction in myocardial infarct size and post-ischaemic inflammation. These beneficial effects were closely related with the inhibition of cardiac neutrophil recruitment and ROS production. On the other hand, in contrast with previously reported data (204), Evasin-3 was ineffective in a Langendorff perfused heart model, an ex vivo experimental system characterised by absence of circulating leukocytes (202).

In 2012, evidence from our laboratory suggested a pivotal role for another neutrophilic chemokine (i.e. CCL5) in two mouse models of chronic cardiac ischaemia and transient ischaemia followed by reperfusion. This CC chemokine has been shown as a crucial chemoattractant for macrophages both in mouse atherogenesis and MI (198, 199) via the binding to two main transmembrane receptors (such as CCR1 and CCR5). Since several intra-plaque and circulating cell subsets (including platelets, macrophages) are might release CCL5, multiple sources of this chemokine might be considered in atherogenesis. For instance, Drechsler et al. showed that platelet-derived CCL5 was deposited on endothelial cells of the in arteries but not in veins in hypercholesterolaemic mice (60). In addition, the selective activation of receptors has been shown to play different activities in cardiovascular diseases. For instance, Liehn et al. showed that the main receptor for CCL5-mediated neutrophil recruitment after MI was CCR1 and not CCR5 (205). Considering these premises, we firstly confirmed that CCL5 was upregulated both locally (within the infarcted myocardium) and systemically early after the onset of myocardial chronic ischaemia onset (201) and during reperfusion (198). Then, in the first 24 hours, we focused on the pharmacological inhibition of CCL5. This approach potently reduced neutrophil recruitment and inflammation within infarcted hearts, improving infarct size when compared to control vehicle. At 21 days of chronic ischaemia, the pharmacologic inhibition of CCL5 (in the first three days after ischaemia onset) was associated with improved mouse survival, cardiac myocyte mass and function (201).

More recently, evidence from our research group also showed that treatment with FK866 (a nicotinamide phosphoribosyltransferase [Nampt] inhibitor that lowers NAD⁺ levels) potently reduces CXC chemokine serum levels, neutrophil infiltration in the infarcted heart and myocardial injury following ischaemia-reperfusion in mice. This treatment approach also ROS formation in the heart (likely as a consequence of the reduced PMN infiltration of the ischaemic tissue), thus suggesting reducing the levels of CXC chemokines may induce benefits that are similar to those achieved with strategies that inhibit chemokine bioactivity instead (206). The mechanisms underlying FK866 ability to prevent CXC chemokine production appears to entail a reduced activity of SIRT6, an NAD⁺-dependent deacetylase which we have recently shown to promote inflammatory cytokine expression by boosting...
Ca²⁺ responses and Nuclear Factor of Activated T-cells (NFAT) activity (207).

Conclusions and perspectives

Amongst the different inflammatory cell types, PMNs are now increasingly appreciated as pivotal players in coronary plaque vulnerability, neointima formation and the first phases of myocardial reperfusion injury and adverse remodelling. Compelling in vivo studies have demonstrated a strong therapeutic potential for PMN-targeting treatments, including those aimed to dampen chemokine levels or bioactivity. Although human clinical studies are still lacking, selective treatments targeting chemokine-mediated PMN recruitment and activation within atherosclerotic and infarcted myocardium are predicted to have a strong impact on cardiovascular disorders and to help improve clinical outcomes.

Conflicts of interest

None declared.

References


Carbone et al. Neutrophils in acute myocardial infarction

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