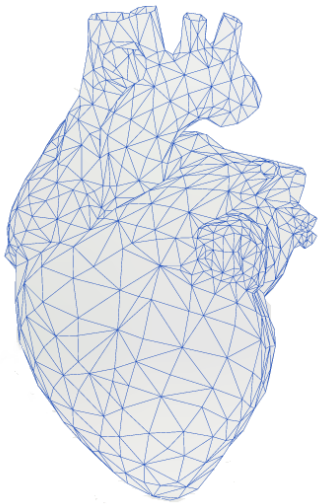
BioLab Assays

Monomeric CRP

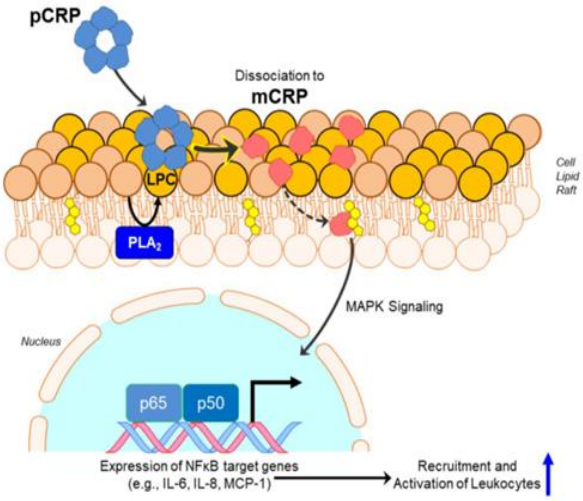


A NEW PLAYER IN EVALUATION OF CARDIOVASCULAR RISK?

BACKGROUND: CRP AND MONOMERIC CRP (mCRP) IN INFLAMMATION

Although CRP is an independent risk factor for CVD and offers a prognostic advantage over measurement of lipids alone, the precise mechanism by which CRP is related to CVD pathogenesis is poorly understood. It is generally accepted, that CRP plays an active role in endothelial dysfunction, and induces complement activation. However, there is evidence that natural CRP is not a direct mediator of cardiovascular events. The modest association between risk evaluation and CVD was inappropriately conflated with causality, and it has been claimed that CRP is proatherogenic. The reported proinflammatory effects of human CRP *in-vitro* or *in-vivo* resulted from impurities of CRP preparations and above that, it was revealed that pharmaceutical graded natural CRP is not proinflammatory in healthy human adults.

There are distinct isoforms of CRP, pCRP (pentameric CRP) and mCRP (monomeric CRP), and the pCRP isoform can irreversibly dissociate at sites of inflammation, tissue damage, and infection into five mCRP subunits. Evidence indicates that pCRP often tends to exhibit more anti-inflammatory activities compared to mCRP, which contrary shows pro-inflamatory and pro-thrombotic effects. The pCRP isoform activates the complement pathway, induces phagocytosis, and promotes apoptosis, whereas mCRP promotes the chemotaxis and recruitment of circulating leukocytes to areas of inflammation and can delay apoptosis. In terms of pro-inflammatory cytokine production, mCRP increases IL-8 and MCP-1 production, while pCRP has no detectable effect on their levels. These findings suggest the differential roles of each CRP isoform in inflammation and infection.



C-reactive protein (CRP) undergoes conformational changes between circulating native pentameric CRP (pCRP), pentameric symmetrical forms (pCRP*) and monomeric CRP (mCRP) forms. mCRP exhibits strong pro-inflammatory activity and activates

Reference: Rajab, Ibraheem & Hart, Peter & Potempa, L.A.: How C-Reactive Protein Structural Isoforms With Distinctive Bioactivities Affect Disease Progression. Frontiers in Immunology; 11. 2126 (2020)

platelets, leukocytes, and endothelial cells. Abundant deposition of mCRP in inflamed tissues plays a role in several disease conditions, such as ischemia/reperfusion injury, Alzheimer's disease, and cardiovascular disease.

Conversion of pCRP to mCRP induces inflammatory signalling. Monoacyl phosphatidylcholine, generated by PLA_2, or by oxidation lipid acyl chains, promotes binding and dissociation of pCRP to mCRP, which exposes cholesterol binding sequence. The hydrophobic element allows traffic through the plasmatic membrane into cells and activates NF-κβ signaling pathway. mCRP gains functionally active neoepitopes that carry out highly pro-inflammatory and pro-thrombotic features. Deposition of mCRP, which has significantly lower water solubility than pCRP, has been demonstrated in the brain in infarcted areas of Alzheimer's disease and in areas of amyloid burden, in atherosclerotic plaques in vascular disease and in other foci of inflammatory tissue damage.

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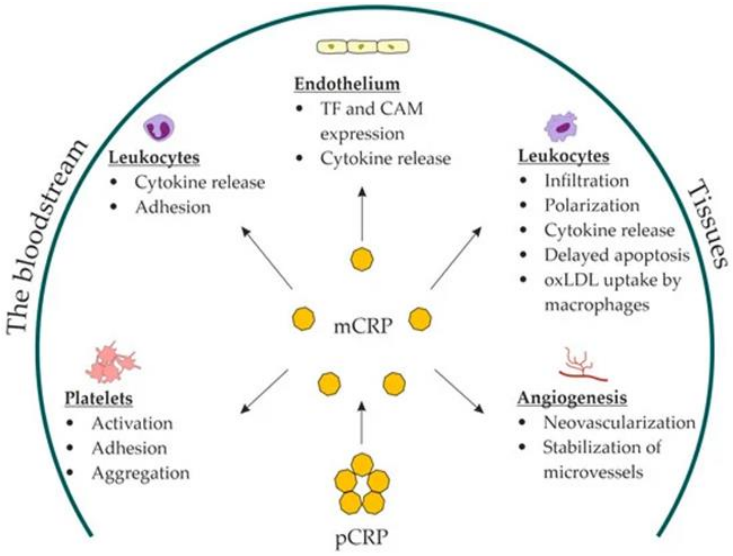
CARDIOVASCULAR RISK AND mCRP

The cardiovascular risk that persists despite aggressive lipid-lowering therapy-such as anti-PCSK-9 therapy- and correction of modifiable risk factors is called "residual cardiovascular risk" [1]. One of its main types is the residual inflammatory risk resulting from low-grade inflammation in atherosclerotic plaques [2]. It is determined by the level of the main inflammatory biomarker C-reactive protein (CRP), measured using a high-sensitivity assay (hsCRP), with a value of 2.0 mg/L or more [3]. The hsCRP assay measures the level of the pentameric form of CRP (pCRP), which is produced in the liver under the stimulation by interleukin (IL)-6 [4]. The USPSTF meta-analysis that explored studies published from 1966 to 2007 demonstrated that relative cardiovascular risk is 1.58-fold higher in individuals with a CRP level more than 3.0 mg/L than in those with a CRP level less than 1.0 mg/L [5].

Recent in-vitro and animal-model studies have suggested a task for mCRP in cardiovascular risk initiation and development, and show its active role in platelet activation, adhesion, and aggregation; endothelial activation; leukocyte recruitment and polarization; foam-cell formation; and neovascularization. mCRP contributes to the complex interplay between blood coagulation and inflammation, which is called thromboinflammation [6]. Bound on a collagen substrate, mCRP substantially increases platelet adhesion and thrombus growth rate. Unlike pCRP, mCRP induces platelet glycoprotein (GP) IIb/IIIa activation in a dose-dependent manner, and facilitates platelet adhesion via activation of GP IIb/IIIa receptors. Additionally, mCRP stimulates platelet adhesion to the endothelial cells [7] and induces tissue-factor expression and fibrin formation on endothelial cells [8]. When



Reference: Melnikov, I.; Kozlov, S.; Saburova, O.; Avtaeva, Y.; Guria, K.; Gabbasov, Z. Monomeric C-Reactive Protein in Atherosclerotic Cardiovascular Disease: Advances and Perspectives. Int. J. Mol. Sci., 24, 2079 (2023)

dissociated on platelets and adhering to the vessel wall, mCRP enhances endothelial activation and neutrophil attachment to the endothelium [7,9]; monocyte adhesion to the collagen [10], fibrinogen [11], and fibronectin matrix [12]; and T-lymphocyte extravasation [13]. In vitro, mCRP decreased nitric-oxide release and increased production of proinflammatory IL-8 and monocyte chemoattractant protein-1 by endothelial cells via the NF-kB pathway [14]. Moreover, mCRP stimulated leukocyte recruitment to the vessel wall, inducing the expression of vascular cell adhesion molecule-1, intercellular adhesion molecule-1, and E-selectin, as well as the production of IL-6 and IL-8 by the endothelium [7,14,15]. mCRP can also stimulate oxidized LDL uptake by macrophages [16]. The in vivo evidence that mCRP can stimulate monocyte infiltration into damaged tissues was obtained from recent animal studies [17]. In addition, mCRP has been shown to stimulate neoangiogenesis and stabilize novel microvessels [18,19]. mCRP deposition into atherosclerotic plaques has been addressed in several immunohistochemical studies. In human tissues, mCRP deposits have been detected in atherosclerotic plaques of the aorta [10], carotid [10,11,20], coronary [21,22], and femoral arteries [23], as well as diseased coronary artery venous bypass grafts [24] or infarcted myocardium [11]. In contrast, no mCRP deposits have been found in intact arteries or fibrous or calcific plaques [10,11,20,21,23,24]. mCRP can cross the endothelial barrier after dissociation [11] or be synthesized locally. Nevertheless, it is still unclear the contribution of local synthesis to the total concentration of mCRP in the tissues and bloodstream. The studies clearly distinguishing between the two forms of CRP confirmed that mCRP, but not pCRP, was deposited into damaged tissues [10,11,22], whereas other studies did not discriminate between CRP forms [20,21,23,24].

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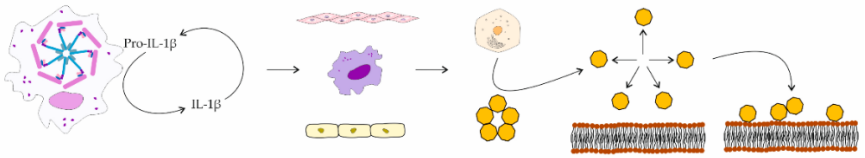
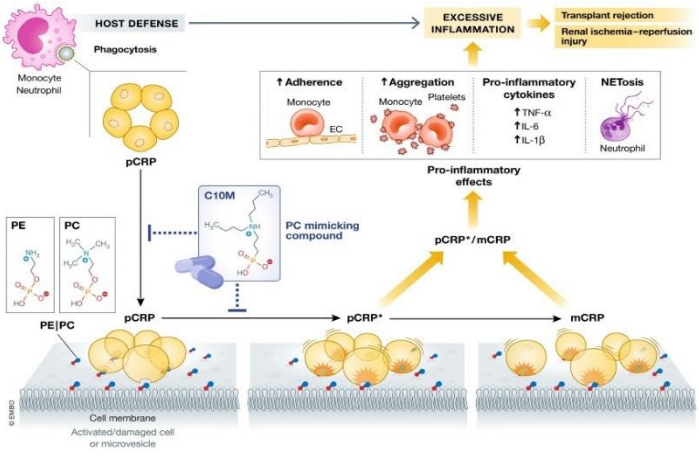
ANTI-INFLAMMATORY THERAPY AND mCRP

In the future, tailored antibodies for inhibiting transformation of pCRP into mCRP or selective inhibition of deposition of mCRP in the injured myocardium could be a promising method for minimizing ischaemia–reperfusion injury in patients with elevated serum CRP. A small-molecule inhibitors of pCRP (e.g. 1,6-bis(phosphocholine)-hexane), which blocks the pCRP–microvesicle interactions, abrogates its proinflammatory effects. The inhibition of the conformational change generating pro-inflammatory CRP isoforms via phosphocholine-mimicking compounds represents a promising, potentially broadly applicable anti-inflammatory therapy, improving the outcome of myocardial infarction, stroke and other inflammatory conditions.

	Upstream			Downstream		
	Inflammasome	Interleukin-1β	Interleukin-6	CRP translation	CRP dissociation	mCRP
Therapeutic target						
Potential agents	Colchicine	Canakinumab	Ziltivekimab	Antisense oligonucleotides	Phosphocholine mimetics	Anti-mCRP antibodies
Evidence	Large RCTs: COLCOT LoDoCo2	Large RCT: CANTOS	Large RCTs: RESCUE ZEUS (ongoing)	Small RCTs (As of 2022, efficacy was not proven)	Animal models	Animal models

Recently, Zeller et al designed a low molecular weight compound that targets the PC/PE (phosphatidylcholine/ phosphatidylethanolamine) binding pocket on pCRP and thereby has the potential to prevent the formation of the pro-inflammatory pCRP* and mCRP species. The compound labelled C10M (3-(dibutylamino)propyl)phosphonic acid) did not show immunosuppression activities, and might represents a successful anti-inflammatory treatment strategy.



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PMID: 36465053

BioLab Assays

Human Monomeric CRP ELISA

BioLab Assays

Cat.No. BA1026

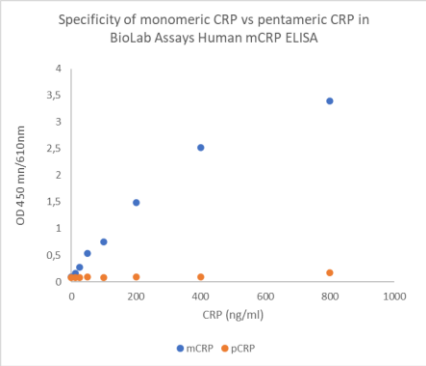
Sandwich ELISA: capture MAb specific to mCRP

Size: 96 wells

Measuring range: 1.25 - 80ng/ml

Sample type: serum, plasma

Regulatory status: RUO



Human Monomeric CRP ELISA specifically determines monomeric CRP (mCRP) without any reactivity with pentameric CRP (pCRP)

POPULATION AND CLINICAL DATA

Typical mCRP values in human serum were obtained with BioLab Assays Human Monomeric CRP (mCRP) ELISA kit

Clinical area	Serum mCRP range (median)
Individuals with hsCRP within 1-5mg/L	1.2- 4.8 ng/ml (median 2.6 ng/ml)
Individuals with pancreatic cancer	18.3- 73.9 ng/ml (median 36.5 ng/ml)
Individuals with bacterial infection, CRP over 50mg/L	24.1- 98.3 ng/ml (median 47.7 ng/ml)

According to the health status, mCRP next to the ratio mCRP/pCRP should be consider for a risk value assessment.

Manufactured by **BioLab Assays s.r.o.**

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