The role of inflammatory biomarkers in developing targeted cardiovascular therapies: lessons from the cardiovascular inflammation reduction trials

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Abstract

Anti-inflammatory add-on therapy to conventional cardiovascular prophylaxis has been proposed as a novel therapeutic approach to potentially reduce residual cardiovascular risk. This hypothesis has been challenged by a series of unsuccessful Phase III studies testing the impact on clinical outcomes of novel agents with immunomodulatory actions. Specifically, the apparent ability of phospholipase A2 (PLA2) inhibitors and of antioxidants to ameliorate inflammation and to reduce coronary disease in Phase II trials did not translate into improved secondary cardiovascular prevention in larger population-based studies. Other anti-inflammatory agents are still under scrutiny. However, studies to date have lacked information on the inflammatory profile of the participants, both at baseline and at follow-up, thereby limiting the possibility of identifying subgroups of patients in whom ‘residual inflammation’ can be detected despite optimal conventional therapy, and who could therefore benefit from a cardiovascular prevention strategy specifically targeting inflammation. This has also rendered it difficult to interpret the results as a conclusive demonstration of inefficacy of the tested anti-inflammatory strategies in the treatment of atherosclerosis. We here discuss the importance of better patient characterization to minimize heterogeneity of the study population, so that effectiveness of different anti-inflammatory strategies can be evaluated in targeted subgroups of patients. We also illustrate how specific inflammatory biomarkers could assist in this process, with particular emphasis on the roles of high-sensitivity C-reactive protein and circulating monocyte phenotype.

Keywords

Atherosclerosis • Biomarkers • Inflammation

1. Introduction

A new trend has emerged in cardiovascular pharmacotherapy over recent years, with the advent of anti-inflammatory strategies as add-on therapy to conventional prophylaxis to reduce residual cardiovascular risk. A number of novel molecules targeting selected inflammatory pathways have been tested in Phase III secondary prevention trials, including lipoprotein-associated phospholipase A2 (Lp-PLA2) inhibitors,1,2 soluble (s)PLA2 inhibitors,3 and antioxidants.4 Despite promising results as regards anti-inflammatory efficacy being obtained in Phase II trials (Tables 1 and 2), these agents have proved unsuccessful in reducing cardiovascular event rates in large population-based studies (Table 3). Other molecules targeting different inflammatory pathways are still under scrutiny, and Phase III trial results with these are awaited to determine whether their pharmacological action translates into improved cardiovascular prophylaxis.

The rationale underlying this approach is based upon the large experience accumulated with the use of statins in primary and secondary prevention, which has suggested that the magnitude of their efficacy in preventing cardiovascular events is not linearly and simply related to their hypolipidaemic action: but rather, over and above this, their efficacy can also be partly attributed to their ability to lower C-reactive protein.18–22 The question then has arisen of whether reduction of inflammation per se is protective against atherosclerosis progression and positively impacts on clinical outcomes, independently of traditional cardiovascular risk factor correction.

The disappointing results obtained with the anti-inflammatory strategies alluded to above seem at first sight to stand in stark contrast to the hypothesis that ‘residual CV risk’ implies ‘residual inflammation’ as a casual factor. However, it is clear and noteworthy that these studies to date have not characterized the inflammatory profile of the study population (Table 3). This is likely to have led to the recruitment of a
<table>
<thead>
<tr>
<th>Phase II studies</th>
<th>Dose</th>
<th>Study population and sample size</th>
<th>Endpoints</th>
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<th>Baseline hs-C-reactive protein levels and statin use</th>
<th>Outcomes</th>
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</thead>
<tbody>
<tr>
<td>Atreleuton(^5) VIA-2291</td>
<td>25 mg daily</td>
<td>ACS &lt; 3 weeks prior to recruitment 191 pts</td>
<td>Primary: plasma LTB4; Secondary: urine LTE4 and hs-C-reactive protein</td>
<td>12 weeks; 24 weeks for subgroup undergoing coronary CT</td>
<td>Median hs-C-reactive protein ≤ 2 mg/L</td>
<td>Significant plasma LTB4 reduction in a dose-dependent manner at 12 and 24 weeks. No effects on hs-C-reactive protein at 12 weeks. 67% decrease in hs-C-reactive protein vs. baseline in the 100-mg group and at 24 weeks (no effects at lower doses). Significant reduction in non-calcified plaque volume vs. placebo in the 50 mg group and at 24 weeks.</td>
</tr>
<tr>
<td>Velipaplon (DG-031)(^6)</td>
<td>Cross-over with 250 mg daily 500 mg daily 750 mg daily or placebo</td>
<td>Prior MI in patients carrying at-risk FLAP and LTA4 gene variants 191 pts</td>
<td>Primary: plasma LTB4, MPO, hs-C-reactive protein associated with risk of MI. Secondary: to establish dose dependence</td>
<td>4 weeks</td>
<td>Mean hs-C-reactive protein &lt; 1 mg/L. Around 80% patients on statins</td>
<td>Dose-dependent reduction in plasma LTB4 and MPO vs. baseline that reached statistical significance in the group of 750 mg daily. No effects on hs-C-reactive protein and hs-C-reactive protein reduction vs. placebo over the first 7 days. hs-C-reactive protein reduction vs. placebo at 7 and 14 days; rebound effect in hs-C-reactive protein and 30 days with significant increase in anakinra group vs. placebo. No effects on other variables. MACE at 1 and 3 months similar between groups; at 1 year, there was a significant excess of events in the IL-1ra group.</td>
</tr>
<tr>
<td>Anakinra(^7)</td>
<td>100 mg daily or placebo during the first 14 days post-MI</td>
<td>NSTE-ACS &lt; 48 h prior to recruitment 182 pts</td>
<td>Primary: hs-C-reactive protein over the first 7 days. Secondary: hs-C-reactive protein at 7, 14, and 30 days post-MI; troponin-I, vWF and IL-6, ST-depression on ECG-Holter, LVF on MRI MACE: death, stroke, and new MI at 1, 3, and 12 months</td>
<td>1 year</td>
<td>Mean hs-C-reactive protein &gt; 5 mg/L 95% patients on statins</td>
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</tr>
<tr>
<td>Darapladib(^8)</td>
<td>40 mg daily, 80 mg daily, 160 mg daily, or placebo</td>
<td>Stable CHD or CHD-risk equivalent 959 pts</td>
<td>Primary: inhibition of plasma Lp-PLA2 activity. Secondary: dose–response of darapladib and changes in inflammatory and platelet-related biomarkers</td>
<td>12 weeks</td>
<td>Mean hs-C-reactive protein of 1.17 mg/L 100% patients on atorvastatin (20 or 80 mg).</td>
<td>Dose-dependent reduction in plasma Lp-PLA2 activity at 4 and 12 weeks. hs-C-reactive protein reduction in the 160 mg group only at 12 weeks; no effects on P-selectin, CD40 ligand, urinary 11-dehydrothromboxane B2.</td>
</tr>
<tr>
<td>Treatment</td>
<td>Dose/Regimen</td>
<td>Condition</td>
<td>Primary/Secondary Endpoints</td>
<td>Follow-up</td>
<td>Results</td>
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<tr>
<td>Darapladib (IBIS)</td>
<td>160 mg daily or placebo</td>
<td>ACS and coronary stenosis &gt;50% 330 pts</td>
<td>Coronary atheroma deformability (assessed by intravascular ultrasound palpography) and hs-C-reactive protein</td>
<td>12 months</td>
<td>Median hs-C-reactive protein of 2.4 mg/L 90% of patient on statins No significant differences between groups in plaque deformability and total volume Darapladib significantly counteract necrotic core expansion vs. placebo group No effects on hs-C-reactive protein</td>
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<tr>
<td>Darapladib (PLASMA I)</td>
<td>50 mg bd, 100 mg bd, 250 mg bd, 500 mg bd or placebo</td>
<td>Stable CHD 393 pts</td>
<td>Primary: sPLA2 concentration or activity. Secondary: inflammatory markers, lipid profile</td>
<td>8 weeks</td>
<td>hs-C-reactive protein between 14.3 and 22.7 nmol/L (equal to 1.5 and 2.38 mg/L) &gt;60% on statins Dose-dependent reduction in sPLA2</td>
<td>55.6% reduction in hs-C-reactive protein at Week 8, compared with 24.8% reduction in the placebo arm</td>
</tr>
<tr>
<td>Darapladib (PLASMA II)</td>
<td>250 mg daily, 500 mg daily or placebo</td>
<td>Stable CHD 135 pts</td>
<td>sPLA2 concentration, lipid profile and inflammatory biomarkers</td>
<td>8 weeks</td>
<td>Mean hs-C-reactive protein at baseline ranged from 1.1 to 1.3 mg/L in the different groups ~90% patient on statins Dose-dependent reduction in sPLA2 concentration LDL-cholesterol, and non-HDL cholesterol No effect on hs-C-reactive protein</td>
<td></td>
</tr>
<tr>
<td>Darapladib (FRANCIS)</td>
<td>500 mg daily or placebo</td>
<td>ACS within 96 h 652 pts</td>
<td>Primary: LDL-cholesterol and hs-C-reactive protein Secondary: MACE (unstable angina, non-fatal MI and stroke, urgent revascularization, and death)</td>
<td>6 months</td>
<td>Median hs-C-reactive protein 8.2 and 8.9 mg/L in varespladi and placebo groups, respectively 100% patients on atorvastatin 80 mg daily Significant reduction in LDL-cholesterol and hs-C-reactive protein vs. placebo arm at all time points (Weeks 8, 16, and 24). No effects on MACE at 6 months</td>
<td></td>
</tr>
<tr>
<td>Succinobucol CART-2</td>
<td>280 mg qd or placebo</td>
<td>Stable CHD 232 pts</td>
<td>Atherosclerosis progression assessed by IVUS and plasma lipids, MPO, and hs-C-reactive protein</td>
<td>12 months</td>
<td>hs-C-reactive protein not specified &gt;90% patients on statins Significant regression in plaque volume vs. baseline in the active arm vs. Placebo No effects on hs-C-reactive protein</td>
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</tr>
</tbody>
</table>

LTB4, leukotriene B4; LTE4, Leukotriene E4; FLAP, 5-lipoxygenase-activating protein; LTA4H, leukotriene-A4 hydrolase; hs-C-reactive protein, high-sensitivity C-reactive protein; Lp-PLA2, lipoprotein-associated phospholipase A2; sPLA, secreted phospholipase A2; MACE, major adverse cardiovascular events; MI, myocardial infarction; NSTE-ACS: non-ST elevation acute coronary syndrome; CHD, coronary heart disease; IVUS, intravascular ultrasonography.
### Table 2: Effect of anti-inflammatory molecules on metabolic profile and hs-C-reactive protein

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Targeted pathway</th>
<th>Mechanism of action</th>
<th>Study population</th>
<th>Effects on lipid profile and glucose metabolism</th>
<th>Effects on hs-C-reactive protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atreleuton6</td>
<td>Leukotriene</td>
<td>Inhibitor of 5-LO</td>
<td>ACS &lt;3 weeks prior to randomization</td>
<td>LDL-cholesterol increase by 8% with 750 mg daily; no effects at lower doses (250 and 500 mg daily); Effects on glucose metabolism not addressed</td>
<td>67% reduction vs. baseline in response to 100 mg daily for 24 weeks, but no effects at lower doses (25 mg or 50 mg daily)</td>
</tr>
<tr>
<td>Veliflapon6</td>
<td>Leukotriene</td>
<td>Inhibitor of FLAP</td>
<td>Prior MI in patients carrying at-risk FLAP and LTA4 gene variants</td>
<td>LDL-cholesterol increase by 8% with 750 mg daily; no effects at lower doses (250 and 500 mg daily); Effects on glucose metabolism not addressed</td>
<td>25% reduction vs. baseline in response to 750 and 500 mg daily for 2 weeks and following 2 weeks of wash-out</td>
</tr>
<tr>
<td>Anakinra14</td>
<td>IL-1 pathway</td>
<td>Inhibitor of IL-1RI</td>
<td>Type 2 diabetic patients</td>
<td>Effect on lipid profile not explored; 0.33% reduction of HbA1c vs. baseline with 100 mg daily for 13 weeks</td>
<td>4% reduction vs. baseline in response to 100 mg daily for 4 and 13 weeks of treatment</td>
</tr>
<tr>
<td>Gevokizumab15</td>
<td>IL-1 pathway</td>
<td>Humanized mAb targeting IL-1 receptor</td>
<td>Type 2 diabetic patients</td>
<td>Effect on lipid profile not addressed; 0.11, 0.44, and 0.85% reduction of HbA1c vs. baseline after 1, 2, and 3 months of treatment in the combined intermediate-dose group (single doses of 0.03 and 0.1 mg/kg)</td>
<td>Around 2 mg/L reduction vs. baseline after 14 days of treatment in the combined intermediate-dose group (single doses of 0.03 and 0.1 mg/kg)</td>
</tr>
<tr>
<td>Canakinumab16</td>
<td>IL-1 pathway</td>
<td>Humanized mAb targeting IL-1 receptor</td>
<td>Type 2 diabetic patients</td>
<td>Neutral on LDL; 10% increase in triglycerides with 50 and 150 mg monthly for 4 months (but not at lower doses of 5 and 15 mg daily)</td>
<td>36.4, 53.0, 64.6, and 58.7% reduction vs. baseline for 5, 15, 50, and 150 mg monthly, respectively, for 4 months</td>
</tr>
<tr>
<td>Tocilizumab17</td>
<td>IL-6 pathway</td>
<td>Humanized mAb targeting IL-6 receptor</td>
<td>Patients with RA</td>
<td>Increase of total and LDL-cholesterol, and triglycerides</td>
<td>19.02 mg/L vs. placebo in response to 8 mg/kg every 4 weeks, treatment duration between 12–52 weeks</td>
</tr>
<tr>
<td>Darapladib8</td>
<td>Ox-LDL</td>
<td>Inhibitor of Lp-PLA2</td>
<td>CHD or CHD-risk equivalent coronary disease angiographically documented</td>
<td>Neutral on lipid profile</td>
<td>20.2% reduction vs. baseline with 160 mg once daily for 12 weeks; no effects at lower doses neutral with 160 mg once daily for 12 weeks</td>
</tr>
<tr>
<td>Darapladib IBIS12</td>
<td>Ox-LDL</td>
<td>Inhibitor of Lp-PLA2</td>
<td>CHD or CHD-risk equivalent coronary disease angiographically documented</td>
<td>Neutral on lipid profile</td>
<td>20.2% reduction vs. baseline with 160 mg once daily for 12 weeks; no effects at lower doses neutral with 160 mg once daily for 12 weeks</td>
</tr>
<tr>
<td>Varespladib10</td>
<td>Ox-LDL</td>
<td>Inhibitor of sPLA2</td>
<td>Stable CHD: Stable CHD as above ACS &lt; 96 h prior to randomization</td>
<td>6.6% reduction in LDL</td>
<td>55.6% reduction vs. baseline (combined dose groups 50, 100, 250, and 500 mg twice daily for 8 weeks) Neutral with either 250 or 500 mg once daily; however, 22% increase in placebo group was observed 79.8% reduction vs. baseline with 500 mg once daily for 24 weeks</td>
</tr>
<tr>
<td>Succinobucol13</td>
<td>Antioxidant</td>
<td>Antioxidant</td>
<td>Stable CHD</td>
<td>Neutral with 280 mg qd for 12 months</td>
<td>Neutral with 280 mg qd for 12 months</td>
</tr>
</tbody>
</table>

Data are extrapolated from Phase II trials, with the exception of tocilizumab for which the reported information are from a meta-analysis. 5-LO, arachidonate 5-lipoxygenase; FLAP, 5-LO-activating protein; CCL2, chemokine CC motif ligand 2; CCR2, chemokine CC motif receptor 2; IL-1RI, IL-1 type I receptor; IL-1Rb, IL-1 beta receptor; Lp-PLA2, lipoprotein-associated phospholipase A2; sPLA2, secreted phospholipase A2.
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<th>Baseline hs-C-reactive protein and statin use</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Darapladib SOLID-TIMI52¹</td>
<td>160 mg daily or placebo</td>
<td>ACS 30 days prior to recruitment 13 026 pts</td>
<td>Primary: major coronary events (composite of CHD death or urgent revascularization for myocardial ischaemia) Secondary: composite of CV death, MI, and stroke</td>
<td>Median of 2.5 years</td>
<td>hs-C-reactive protein unknown &gt;90% patients on statins</td>
<td>No difference between groups in primary and secondary endpoints</td>
</tr>
<tr>
<td>Darapladib STABILITY²</td>
<td>160 mg daily or placebo</td>
<td>Stable CHD 15 828 pts</td>
<td>Primary: composite of CHD death, MI, or stroke Secondary: coronary events (a composite of CHD death, MI, or urgent revascularization)</td>
<td>Median of 3.7 years</td>
<td>hs-C-reactive protein unknown 96% patients on statins</td>
<td>No difference between groups in primary and secondary endpoints, with the exception of mild reduction in major coronary events (NNT = 100)</td>
</tr>
<tr>
<td>Varespladib VISTA-16³</td>
<td>500 mg daily or placebo for 6 months</td>
<td>ACS &lt; 96 h prior to recruitment 5 145 pts</td>
<td>Primary: composite of CV mortality, non-fatal MI, non-fatal stroke, or hospitalization for angina with evidence of ischaemia Secondary: composite of CV mortality, each component of primary outcomes and inflammatory markers</td>
<td>16 weeks</td>
<td>Median hs-C-reactive protein of 10.4 and 11.4 mg/L in varespladib and placebo group, respectively &gt;100% patients on statins</td>
<td>Increased CV mortality due to higher MI event rates in the active treatment compared with placebo. The trial was stopped for safety issues</td>
</tr>
<tr>
<td>Succinobucol ARISE⁴</td>
<td>300 mg daily or placebo</td>
<td>Stable CHD 6144 pts</td>
<td>Primary: CHD death, resuscitated cardiac arrest, non-fatal MI, non-fatal stroke, hospitalization for unstable angina with evidence of ischaemia, or coronary revascularization Secondary: primary endpoints including all death instead of CHD death Tertiary: hospitalization for heart failure, glycaemic control in diabetes, new-onset diabetes, and atrial fibrillation</td>
<td>Mean of 2 years</td>
<td>hs-C-reactive protein unknown 91% patients on statins</td>
<td>No difference between groups in primary and secondary endpoints</td>
</tr>
</tbody>
</table>
heterogeneous study population, solely characterized on the basis of clinical assessment, within which one would expect considerable heterogeneity in effectiveness of anti-inflammatory therapy depending on subjects’ initial inflammatory profile, thereby diluting any effect that may have occurred in subgroups of patients in whom ‘residual inflammation’ may persist despite standard of care.

Another important consideration relates to the most appropriate method to use in quantifying inflammation in cardiovascular disease. C-reactive protein has been the most widely used biomarker in this setting, in line with the approach used in clinical trials of statins, and also in recognition of the fact that this is the only reproducible indicator for which a standardized assay has been developed. Ridker and Lüchner have emphasized the usefulness of this biomarker in the development of immunomodulatory strategies for the treatment of atherosclerosis, so that they have recently proposed a classification of anti-inflammatory agents based upon their ability to reduce C-reactive protein levels, as seen in Phase II trials (Table 2). Hence, a distinction has been made between strategies directly targeting the IL-6 signalling pathway that ultimately results in powerful C-reactive protein modulation (namely anti-cytokines and MTX) and those inhibiting alternative pathways (PLA2 inhibitors and antioxidants), which have exhibited variable degrees of efficacy in reducing C-reactive protein concentration. However, such categorization of these agents may generate a misleading interpretation of the results. Indeed, C-reactive protein has limited specificity for atherosclerosis-related inflammation, and its prognostic value for future cardiovascular events in patients who are on optimal prophylactic medications (including anti-platelet agents and statins) is particularly weak in secondary prevention. Modulation of this biomarker in response to a particular drug in cardiovascular patients could merely reflect an anti-inflammatory action unrelated to atherosclerosis; on the other hand, lack of therapeutic effect on C-reactive protein may not necessarily imply failure in modulating arterial inflammation. We here comment on the adequacy of C-reactive protein to monitor the effect of anti-inflammatory strategies in cardiovascular prevention and highlight those aspects that limit its diagnostic value in this clinical setting. Novel biomarkers indicative of a deregulation of the immune system in the context of atherosclerosis are emerging, but the major challenge remains to identify those that more closely reflect the inflammatory status of the arterial wall that ultimately results in plaque destabilization and cardiovascular events (Figure 1).

One particularly promising candidate, which has demonstrated superiority over C-reactive protein, particularly in detecting plaque

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**Figure 1** Emerging biomarkers for atherosclerosis. A number of biomarkers indicative of increased oxidative stress and systemic inflammation are detectable in the peripheral blood of cardiovascular patients. Their increase in the circulation throughout the atherosclerotic process has been reported in many independent clinical trials. However, their ability to reflect stage of disease, with particular reference to plaque destabilization that mainly derives from the degree of arterial inflammation, remains poorly established (An extensive review on the inflammatory biomarkers reported in the diagram has been presented by Stoner et al. [26]).
vulnerability\textsuperscript{27,28} is circulating monocyte phenotype. Indeed, a change towards a pro-atherogenic profile by peripheral monocytes has been widely reported in cardiovascular patients, and a number of lines of experimental evidence collected in animal models of atherosclerosis suggest a potential involvement of monocyte phenotypic change in the perpetuation of systemic as well as of arterial inflammation (Figure 2).

We propose that the adoption of this biomarker may more specifically address the role of systemic inflammation in residual cardiovascular risk and, in turn, establish more precisely the place of immunomodulatory agents in the treatment of atherosclerosis.

2. Inflammatory biomarkers, plaque vulnerability, and cardiovascular events: usefulness and limitations of C-reactive protein

Measurement of C-reactive protein has been the test most largely applied in Phase II cardiovascular inflammation reduction trials (Tables 1 and 2), due to the availability of a standardized methodology and the fact that it is relatively inexpensive to measure.\textsuperscript{23} However, the true value of C-reactive protein as indicator of atherosclerosis-related

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{image.png}
\caption{Monocyte and macrophage heterogeneity. The ‘classical’ (CD14$^{++}$CD16$^{-}$) and ‘intermediate’ (CD14$^{++}$CD16$^{+}$) subsets of circulating monocytes display a distinct pattern of expression of surface molecules that mediate their interaction with the activated endothelium and that are involved in cell activation in response to pro-atherogenic stimuli (i.e. Ox-LDL).\textsuperscript{29} This may lead to a distinct ability of the different subsets to infiltrate the arterial wall and to sustain systemic inflammation. Compared with classical monocytes, CD14$^{++}$CD16$^{-}$ cells express higher levels of scavenger receptors (i.e. CD36 and TLR)\textsuperscript{30,31} that, upon activation, lead to release of pro-inflammatory mediators. However, classical monocytes mainly produce MPO in response to TLR activation, while the intermediate subset releases pro-inflammatory cytokines (IL-1, IL-6, and TNF$\alpha$).\textsuperscript{32} Also, these latter express increased levels of adhesive molecules (i.e. CD11b and CD11c) compared with CD14$^{++}$CD16$^{-}$ cells,\textsuperscript{30} which are able to interact with ICAM-1 and VCAM-1 expressed on dysfunctional endothelium. Distinct monocyte subsets also respond differently to chemotactic molecules such as MCP-1 and RANTES that specifically engage CCR2 and CX3CR1 expressed on classical and intermediate subsets, respectively. Heterogeneity in the macrophage component of plaques also has been detected. The M1 subtype is primarily involved in ox-LDL uptake with consequent formation of foam cells and release of pro-inflammatory cytokines; while the M2 subtype displays iron-handling capacities and the ability to synthesize anti-inflammatory cytokines.\textsuperscript{33} Anti-inflammatory drugs have the potential to modulate both monocyte and macrophage biological activity by targeting specific pro-inflammatory mediators as indicated in the diagram, thus modulating both systemic and local arterial inflammation. CV, cardiovascular; NO, nitric oxide; ox-LDL, oxidized low-density lipoprotein; sPLA$2$, soluble phospholipase A$_2$; TLR, toll-like receptor; CCR2, C-C chemokine receptor type 2; VCAM, vascular cell adhesion molecule; ICAM, intracellular adhesion molecule; PSGL-1, P-selectin glycoprotein ligand-1; CX3CR1, CX3C chemokine receptor 1; RANTES, Regulated on Activation, Normal T Expressed and Secreted.}
\end{figure}
inflammation is controversial. Level of C-reactive protein has been shown to predict future cardiovascular events in primary prevention trials, although this has not been a universal finding. Addition of C-reactive protein to standard Framingham risk calculation was found not to improve the estimation of future risk consistently. Importantly also, C-reactive protein level is a poor predictor of atherosclerotic burden in asymptomatic patients, and this undermines its utility in clinical decision-making, because of the inability to identify patients for whom detection of silent atherosclerosis has important implications as regards their clinical management.

In the context of secondary prevention, the role of C-reactive protein as an indicator of cardiovascular risk and of disease progression appears even more contentious. A study conducted by Riedel et al. showed that levels of C-reactive protein in patients with stable acute coronary syndrome (ACS) are not predictive of recurrence of total cardiovascular events over 22 months of follow-up where preventative pharmacological treatments have been optimized. Moreover, Phase II cardiovascular inflammation trials that have combined measurement of circulating biomarkers with imaging modalities able to characterize atherosclerotic lesions, have highlighted a lack of correspondence between C-reactive protein reduction and changes in plaque size or composition in response to tested anti-inflammatory therapies. Specifically, the leukotriene inhibitor atreleuton (VIA-2291) 50 mg daily was able to decrease non-calciﬁed plaque volume as detected by coronary CT in the absence of any effect on C-reactive protein levels at the end of a 24-week treatment period; similarly, the beneﬁcial action of the LP-PLA2 inhibitor darapladib 160 mg daily on lipid core expansion as evaluated by intravascular ultrasound palpography was not associated with a decrease in C-reactive protein in the IBIS-2 study, and succinobucol 280 mg daily, a drug with antioxidant, lipid-lowering and anti-inﬂammatory actions, reduced plaque size in patients undergoing percutaneous coronary intervention (PCI) at the end of a 12-month treatment period, but had no effect on C-reactive protein levels.

As previously mentioned, PLA₂ inhibitors, anti-leukotrienes, and antioxidants have been classiﬁed by Ridker and Lüchner as anti-inﬂammatory agents not directly targeting the IL-6 pathway that gives rise to C-reactive protein generation from the liver, unlike anti-cytokines and MTX. The reported effects of these drugs on C-reactive protein have not been consistent across the Phase II trials. However, it is worth noting that the PLASMA trial showed a 55.6% reduction in C-reactive protein compared with baseline levels in patients with stable coronary heart disease (CHD) receiving the inhibitor of secretory PLA₂ varespladib (at doses ranging from 50–500 mg twice daily), while a 24.8% reduction of C-reactive protein was observed in the placebo arm; this effect was not conﬁrmed in the PLASMA II trial, but it is important to note that in this trial (i) lower doses of the drug were administered (250 and 500 mg once daily), (ii) a 22% increase in C-reactive protein was observed in the placebo group, and (iii) baseline C-reactive protein levels in the study population were lower than those seen in the PLASMA trial (Table 1). In addition, varespladib 500 mg once daily proved effective in reducing C-reactive protein in the FRANCIUS trial conducted in ACS patients on a background of atorvastatin 80 mg daily. In a study of 939 patients with CHD or CHD-risk equivalent, also including statin-treated patients, LP-PLA₂ inhibition with darapladib produced signiﬁcant 12% and 13% reductions of IL-6 and C-reactive protein, respectively. These ﬁndings were not conﬁrmed in the IBIS-II trial, that was restricted to patients with CHD as documented by angiography; however, darapladib counteracted the lipid core expansion that was observed by virtual histology in the placebo arm. These results point at the need to better understand the link between systemic inﬂammation, as determined by C-reactive protein measurement, plaque composition/vulnerability, and cardiovascular event rates. Imaging modalities would certainly support advances within this area. However, their applicability in large multicentre studies is not practicable because of cost- and technical-related issues. The question therefore arises as to how one can overcome the difﬁculty in testing the inﬂammatory hypothesis of atherosclerosis in the absence of a reliable biomarker of disease progression. C-reactive protein proved very effective as a prognostic marker for future events in the statin trials, in which the pre-treatment baseline level of this biomarker was generally above 2 mg/L, this level being the cut-off value identiﬁed as able to distinguish between sub-optimal and optimal anti-inﬂammatory effects in response to statins that, in turn, predicted more favourable cardiovascular outcomes independently of the LDL target achieved. Refining recruitment to those patients with a C-reactive protein persistently >2 mg/L despite conventional therapy may be a useful strategy to test the efficacy of anti-inﬂammatory agents in secondary cardiovascular prevention. Indeed, this criterion is currently being adopted in the CANTOS study, as discussed later in this review. However, it remains unclear whether C-reactive protein possesses sufﬁcient sensitivity to predict the presence of vulnerable plaques and hence consequent acute cardiovascular events, given the data discussed above, especially in patients already on statin treatment—and it certainly lacks any speciﬁcity in this regard, being a better indicator of more systemic inﬂammatory processes.

3. Emerging inﬂammatory biomarkers: focus on monocyte phenotype

3.1 Prognostic value for cardiovascular events

In recent years, there has been increasing interest in the determination of monocyte function and phenotype because of the primary role of monocytes in atherogenesis and in plaque progression towards vulnerability. Assessment and characterization of the proﬁle of circulating monocytes, which carry the pattern recognition receptor CD14, have led to the identiﬁcation of functionally distinct subsets of CD14<sup>++</sup> cells with variable degrees of inﬂammatory activity: the so-called intermediate CD14<sup>++</sup>CD16<sup>−</sup> cells, but not of C-reactive protein, has been found to correlate with plaque vulnerability, as detected by coronary CT in patients with stable angina pectoris and by optical coherence tomography (OCT) in patients with unstable angina undergoing PCI. In keeping with this, the prevalence of CD16<sup>++</sup> monocytes has been demonstrated to be an independent risk factor for future cardiovascular events in several population studies. A shift in the inﬂammatory phenotype of circulating monocytes towards a more
pro-atherogenic CD16⁺ profile seems to accompany atherosclerosis progression from its earliest stages of development. Indeed, patients with cardiovascular risk factors but without prior clinical cardiovascular events display an expansion of CD16⁺ monocytes compared with healthy subjects. In addition, within asymptomatic but at-risk patients, the level of this monocyte subset strongly correlates with the presence of subclinical atherosclerosis, as detected by increased carotid intima-media thickness (IMT).

### 3.2 Pro-atherogenic implications

The phenotype of circulating monocytes as defined by CD14 and CD16 positivity is believed to have functional atherogenic implications. These can be primarily ascribed to differing abilities, between subsets, to synthesize pro-inflammatory cytokines as well as to infiltrate the arterial wall, this latter by virtue of a different pattern of expression of chemokine receptors and vascular adhesion molecules mediating cell recruitment and sub-endothelial infiltration. How these experimental findings translate into human disease remains an unanswered question, since a multi-

Experimental inves-

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The distinct abilities of different human cell subpopulations to produce either pro- or anti-inflammatory cytokines have been demonstrated in isolated cells following in vitro exposure to specific agonists, such as lipopolysaccharide (LPS). Similarly, the degree of interaction of different monocyte subtypes with the vasculature is a notion extrapolated from in vitro assays, demonstrating that recruitment of the classical subset is mainly monocyte chemoattractant protein (MCP)-1 mediated while the intermediate population more strongly responds to CXCR stimuli (RANTES). This suggests that the stage of disease and differences in shear stress and/or tissue microenvironment are likely to favour preferential recruitment of one mononcytic type over another through the selective modulation of specific endothelial surface integrin expression, MCP production, and chemokine ligand accumulation (RANTES). For instance, early atherosclerotic lesions are rich in endothelium-derived MCP-1, while in advanced stages of disease the high level of activated platelets at plaque sites is responsible for local release of RANTES. How these experimental findings translate into human disease remains an unanswered question, since a multitude of inflammatory stimuli co-exist in vivo, and there are no available diagnostic techniques applicable to the clinical setting that enable the tracking of different circulating monocytes from the bloodstream into atherosclerotic plaques in humans. The picture is rendered even more complex by the heterogeneity that also characterizes the macrophage compartment. Indeed, the local arterial microenvironment influences the phenotype of resident macrophages, that can be distinguished into two main subtypes: the classical activated M1 lipid-triggered foam cells and the anti-inflammatory M2 involved in healing and repair (the phenotypic and functional characterization of macrophage subsets has been recently reviewed by Italiani and Boraschi). The phenotypic profile of these two major macrophage subsets has been characterized via transcriptome profiling of in vitro differentiated macrophages subsequent to exposure of isolated human monocytes to M1- and M2-specific polarizing factors, such as LPS and IL-4, respectively. While selective signature molecules have been identified that enable histology-based identification of the M1 or M2 subtype within plaques, the hierarchic relationship between macrophage subpopulations and the distinct subsets of circulating monocytes remains unclear.

### 3.3 Relationship between the phenotype of circulating monocytes and degree of plaque inflammation

Investigations in the ApoE⁻/⁻ mouse model of atherosclerosis have demonstrated a predominant colonization of arterial lesions by the M2 subtype in the early stages of disease that is followed by enrichment in the M1 macrophage phenotype in more advanced phases. Using the atherosclerotic Reversa mouse model, Feig et al. have demonstrated that the total plaque content of macrophages is reduced when hyperlipidaemia is normalized by inactivating the gene for microsomal triglyceride transfer protein, and the concomitant administration of the peroxisome proliferator-activated receptor-γ agonist pioglitazone reverses the predominance of the M1 phenotype observed in advanced lesions towards M2 polarization. Histopathological analyses of human plaques have revealed that the main difference between M1 and M2 subtypes during disease progression is mainly in their spatial distribution within the atheroma, rather than in their abundance which is similar in both stable and unstable plaques. How circulating monocytes specifically determine the inflammatory profile of atherosclerotic...
lesions remains elusive. The murine counterpart of classical monocytes, namely Ly6C<sup>high</sup> cells, gives rise to foam cell accumulation within the atheroma, suggesting that CD14<sup>++</sup> CD16<sup>−</sup> could be the precursor of the M1 macrophage subtype; the role in atherogenesis of Ly6-C<sup>low</sup> cells, which are the murine counterpart of the human CD16<sup>+</sup> subpopulation, remains uncertain but compelling evidence has emerged demonstrating their patrolling behaviour, and this may support the concept that intermediate human monocytes could preferentially polarize towards an M2 phenotype. However, the clinical translatability of these experimental findings remains controversial. Indeed, although correspondence has been identified between the murine and human species in the phenotypic pattern of monocytic cell subsets, major differences exist between the two species in their inflammatory pathways. For instance, lymphocytes prevail over neutrophils in murine blood even under physiological conditions, while humans exhibit predominance of polymorphonuclear over mononuclear cells. Moreover, ApoE<sup>−/−</sup> mice develop monocytosis during atherosclerosis progression, which is absent in the human disease. Additionally, although not confirmed by functional assays, discrepancies have been identified between murine and human monocytes in the expression profile of genes involved in phagocytosis of apoptotic cells and lipid-dependent activation. Such differences in white cell count and monocyte function can alter the inflammatory milieu and give rise to distinct inflammatory responses. Moreover, the human immune system possesses a higher level of complexity than non-human species, particularly as regards the human CD16 signalling molecule that exhibits distinct cell-specific isoforms and polymorphisms not shared by non-human species, and this could underlie specific biological functions of the human CD16<sup>+</sup> mononuclear subset not entirely exploitable in animal models. In addition, a phenomenon of monocyte recruitment into and macrophage egress from atherosclerotic plaques has been described, and the balance between these two events is believed to regulate the accumulation of myeloid cells within lesions, at least in animal models of disease. Moreover, we recently demonstrated that currently used anti-platelet drugs (aspirin and clopidogrel) are able to influence the phenotype of circulating monocytes during atherosclerosis progression with a marked inhibitory effect on the atherosclerosis-associated monocytosis that develops in the ApoE<sup>−/−</sup> animal model; however, their beneficial effect on plaque inflammation differed depending on their specific ability, at the plaque level, to enhance the endothelial barrier against monocyte arterial infiltration. Hence, the correspondence between the pattern of circulating monocytes, which may contribute to systemic immune deregulation, and the inflammatory profile of atherosclerotic plaques, which is strongly influenced by the local microenvironment, requires further investigation. Combining histology-based plaque analysis and blood monocyte characterization in cardiovascular patients will shed new light on the role of systemic inflammation in determining arterial inflammation in human disease. Of note, classical and intermediate monocytes also present differences in the level of expression of proteins that are key regulators of the innate immune response, such as scavenger receptors involved in the binding to and phagocytosis of immunogenic molecules (ox-LDL), and the MHC responsible for antigen presentation to T and B cells (Table 4).

The different biological function of monocyte subsets, in terms of cytokine production, antigen presentation, endothelial adhesion and response to chemotactic stimuli, might be ascribed to a distinct cell phenotype as determined by the different level of expression of key molecules [scavenger receptors (TLR, LRP1, and CD36), MHC class II, and adhesive molecules (CD11b, CD11c, CX3CR1, CCR5, CCR2 and CCR4)] on the distinct cell types.

The higher expression of these molecules on the extracellular surface of intermediate monocytes compared with the classical subpopulation is highly suggestive of a central role played by the CD14<sup>++</sup> CD16<sup>−</sup> subset in the innate immune system activation that characterizes atherosclerosis. Indeed, an autoinflammatory component has been identified in atherosclerosis, where pro-atherogenic molecules such as, but not limited to, ox-LDL particles have immunogenic properties able to trigger an innate immune response that further evolves towards activation of the adaptive immune system. Although the parental development of one monocyte subset from another is still the subject of much discussion, the increment in CD16<sup>−</sup> cells with reduction in the prevalence of classical monocytes has been described as a dynamic process occurring in response to specific inflammatory stimuli, including platelet activation or lipid dysmetabolism, and the clinical evidence discussed above strongly points to the plasticity in the inflammatory pattern of circulating monocytes throughout atherosclerosis progression. Hence, the acquisition of a CD16<sup>−</sup> phenotype by circulating cells could act as a bridge between the innate and adaptive immune systems and could be directly involved in the instigation of a specific immune response against pro-atherogenic stimuli that ultimately gives rise to disease progression. This hypothesis needs to be proved, and pharmacological interventions specifically targeting ox-LDL formation (such as PLA<sub>2</sub> inhibitors) or anti-cytokines might all have the potential to modulate the phenotype and activation status of circulating monocytes, as well as the inflammatory profile of macrophages resident within arterial lesions, with consequent effects on both systemic and arterial inflammation (Figure 2). Investigating the action of immunomodulatory interventions on monocyte phenotype in vivo and establishing the relationship between this effect and clinical outcomes would elucidate the causal role of this inflammatory marker in atherosclerosis progression. Based on this, we suggest that large prospective studies are now indicated to verify the utility of monocyte phenotype determination in predicting the presence and extent of atherosclerotic disease as well as response to therapy. Moreover, given the demonstrated ability of CD16<sup>−</sup> monocytes to reflect coronary plaque vulnerability in patients with prior events, it is reasonable to hypothesize that assessment of monocyte phenotype can be considered a useful adjunct for risk stratification in cardiovascular inflammation reduction trials and is likely to be more informative as regards plaque inflammation/vulnerability than C-reactive protein measurement; or at the very least, that the two measurements taken together are likely to be more useful in this regard than C-reactive protein in isolation.

### 3.4 Inflammation and classical cardiovascular risk factors

Given the mutual relationship between metabolic parameters and systemic inflammation, another important aspect to be considered is regards the inflammatory hypothesis of atherosclerosis is the effect of immunomodulatory agents on classical cardiovascular risk factors, which is in turn could affect overall cardiovascular risk profile purely through their effect on the latter. Cardiovascular risk factor correction with conventional therapies, including anti-hypertensive drugs, statins, hypoglycaemic agents, and anti-platelet therapies may reduce systemic inflammation. However, the anti-inflammatory efficacy of multi-drug conventional preventative schemes in cardiovascular patients is unclear. More importantly, the extent to which the
beneficial effect of classical therapies on cardiovascular outcomes is attributable to the modification of the inflammatory profile remains dubious. Assessing the efficacy of anti-inflammatory strategies in cardiovascular prevention trials represents a valuable experimental approach to address the relevance of immune system deregulation in the pathogenesis of atherosclerosis, assuming that no effects of the tested immunomodulatory drugs are observed on classical cardiovascular risk factors. We here focus on three main classes of drugs that have already entered Phase III trials, namely anti-cytokines, PLA2 inhibitors, and anti-oxidants, to discuss their differential C-reactive protein-lowering effects as well as their actions in parallel on lipid profile and glucose metabolism in relation to clinical outcomes. The ability of these molecules to modify classical cardiovascular risk factors has already emerged. Hence, the question arises as to whether their potential benefit over conventional approaches may derive, to some extent, from improved cardiovascular risk correction rather than a direct immunomodulatory action.

3.5 Anti-cytokines

TNF alpha (TNFα-antagonists) and IL-6 blockers are already in clinical use as FDA-approved DMARDs. Data from observational Phase IV studies suggest that they protect against acute cardiovascular events in patients with RA. However, further studies specifically designed to address the cardiovascular protective role of TNFα-antagonism in comparison to traditional DMARDs other than biological agents in RA patients have not produced evidence of advantage of the former over the latter approach, at least so far as clinical cardiovascular outcomes are concerned. The benefit of TNFα-antagonists in reducing C-reactive protein levels has been consistent among these studies, but, given the strong inflammatory component of RA, this biomarker could merely reflect RA disease control rather than cardiovascular risk. Of note, several authors have reported that TNFα-antagonism induces an increase in both LDL-cholesterol and triglyceride levels, although this finding was not confirmed by others. A Phase IV trial [ClinicalTrials.gov Identifier: NCT01331837] is currently recruiting patients with moderate and severe RA to evaluate cardiovascular protection, over a 5-year follow-up period, in response to the TNFα-antagonist etanercept or the IL-6 blocker tocilizumab, which also has been shown to adversely impact on lipid profile. Results from this study should clarify the effect of these anti-cytokines on metabolic parameters and their interrelation with anti-inflammatory efficacy and cardiovascular prophylaxis in this specific population of high-risk patients.

The action of IL-1β antagonists on metabolic profile is more defined, and the evidence shows that they exert a hypoglycaemic effect through enhancement of β-cell secretory function. Gevokizumab, a recombinant engineer human mAb that neutralizes IL-1β, efficiently reduces inflammatory biomarkers in diabetics. A proof-of-concept (POC) study investigating its action on arterial inflammation in patients with ACS has recently concluded and the results are awaited (ClinicalTrials.gov Identifier: 2012-002677-53). The IL-1 receptor antagonist (IL-1RA) anakinra also proved effective in improving glycaemic control in type 2 diabetic patients, and a parallel reduction in hs-C-reactive protein was observed in the study population. The CANTOS study is currently testing the efficacy of another IL-1β-targeted humanized mAb, canakinumab, in reducing the risk of major recurrent cardiovascular events in stable patients post-myocardial infarction (MI). This is the only Phase III cardiovascular inflammation reduction trial to date that restricts recruitment to patients with a level of C-reactive protein > 3 mg/L, thus ensuring certain homogeneity in terms of inflammatory profile of the study population. A subgroup analysis is also planned to determine the hypoglycaemic effect of canakinumab in diabetic patients. A previous Phase II trial in type 2 diabetic patients showed no effect on glucose control; however, the baseline median value of HbA1c in the study population was already optimal (<7.5%). Thus, CANTOS will elucidate the clinical benefit of IL-1β antagonism on cardiovascular outcomes in both diabetics and non-diabetics, as well as its action on insulin resistance, which is a well-established stimulus of rise in C-reactive protein.

3.6 Anti-inflammatory drugs targeting oxidized LDL

Uptake of oxidized LDL (ox-LDL) cholesterol particles by monocytes enhances pro-inflammatory cytokine release and, when internalized by macrophages within plaques, promotes their apoptosis, thus contributing to plaque necrotic core formation and growth. Recognition of the crucial importance of oxidative stress in this setting has promoted the study of antioxidants in the treatment and prevention of coronary disease. Succinobucol, an antioxidant related to probucol, exerts antioxidant, anti-inflammatory, anti-hyperglycaemic, and anti-platelet effects in preclinical studies. Encouraging results were also obtained in humans in Phase II trials as regards coronary atherosclerosis regression and anti-inflammatory activity—this latter as assessed by reduction in MPO, white blood cell count, and fibrinogen, but not in C-reactive protein. However, the subsequent Phase III trial ARISE failed to demonstrate efficacy of succinobucol in the prevention of cardiovascular events over a 2-year follow-up period. This study, which recruited 6144 patients with recent ischaemia (MI or unstable angina) within the period 2 weeks to 1 year prior to recruitment, not only emphasized lack of efficacy of the drug on primary endpoints (composite of time to cardiovascular death, resuscitated cardiac arrest, MI, stroke, unstable angina, or coronary revascularization), but also reported a detrimental effect of succinobucol on lipid profile, with increase in LDL and decrease in HDL levels in the treatment arm compared with placebo, despite similar usage of statins between groups. A modest advantage of succinobucol was found for secondary and tertiary endpoints including new-onset diabetes.

Reduction of ox-LDL can be achieved with inhibitors of PLA2, including sPLA2 and Lp-PLA2, both of which can modify phospholipids within the LDL particles that generate atherogenic moieties. In a Phase II trial conducted in patients with stable CHD, sPLA2 inhibition with varespladib led to a decrease in LDL-cholesterol levels, as well as in C-reactive protein and other inflammatory biomarkers, within an 8-week follow-up period. However, when tested in patients with ACS in the VISTA-16 clinical Phase III trial, varespladib did not improve lipid profile or C-reactive protein levels compared with placebo and indeed was even found to increase MI and cardiovascular mortality compared with placebo after 16 weeks, leading to its premature termination by the Data and Safety Monitoring Board due to futility and possible harm. The Lp-PLA2 inhibitor darapladib was able to reduce IL-6 and C-reactive protein levels in patients with CHD, and also prevented coronary atherosclerotic lesion necrotic core expansion as evaluated by OCT, in Phase II trials. However, the SOLID-TIMI 52 Phase III trial showed inefficacy of darapladib in reducing the composite risk of CHD death, MI, and urgent coronary revascularization in 13,026 patients following an ACS event and over 2.5 years of follow-up. Darapladib also missed its primary endpoint—a reduction in the risk of cardiovascular...
death, MI, or stroke—in the STABILITY trial, in which over 15,000 patients with stable CHD were treated with the drug for a median of 3.5 years. No information is available as regards any effects of darapladib on classical inflammatory biomarkers in either of these Phase III trials. However, given that baseline LDL levels were extremely good in these study populations and that statins (taken by most of these patients) also favourably act on PLA2 activity, it has been proposed that PLA2 inhibition does not offer additional benefit as add-on to conventional treatment in secondary prevention. The SOLID-TIMI study also reported failure of darapladib to reduce cardiovascular event rates in a subgroup analysis of patients stratified according to LDL-cholesterol concentrations. It remains to be established whether such therapy may be beneficial in patients exhibiting persistent inflammation despite standard of care. Novel biomarkers such as monocyte phenotype may, as discussed above, aid considerably in identifying such patients.

### 3.7 Future perspectives

Distinct from the immunosuppression discussed so far, an immunomodulatory approach that is currently under development is a vaccination strategy. Such an approach in animal models of atherosclerosis has yielded evidence for a protective anti-atherogenic action of therapies which drive the immune system towards an antigen-specific response against targets that are involved in plaque development. Immunization experiments with LDL particles (in native or modified forms) or with cholesterol-related antigens, mainly identified within the apoB-100 protein sequence via peptide library screening, have been conducted in hypercholesterolaemic mice and proved successful in reducing lesion growth. The precise mechanisms underlying the anti-atherogenic action of these vaccine approaches remain to be established, but facilitated clearance of lipoproteins from the circulation before they accumulate within the arterial wall is believed to be involved, due to the presence of protective specific antibodies that opsonize circulating LDL to form immunocomplexes, ultimately phagocytosed by myeloid cells via the complement receptors. In addition, activation of CD8 T-cells is involved in the response to antigens targeting the ApoB-100 protein, with particular reference to the p210 peptide. Promising results also have been achieved by immunization against the cholesteryl ester transfer protein (CETP), a pivotal enzyme involved in HDL metabolism. Anti-CETP vaccine administration in rabbit models of atherosclerosis has been found to induce the synthesis of neutralizing antibodies against CETP, resulting in HDL increase and concomitant reduction in plaque formation within the aorta. The safety profile of CETi-1 vaccine has been demonstrated in a Phase I trial run by Avant Immunotherapeutics, although its efficacy in inducing neutralizing antibodies in humans was below expectation. Preclinical work continues to produce a CETP-target vaccine able to elicit a more powerful immunogenic response, that can be potentially tested in future clinical trials. Other immunogenic therapies that have used cholesterol-unrelated targets, such as those directed against heat-shock proteins (hsp), namely hsp60/65, have produced inconsistent results, and this has limited the further development of these strategies. On the other hand, a valuable prospective target for

**Figure 3** The relevance of inflammatory biomarkers in the development of immunomodulatory strategies for secondary cardiovascular prevention. Evaluation of inflammatory biomarkers, such as high-sensitivity C-reactive protein (hs-C-reactive protein), and monocyte phenotype, could support stratification of patients accordingly to their inflammatory profile. Anti-inflammatory strategies could be tested in selected subgroups of patients in whom ‘residual’ inflammation (as defined by increased hs-C-reactive protein and/or CD14++CD16+ levels) persists despite standard of care. The efficacy of immunomodulatory agents can be established in selected subpopulations in terms of impact on both clinical outcomes and inflammatory biomarkers, thus clarifying the relationship between ‘residual’ inflammation and ‘residual’ cardiovascular risk.
immunogenic therapies has been recognized in the vascular–endothelial growth factor receptor (VEGFR)-2 expressed by proliferating endothelial cells that participate in neangiogenesis, a process involved in plaque growth; VEGFR2 vaccination has prevented disease development in LDL−/− mice through activation of a cytotoxic CD8+ T-mediated response against VEGFR2-expressing cells.14 However, an important issue that would require consideration in future studies is the timing for therapy. Indeed, in all the preclinical experiments conducted so far, immunization against the different targets was performed before animals developed atherosclerosis and/or were exposed to pro-atherogenic factors, i.e. high-fat diet. The question is whether later intervention results in a similar anti-atherogenic action, as this may have important implications in human disease and the potential translatable utility of these immunomodulatory strategies in either primary or secondary cardiovascular prevention. Furthermore, safety and efficacy in humans of such experimental vaccines need to be established.

4. Conclusion

Further clinical studies are required to define the place of immunomodulation in secondary cardiovascular prophylaxis. Characterization of the inflammatory profile in study participants, and quantification of the anti-inflammatory efficacy of immunomodulatory therapies, should not be restricted to Phase II trials but also be included in Phase III study design to enable identification of residual inflammation and to determine the relationship between immunomodulation and cardiovascular protection. C-reactive protein is a useful tool to consider in this setting; however, because of its limitations both as an indicator of disease burden and as a prognostic marker in secondary cardiovascular prevention, especially in statin-treated patients, additional and more specific biomarkers of atherosclerosis-related inflammation and plaque vulnerability should be considered. Assessment of monocyte phenotype is emerging as a useful tool in this respect, with the potential to support major advances within this area. However, further work is necessary to fully characterize the contribution of each monocyte subset to plaque inflammation, particularly their relationship (if any) with the distinct macrophage populations that colonize arterial lesions (Figure 3).

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