Circulating microparticles generate and transport monomeric C-reactive protein in patients with myocardial infarction

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Aims
Elevated serum C-reactive protein (CRP) following myocardial infarction (MI) is associated with poor outcomes. Although animal studies have indicated a direct pathogenic role of CRP, the mechanism underlying this remains elusive. Dissociation of pentameric CRP (pCRP) into pro-inflammatory monomers (mCRP) may directly link CRP to inflammation. We investigated whether cellular microparticles (MPs) can convert pCRP to mCRP and transport mCRP following MI.

Methods and results
MPs enriched in lysophosphatidylcholine were obtained from cell cultures and patient whole-blood samples collected following acute MI and control groups. Samples were analysed by native western blotting and flow cytometry. MPs were loaded with mCRP in vitro and incubated with endothelial cells prior to staining with monoclonal antibodies. In vitro experiments demonstrated that MPs were capable of converting pCRP to mCRP which could be inhibited by the anti-CRP compound 1,6 bis-phosphocholine. Significantly more mCRP was detected on MPs from patients following MI compared with control groups (P = 0.0005 for association). MPs containing mCRP were able to bind to the surface of endothelial cells and generate pro-inflammatory signals in vitro, suggesting a possible role of MPs in transport and delivery of pro-inflammatory mCRP in vascular disease.

Conclusion
Circulating MPs can convert pCRP to pro-inflammatory mCRP in patients following MI, demonstrating for the first time mCRP generation in vivo and its detection in circulating blood. MPs can bind to cell membranes and transfer mCRP to the cell surface, suggesting a possible mCRP transport/delivery role of MPs in the circulation.

Keywords
CRP • Microparticles • Myocardial infarction • Peripheral sample • Monomeric CRP

1. Introduction
The prototypic acute-phase reactant C-reactive protein (CRP) has a long association with cardiovascular disease. Clinical studies have demonstrated a consistent correlation between CRP elevation and adverse outcomes following MI including left ventricular failure1 and death.2 More recently, it has been recognized that chronic, minor elevations of CRP are associated with an increased risk of cardiovascular events independent of the traditional Framingham risk criteria.3 CRP recognizes a wide variety of endogenous and exogenous ligands in its function as part of the innate immune system.4 Given these properties and observational data, it was widely expected that CRP would play a direct pathogenic role in atherosclerosis. However, clinical evidence to support this notion has not been available to date.

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It has been shown in an elegant animal model of MI that CRP leads to a complement-mediated increase in infarct size. It is only recently that a plausible biological hypothesis has been proposed to explain this observed conflict in the current CRP data; it appears to be a marker correlated with the development of atherosclerosis. However, in acute inflammation such as that found after MI, it directly mediates tissue damage.

A possible resolution to this apparent paradox becomes clear if we consider that pCRP undergoes a structural change to a pro-inflammatory monomeric isof orm (mCRP). Although mCRP has been identified previously, it is only recently that a plausible biological mechanism for dissociation on the surface of activated cell membranes has been identified. However, mCRP has not been identified in the peripheral circulation, presumably due to its limited solubility. We hypothesize that circulating MPs, which are predominantly derived from activated cell membranes, are capable of converting pCRP to mCRP, with mCRP residing on the MP surface. Furthermore, in contrast to activated platelets, MPs are plentiful in circulating blood. This could potentially represent a transport mechanism for mCRP and also enable mCRP detection in peripheral blood samples.

In this study, we investigated the interaction of the inflammatory phospholipid membranes of MPs with pCRP. Our experiments demonstrate that MPs can convert pCRP to mCRP, consistent with the hypothesis that this process is catalysed by ‘activated’ membranes containing lysophospholipids. We describe for the first time the detection of mCRP in the circulation of patients following MI. We also demonstrate that MPs are able to bind and transfer mCRP to endothelial cells, thereby stimulating endothelial cell activation in an mCRP-dependent fashion. These findings indicate that MPs play a significant role in disseminating inflammation through the circulation.

2. Materials and methods

2.1 Antibodies and CRP preparations

Antibodies to mCRP (clone 9C9) were a gift from Dr Potempa (College of Pharmacy, Roosevelt University, IL, USA). The secondary antibody Alexa Fluor 488-labelled goat anti-mouse used for immunofluorescence staining was purchased from Invitrogen (Eugene, OR, USA). PE-labelled antibody against human VCAM-1 was purchased from CHEMICON International (Temecula, CA, USA). pCRP was purchased from Merck (Darmstadt, Germany). mCRP was a gift from Dr Potempa.

2.2 Cell culture

Monocyte cells derived from the THP-1 line (ATCC, Manassas, VA, USA) were grown in RPMI 1640 medium (Invitrogen, Carlsbad, CA, USA) with 10% foetal calf serum, 1% streptomycin, 1% penicillin, 1% L-glutamine, and 1% non-essential amino acids (Invitrogen).

Human umbilical vein endothelial cells (HUVECs) (ATTC) were grown in endothelial basal medium (EBM)-2 medium with endothelial growth medium-2 SingleQuots (Lonza, Basel, Switzerland). Experiments were performed between cell passages 3 and 6.

2.3 MP preparation

MP preparation is described in Supplementary material online. Briefly, MPs were obtained with slight modification of the method previously described. Cells were separated by centrifugation and MPs concentrated by further centrifugation at 16 100g for 90 min. The MP pellet was then resuspended in PBS and stored at 4°C until use. For polyacrylamide gel electrophoresis (PAGE), patient’s MPs were pelleted with an additional centrifugation step at 16 100g for 60 min at 4°C.

2.4 MP lipidomic analysis

MPs were isolated from activated platelets of healthy volunteers as outlined in Supplementary material online. Then, 10 μL of MP isolates underwent total lipid extraction, using a single-phase chloroform:methanol (2:1) technique as described previously. Each analysis was performed in triplicate and the median result used for data analysis.

Lipid analysis was performed by liquid chromatography, electrospray ionization–tandem mass spectrometry using an Agilent 1200 liquid chromatography system combined with an Applied Biosystems API 4000 Q/ToF mass spectrometer with a turbo ion-spray source (350°C) and the Analyst 1.5 data system. Results were then given in pmol/L. Values displayed for each lipid class were calculated as the sum of each individual species. More detailed protocol information is provided in Supplementary material online.

2.5 MPs in flow cytometry

MP analysis was performed using a FACS Canto II flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA). The MP gate was defined by size, using 0.8 and 1.1 μm beads (Sigma, St Louis, MO, USA), and the forward scatter threshold set to 400 to exclude exosomes.

2.6 Dissociation of pCRP to mCRP by THP-1-derived and HUVEC-derived MPs

THP-1- and HUVEC-derived MPs were incubated with pCRP (Merck, Darmstadt, Germany) at a final concentration of 20 μg/mL for 60 min at 37°C. Samples were run under native PAGE conditions and blotted onto nitrocellulose membranes as previously described. Membranes were stained with the mCRP-specific antibody 9C9.
2.7 Inhibition of mCRP formation by 1,6 bis-phosphocholine

Citrated whole blood was collected from healthy volunteers and stimulated with 20 μM adenosine diphosphate (ADP) for 20 min. Whole blood was spun at 1500g for 15 min. The top two-thirds of plasma were collected and remaining cells pelleted by a 2 min spin at 13 000g. Concentrated MPs were then prepared by centrifugation at 30 000g for 90 min. After centrifugation, an MP-depleted plasma sample was collected from the top of the preparation and the remainder discarded. The concentrated MP preparation was resuspended in 100 μL of PBS with calcium and magnesium for experimental use.

The MP and control preparations were incubated with pCRP (25 μg/mL) or pCRP with 1,6 bis-phosphocholine (bisPC, Syngene International, Bangalore, India). bisPC is a specific pCRP inhibitor which combines two pCRP molecules preventing membrane binding. Incubation was performed with pCRP bisPC molar ratios as shown. All incubation steps were performed for 60 min at 37°C. Samples were run on a native PAGE and blotted as described earlier. The membrane was stained with the specific mCRP antibody clone 9C9.

2.8 Study population

The study population was divided into four clinical groups. Patient groups were defined on the basis of clinical presentation and angiographic findings. Patients who presented with acute myocardial infarction (AMI) and underwent primary percutaneous intervention (PCI) of a coronary artery were defined as the AMI group. A second less-acute group of patients who presented with an acute coronary syndrome (ACS) without ST-elevation and underwent PCI were enrolled in the ACS group. The third patient group included patients who were found to have non-obstructive coronary artery disease and did not undergo PCI. The final group were patients with no angiographic evidence of coronary artery disease.

All patients were enrolled 24–48 h after angiography. This was to enable sufficient time for serum pCRP to rise and enhance the probability of mCRP detection. The previously documented rapid time course of pCRP dissociation ensured that there was adequate time for this process to occur.

The clinical inclusion criteria were age 18–80 years, coronary artery disease status established by angiography, and willing and able to provide informed consent. Following enrolment, patients were allocated to the appropriate disease group.

Clinical exclusion criteria were age >80 years, thrombolysis, intra-aortic balloon pump insertion, sepsis (positive blood culture during admission), haemorrhage requiring transfusion or creatinine clearance <60 mL/min.

All patient recruitment was performed following approval from the relevant institutional human ethics committees (ethics review boards, AMREP Project 189/06, Monash Medical Centre HREC 10162A) in accordance with the Declaration of Helsinki. All patients gave informed consent before enrolment.

2.9 mCRP detection in patients with ACS

Patients’ blood samples underwent MP preparation as described earlier and were run under native PAGE conditions, transferred to a nitrocellulose membrane, and probed with the antibody clone 9C9 for mCRP.

Western blot samples were run individually or in pairs, therefore results were not quantified and were recorded as either positive or negative for the presence of mCRP. mCRP quantification on MPs was performed using flow cytometry as described earlier.

2.10 Transfer of mCRP from MPs to HUVECs and subsequent HUVEC activation

MPs were isolated from activated (20 μM ADP for 15 min) whole blood of healthy volunteers as outlined in Supplementary material online. The MP pellet was incubated with 25 μg/mL of mCRP in PBS with Ca/Mg or PBS with Ca/Mg only at 37°C for 1.5 h. MPs were then pelleted by centrifugation at 16 100g for 90 min at 4°C and washed in PBS with Ca/Mg. After three washing steps, the MP pellet was resuspended in EBM-2 medium and incubated with pre-activated HUVECs (10 ng/mL TNF alpha for 6 h) in 24-well plates for 1.5 h at 37°C. The supernatant of the last washing step was incubated with HUVECs as an additional control to detect the binding of free mCRP to HUVECs. After three washes in PBS, HUVECs were stained for mCRP with monoclonal antibody clone 9C9 (1:20) and Alexa Fluor 488-labelled goat anti-mouse secondary (1:100 dilution) (Invitrogen) and fixed in CellFix. Pictures were taken on an Olympus IX81 microscope at ×20 magnification. Microscope settings were obtained with controls in which primary antibody had been omitted.

To assess HUVEC activation, cells were pre-activated with TNF-α (10 ng/mL) for 3 h prior to incubation with MP preparations. MPs were prepared and loaded with mCRP as described earlier and incubated with HUVECs for 2 h at 37°C. After three washing steps in PBS with Ca/Mg, HUVECs were trypsinized and spun down. After re-suspension in 50 μL of PBS, cells were stained with PE-labelled monoclonal antibody against VCAM-1 and fixed in CellFix (BD Biosciences). Samples were analysed on a FACS Calibur flow cytometer (BD Biosciences).

2.11 Statistical analysis

All statistical analyses were performed using GraphPad Prism v5.0 (GraphPad Software, La Jolla, CA, USA). The data are shown as mean ± standard error. A P-value of P ≤ 0.05 was considered as statistically significant. All experiments were performed at least three times.

3. Results

3.1 MPs convert pCRP to mCRP in vitro

We have previously shown that pCRP is converted to mCRP within minutes following binding to activated cell membranes. As MPs are shed from activated or stressed cells, we hypothesized that they should also be capable of mCRP formation.

MPs were derived from THP-1 cells and HUVECs following stimulation with LPS and TNF-α, respectively. Concentrated MPs and MP-depleted supernatant were obtained and incubated with pCRP. MPs from both cell types were able to generate mCRP from pCRP, which is shown in Figure 1. The MP-depleted sample was unable to produce a significant amount of mCRP, consistent with the substantially reduced MP count as measured by flow cytometry.

3.2 Activated MPs have increased amounts of lysophospholipids enabling the binding and conversion of pCRP to mCRP

The lipidomic profile of MPs derived from activated platelets were analysed by mass spectrometry to investigate for the presence of lysophosphatidylcholine (LPC), which has previously been shown to be essential for pCRP binding and conversion to mCRP. The LPC/phosphocholine (PC) ratio in platelet-derived MPs was 0.106 ± 0.027 (n = 3). This ratio is comparable with the LPC/PC ratio of 0.1, which has previously been used in in vitro experiments demonstrating the ability of lipid membranes to catalyse the conversion of pCRP to mCRP.
3.3 bisPC inhibits the conversion of p-C-reactive protein to mCRP

The anti-pCRP compound bisPC binds pCRP at its phosphocholine (PC) binding site. It acts to bind two CRP pentamers together to form a decamer in a molar ratio of 5 bisPC:2 pCRP. Given the central importance of PC binding in the dissociation of pCRP to mCRP, we investigated whether this compound could inhibit the formation of mCRP.

Concentrated MPs were prepared from whole blood following incubation with ADP. They were then incubated for an hour with pCRP or pCRP pre-incubated with bisPC (30:1 bisPC:pCRP molar ratio, 15 min room temperature incubation) and compared with MP-depleted plasma (Figure 2).

The addition of pCRP to mixed whole-blood MPs results in the formation of mCRP. This process did not occur in the MP-depleted supernatant control. Furthermore, pre-incubation of pCRP with bisPC was able to inhibit the formation of mCRP. BisPC prevents pCRP binding by engaging the PC binding site; therefore, this result provides further support to the concept that ligand engagement is required for pCRP dissociation.

3.4 mCRP can be detected on circulating MPs following MI

mCRP has previously been detected in atherosclerotic plaques and in the infarcted myocardium. However, there are no reports of mCRP detection in the circulation or through methods other than histology. Following acute MI, circulating pCRP levels rise consistently in association with the size of MI. Following the in vitro demonstration that MPs can convert pCRP to mCRP, we investigated whether mCRP could be detected on circulating MPs in patients following MI.

For this study, four groups of patients across the spectrum of coronary artery disease were recruited. The first group enrolled patients with documented ST-elevation MI (STEMI), who all underwent primary PCI with successful restoration of coronary flow. Patients who presented with an ACS without ST-elevation and received PCI on the same admission comprised the second group. The final groups consisted of patients with stable coronary artery disease not requiring PCI and patients with angiographically normal coronary arteries.

Patient characteristics were comparable across the groups, as shown in Table 1. Patients presenting with a STEMI had significantly higher troponin, creatinine kinase (CK), and (p)CRP plasma levels. There were no other significant differences between the groups. Collected samples were analysed by western blotting and flow cytometry for the presence or absence of mCRP.

mCRP was detected on the majority of MP isolates from patients with a STEMI (10 out of 13, 77%). A representative blot is shown in Figure 3A. mCRP was also detected in a small number of patients who had undergone PCI (3 out 13, 23%), although with a significant difference between the two groups (P < 0.01, Figure 3B).

MP analysis by flow cytometry was also performed on each of the patient groups. Following a STEMI, the mean mCRP fluorescence was 525 ± 72 (standard error of the mean, SEM). Those with an NSTEMI/unstable angina recorded a value of 282 ± 65 (SEM) (P < 0.05; one-way analysis of variance (ANOVA) and Bonferroni’s multiple comparison test). Patients with stable coronary artery disease and normal coronary arteries also had significantly less mCRP detected with values of 133 ± 43 (SEM) (P < 0.01) and 93 ± 49 (SEM) (P < 0.001).

Figure 1 MPs can convert pCRP to mCRP. Concentrated MPs (MP conc.) and supernatant controls (MP SN) derived from THP-1 cells and HUVECs were prepared as described. Flow cytometry was performed to quantitate the concentration of MPs in each sample (concentrated and depleted). There were significantly more MPs found in the concentrated group when compared with supernatant (P < 0.01, unpaired t-test). Values are expressed as mean ± SEM. Samples were then incubated with pCRP (20 μg/mL) for 1 h and run on native PAGE. Membranes were probed for mCRP using antibody clone 9C9. All results were obtained at least three times with representative western blots shown above.
3.5 MPs can bind to and transfer mCRP to a HUVEC cell monolayer causing subsequent endothelial cell activation in vitro

We next investigated whether MPs could transfer mCRP to intact cells, potentially acting as mediators for the transmission of a pro-inflammatory signal. As can be seen in Figure 5, MPs are able to bind mCRP and transfer this to activated endothelial cells. mCRP can be detected on pre-activated HUVECs after incubation with mCRP-loaded MPs. Staining for mCRP (green) can be seen on HUVECs which had been incubated with mCRP-loaded MPs. No staining is visible on HUVECs incubated with control MPs or the supernatant of the last washing step. This clearly shows that MPs bind mCRP and are able to subsequently transfer it to the activated endothelial cells. No transfer to non-activated HUVECs was detectable (data not shown).

To determine a potential biological effect of this transfer, HUVEC activation caused by MP-transmitted mCRP was investigated. Figure 6 shows that mCRP transferred to pre-stimulated endothelial cells by MPs causes further cell activation as determined by an increase in VCAM-1 cell surface expression (P < 0.01 compared with control groups, one-way ANOVA and Bonferroni’s multiple comparison test).

4. Discussion

In this study, we demonstrate that the dissociation process of pCRP to monomeric subunits occurs following its binding to MPs. This finding is consistent with the previous discovery that this process takes place on activated cell membranes, either in model systems or in vitro. Furthermore, we demonstrate for the first time that mCRP can be detected on circulating MPs isolated from peripheral blood samples from patients following MI. This finding is supported by a recent study that also identified CRP on circulating MPs, however in this study the conformational status of CRP was not assessed. This is the first study to our knowledge in which mCRP specifically has been detected in the peripheral circulation. This is a critical advance in establishing the biological relevance of this isoform and may resolve some of the conflicts currently surrounding the pathological role of CRP in general.

Although circulating (p)CRP levels have been shown to correlate with individuals’ cardiovascular risk, population studies investigating a direct causal role have generally been inconclusive. This is despite the fact that CRP has been detected in atherosclerotic plaques, infarcted myocardium, and has pro-inflammatory effects on the vascular endothelium. Following MI, the peak circulating level of CRP has been shown to correlate with infarct size, development of heart failure, and mortality. In a rat model of MI, infusion of human pCRP increased the infarct size in a complement-dependent manner by 30–50%. Following the development of the novel anti-CRP agent bisPC, the therapeutic potential of this approach was confirmed with complete inhibition of CRP-induced myocardial cell death in a similar rat MI model. However, the mechanism required for CRP activation and complement fixation in this setting remains elusive.

The dissociation of pCRP to mCRP is an appealing mechanism to resolve this dichotomy. The ability of pCRP to dissociate to monomers with unique biological properties was recognized previously, but the relevance of this process to biological systems has not been fully explored. The notion that mCRP is generated in vivo is supported by identification of auto-antibodies directed against mCRP in patients with systemic lupus erythematosus. mCRP itself has been identified in atherosclerotic plaques and infarcted myocardial tissue.

The lipid profiling of platelet MPs provides data supporting a mechanistic basis of mCRP formation. Upon agonist stimulation, platelets undergo marked changes in membrane shape and phospholipid composition. Phospholipase A2 (PLA2) activation (mostly cytosolic PLA2) is essential to enable the production of potent pro-thrombotic eicosanoid mediators such as thromboxane A2, which promote localized platelet recruitment. Conversely, it appears that in CRP biology, it is the formation of LPC which contributes to pro-inflammatory effects promoting pCRP binding and dissociation. It is these rapid changes in the membrane structure of platelets following activation that lead them to shed a large number of MPs. Indeed, the majority of circulating MPs have been found to derive from platelets. It is therefore likely that the majority of MPs responsible for mCRP formation are derived from platelets following thrombus formation.

The demonstration of a plausible mechanism of in vivo formation of mCRP coupled with the discovery that mCRP can be detected on MPs potentially marks a paradigm shift in our understanding of CRP in inflammation. This is based on an increasing body of evidence demonstrating that mCRP has significant pro-inflammatory properties not shared with pCRP. These include complement component C1q fixation and activation of monocytes and platelets, all of which contribute to myocardial ischaemia and reperfusion injury.

The pathological role of circulating MPs has not been well established, although they have been implicated in the pathogenesis of inflammatory arthritis and atherosclerosis. In this study, we demonstrate that MPs containing mCRP on their surface are able to bind to activated endothelial cells and deliver a pro-inflammatory stimulus to these endothelial cells consistent with previous findings. This finding indicates that MPs are not only able to catalyse the production of mCRP, but they are also able to transport and disseminate pro-inflammatory signals. MPs carrying proteins such as mCRP are able to engage cell surfaces through phagocytosis, ligand engagement, or membrane fusion. They have the potential to transport proteins which are not able to circulate freely due to the instability in the circulation or are not soluble. Recent work has shown that MPs play an important transport role for...
microRNAs$^{33}$ and it is plausible that a similar role is played for insoluble proteins such as mCRP. In this way, MPs act to transmit signals from one cell to another and assist in the co-ordination of complex biological processes.$^{34}$

The demonstration that bisPC is capable of inhibiting mCRP formation confirms that pCRP dissociation is dependent on membrane binding, a process specifically inhibited by bisPC.$^{16}$ It also supports the intriguing possibility that bisPC inhibits the activity of pCRP through prevention of mCRP formation, rather than direct inhibition of pCRP. This concept is supported by studies demonstrating the interaction between mCRP and complement.$^{12}$ Importantly, this finding also demonstrates a potential therapeutic approach to limit ischaemic tissue

### Table 1 Clinical and demographic characteristics of patient groups

<table>
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<tr>
<th>Demographics</th>
<th>STEMI (n = 13)</th>
<th>NSTEMI/unstable angina (n = 13)</th>
<th>Stable coronary artery disease (n = 7)</th>
<th>No coronary artery disease (n = 7)</th>
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<tr>
<td>Age (years)</td>
<td>$58.1 \pm 9.5$</td>
<td>$66.7 \pm 7.7$</td>
<td>$67.9 \pm 7.3$</td>
<td>$63.4 \pm 14.4$</td>
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<td>Sex (male), %</td>
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<td>77</td>
<td>100</td>
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<td>CRP (μg/mL)</td>
<td>$35.8 \pm 33.6$</td>
<td>$10.0 \pm 15.5$</td>
<td>$3.1 \pm 0.4$</td>
<td>$6.6 \pm 9.0$</td>
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<td>Troponin (μ/L)</td>
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<td>$0.1 \pm 0.2$</td>
<td>$=0.003^b$</td>
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<td>CK (U/L)</td>
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<td>NA</td>
<td>$&lt;0.0001^b$</td>
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<td>Creatinine (μmol/L)</td>
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$^a$One-way ANOVA.  
$^b$t-Test.

**Figure 3** (A) mCRP band detected from the MPs of a patient following STEMI. There was no mCRP detectable in a sample prepared from a PCI control patient. (B) There was a significant difference between the number of patients with detectable mCRP, with mCRP found more frequently in the circulation of patients following STEMI than PCI ($P < 0.01$, Fisher’s exact test, 13 patients in each group).
Figure 4  (A) MPs were analysed by flow cytometry. There was significantly more mCRP detected on MPs from patients following a STEMI (n = 13) compared with patients who had undergone coronary angiography presenting with stable disease of the coronary arteries (n = 7), healthy coronary arteries (n = 7), or unstable angina (n = 13). (*P < 0.05; **P < 0.01; ***P < 0.001; one-way ANOVA and Bonferroni’s multiple comparison test).  (B) Representative flow cytometry histograms from each patient group with secondary control shown.

Figure 5  MPs transfer mCRP to endothelial cells. MPs were isolated from whole blood after treatment with ADP and loaded with mCRP. These were able to transfer mCRP to HUVECs as can be seen by staining with a monoclonal antibody specific for mCRP (clone 9C9). Positive staining for mCRP (green fluorescence) can be seen on HUVECs incubated with mCRP-loaded MPs (left), whereas no staining was detected in HUVECs incubated with unloaded MPs (centre). The supernatant of the last washing step of the loaded MPs was incubated with HUVECs as a control to demonstrate that no free mCRP was transferred to HUVECs after washing (right). Images are shown as overlays of brightfield and immunofluorescence images.
damage following MI. CRP may represent a potential therapeutic target as serum levels rise in the hours following infarction, by which time the majority of patients have presented for reperfusion therapy. However, current data would suggest that any anti-CRP agent must either inhibit the formation of mCRP as outlined here or inhibit the actions of mCRP. The recent development of an RNA aptamer specifically targeting mCRP offers an alternate approach to mCRP detection and functional inhibition. However, preventing the formation of mCRP (as demonstrated by the use of bisPC) may be a more advantageous upstream approach. Overall, the suitability of mCRP as a therapeutic target is currently a hypothesis that needs to be proved in future studies.

In conclusion, the identification of mCRP on circulating MPs isolated from peripheral blood samples is a significant novel finding. This validates previous work that has postulated a plausible mechanism by which pCRP dissociates to mCRP on ‘activated’ membranes. This is also the first description of inhibition of mCRP formation by blocking membrane binding through the use of the pCRP inhibitor bisPC. Finally, we have demonstrated that MPs transport and deliver mCRP, which is then capable of inducing pro-inflammatory effects in endothelial cells. By invoking an intermediary conformational change from pCRP to mCRP that generates a highly inflammatory agent, it is possible to reconcile previously conflicting observations regarding the role of CRP in inflammation and MI and in particular as a cardiovascular risk factor. Further large-scale clinical studies are warranted to clarify whether mCRP on MPs correlates with the extent of myocardial damage and in particular whether mCRP represents a better marker for cardiovascular risk than pCRP.

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Conflict of interest: none declared.

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