BioVendor R&D®

Monomeric CRP

Unique Marker of Cardiovascular Risk and Alzheimer's Disease

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Monomeric CRP (C-reactive protein)

- promotes vascular and neuronal degeneration processes leading to poststroke dementia

- strong pro-inflammatory activity: mCRP activates platelets, leukocytes, and endothelial cells; abundant deposition of mCRP in inflamed tissues plays a role in ischemia/reperfusion injury, Alzheimer's disease, and cardiovascular disease

- pro-thrombotic activity: involved in pathogenesis of atherothrombosis and venous thromboembolism (VTE) / thromboinflammation

- deposition of mCRP in the brain in infarcted areas of Alzheimer's diseases patients and in regions with amyloid burden, in atherosclerotic plaques in vascular disease and in other foci of inflammatory tissue injuries

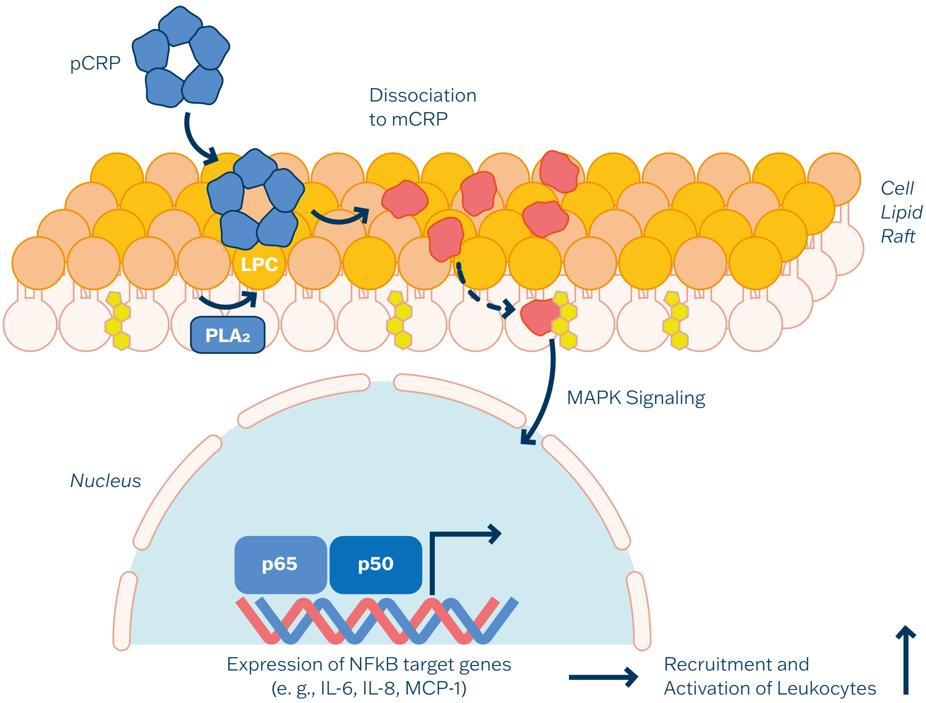
- monomeric C-reactive protein in circulation is associated with the increase in carotid plaque number in patients with subclinical carotid atherosclerosis

- pro-active role in the pathogenesis and progression of autoimmune diseases such as SLE, Alzheimer's disease, psoriasis or rheumatoid arthritis

Background

Although CRP is an independent risk factor for cardiovascular disease (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.



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)

C-reactive protein 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 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 PLA2, 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.

Reference:
Rajab IM, Hart PC, Potempa LA. How C-Reactive Protein Structural Isoforms With Distinctive Bioactivities Affect Disease Progression. Front Immunol. Vol 11:2126 (2020)
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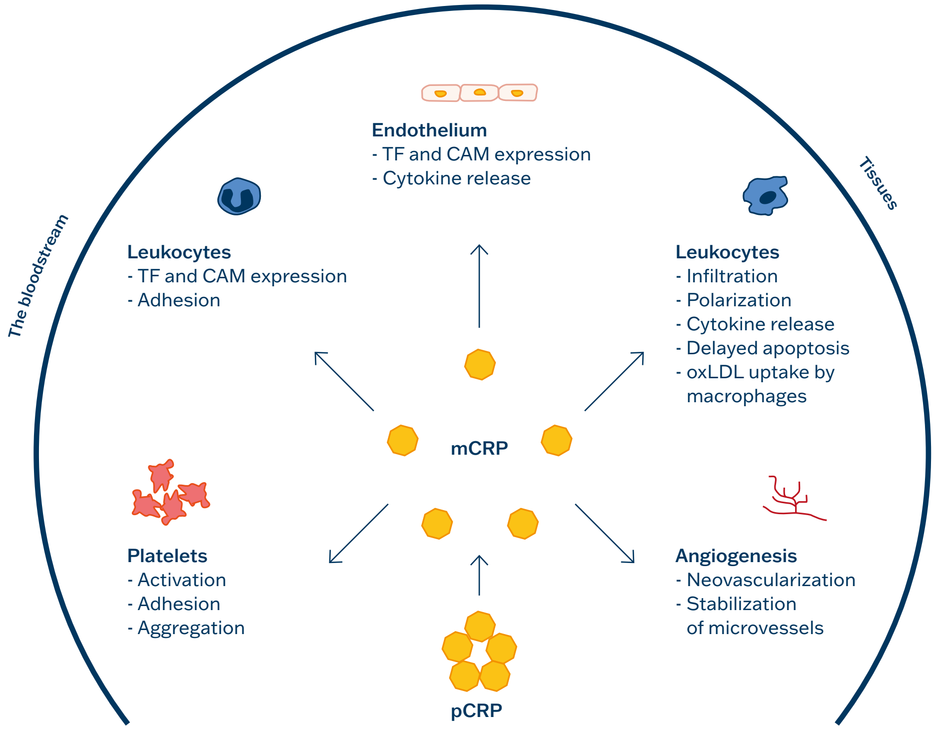
Cardiovascular risk and mCRP

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].



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)

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 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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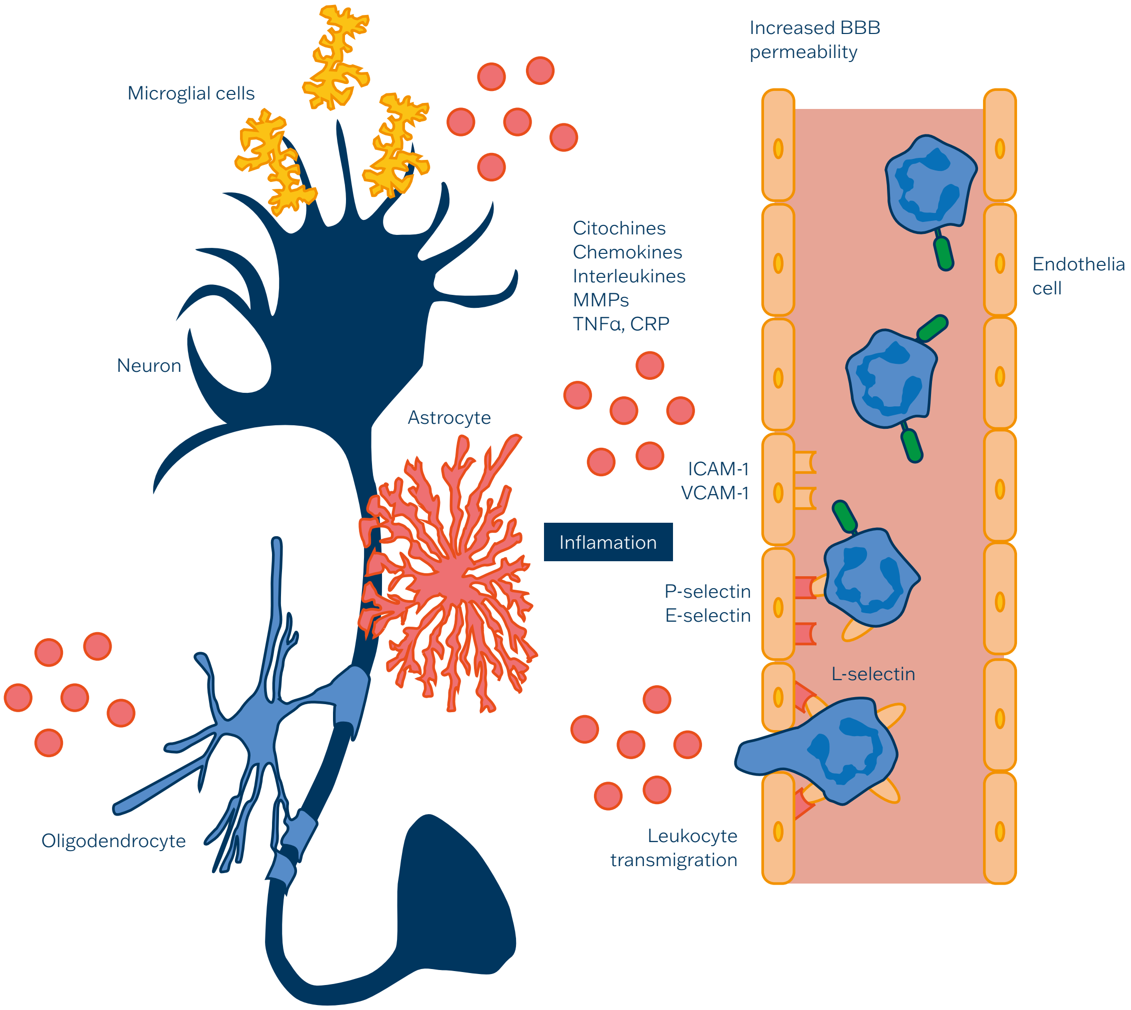
CRP and brain inflammation

CRP is primarily produced by the liver in response to macrophage secreted IL-6. CRP expression, however, may be upregulated in glutamate neurons during specific disease states, such as Alzheimer's dementia. Human and animal studies show that mCRP co-localizes with α-amyloid plaques and with phosphorylated-tau protein in hippocampus. Other studies indicate that CRP is produced in the CNS, either in neurons, glia, and/or microvessel endothelial cells, during immune and homeostatic challenges with some indications that CRP may be neurotoxic. Furthermore, CRP contributes to increasing blood-brain barrier permeability through the endothelial modifications (e.g. after trauma) and thus allowing other inflammatory signaling factors to enter the CNS.



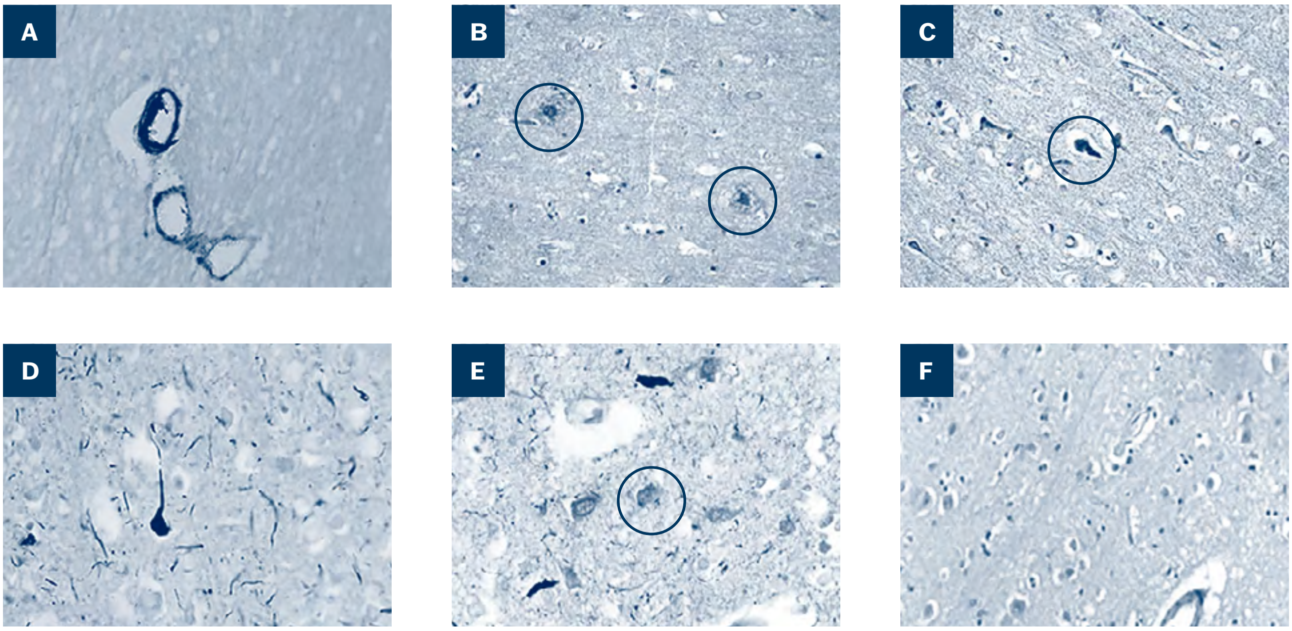
Monomeric CRP and dementia

Inflammatory damage spreading from small blood vessels and linked dysregulation of amyloid β metabolism in the neurons have been implicated in the origin of Alzheimer's disease. It is known that mCRP accumulates in brain micro-vessels after ischemic stroke, where it promotes aberrant angiogenesis, accumulation of amyloid β and probably de novo synthesis of amyloid β. Therefore, mCRP may cause both vascular and neuronal degeneration and underlie the processes leading to poststroke dementia. Specific targeting of mCRP can be a therapeutic approach in areas in which rapid increases in its local generation are expected, such as stroke-affected brain areas, in order to halt subsequent neurodegeneration and dementia. The prevalence of dementia in stroke survivors is about 30%, and a high proportion of these patients suffer from Alzheimer's disease in addition to those with either vascular or mixed Alzheimer's disease together with vascular dementia.

Al-Baradie described mCRP localized in the cerebral tissue of damaged vascular brain regions associated with neuroinflammation and neurodegeneration in an immunohistochemical study. They described co-localization of mCRP with β-amyloid or p-Tau in IHC samples from individuals with neurodegenerative disease.

Co-localization of mCRP with β-amyloid (A–C, microvessels, plaques and neurons, respectively) and co-localization of mCRP with p-Tau in neurons/fibrils (D,E) was shown. Control sample (F) shows a cortical region unaffected (no evidence of neurodegeneration).

Reference:
Al-Baradie RS et al.: Monomeric C-Reactive Protein Localized in the Cerebral Tissue of Damaged Vascular Brain Regions Is Associated With Neuro-Inflammation and Neurodegeneration-An Immunohistochemical study. Front Immunol.; 12:644213 (2021)



Impact of circulating monomeric CRP on Alzheimer's disease

Mouse model: Zhang et al. published a study with mice treated with mCRP, and showed that peripheral mCRP causes cerebrovascular inflammation and damages in ApoE4, but not in ApoE2 or ApoE3, mice via decreasing CD31 and increasing phosphorylated CD31. Garcia-Lara et al. found that anti mCRP antibody was able to completely block mCRP-induced chronic memory loss in a murine model of dementia where mCRP was injected into the hippocampus resulting in symptoms of neurodegeneration.

References:
Zhang Z et al.: Monomeric C-reactive protein via endothelial CD31 for neurovascular inflammation in an ApoE genotype-dependent pattern: A risk factor for Alzheimer's disease? Aging Cell; 20(11) (2021)
Garcia-Lara E, Aguirre S, Clotet N, Sawkulycz X, Bartra C, Almenara-Fuentes L, et al. Antibody Protection Against Long-Term Memory Loss Induced by Monomeric C-Reactive Protein in a Kouse Model of Dementia. Biomedicines 9(7):828 (2021)

Anti-inflammatory therapy and monomeric CRP

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.

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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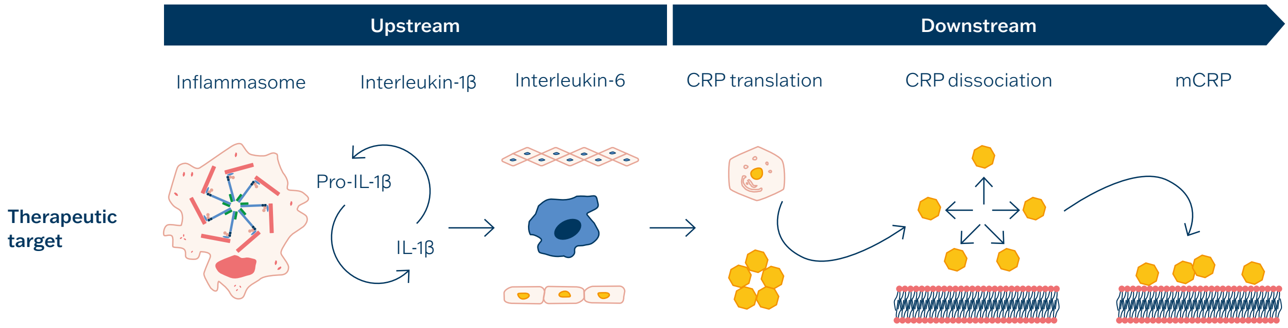
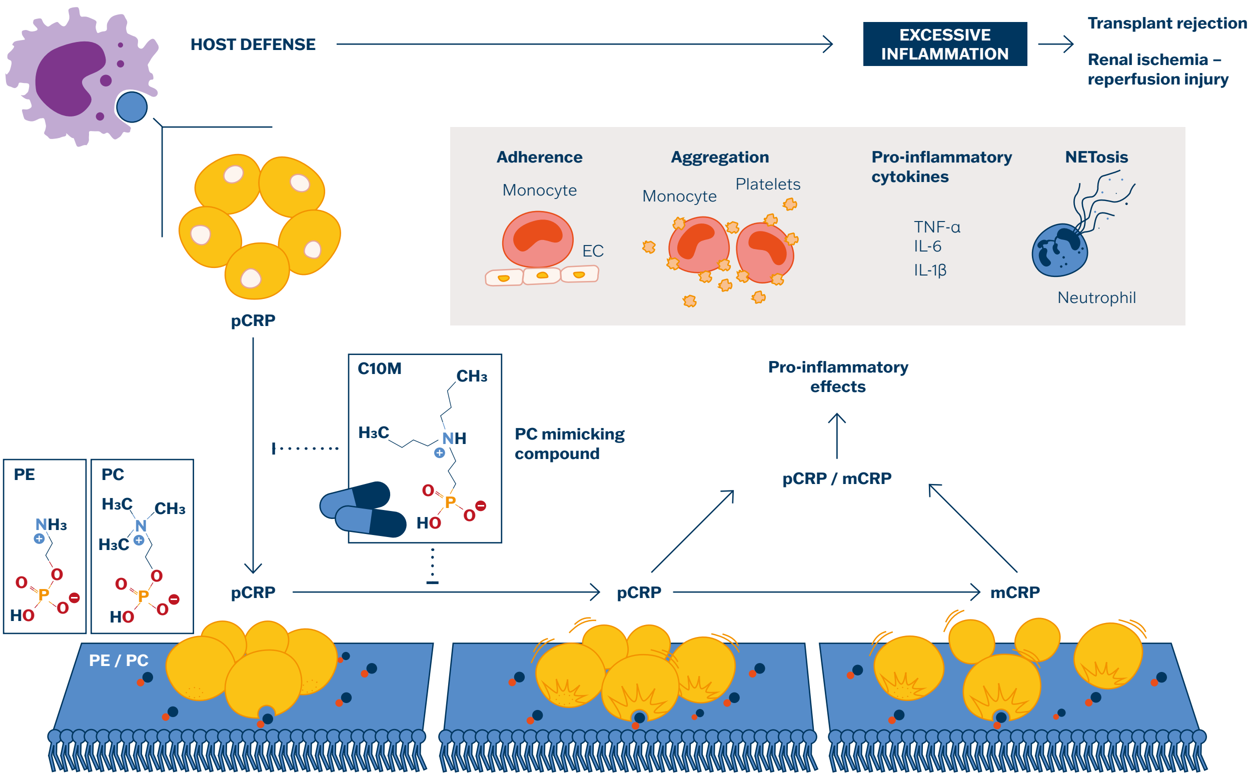
PMID: 36465053
Melnikov, I.; Kozlov, S.; Saburova, O.; Avtaeva, Y.; Guria, K.; Gabbasov, Z. Monomeric C-Reactive Protein in Atherosclerotic Cardiovascular Disease: Advances and Perspectives. Int. J. Mol. Sci., 24, 2079 (2023)
Filep JG. Targeting conformational changes in C-reactive protein to inhibit pro-inflammatory actions. EMBO Mol Med. 11;15(1):e17003 (2023)



	Inflammasome	Interleukin-1β	Interleukin-6	CRP translation	CRP dissociation	mCRP
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



Population and clinical data

Typical mCRP values in human serum were obtained with BioVendor's 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)

Human Monomeric CRP (mCRP) ELISA

Cat.No.	RBL010R
Size	96 wells
Assay type	Sandwich ELISA
Regulatory status	RUO
Validated for samples; recommended sample dilution	Serum, plasma;2x
Assay time	24hours
Quality Control	Serum Control A, Serum Control B
Measuring range	1.25–80 ng/ml
Sensitivity	0.63 ng/ml

Related products – cardiovascular risk

Product	Cat. No.	Regulatory Status
CRP Human ELISA	740001	CE IVD
hsCRP Human ELISA	740011	CE IVD
sICAM-1 Human ELISA	RAF102R	RUO
IL-6 Human ELISA	RD194015200R	CE IVD
IL-8 Human ELISA	RD194558200R	RUO
MCP-1 Human ELISA	RAF081R	RUO
MMP-2 Human ELISA	RBL001R	RUO
MMP-3 Human ELISA	RBL003R	RUO
MMP-9 Human ELISA	RBL002R	RUO

CE IVD The kits are CE-IVD certified and intended for professional use.

Related products – neural tissue damage

Product	Cat. No.	Regulatory Status
Amyloid beta (Aggregated) Human ELISA	RIG018R	RUO
Amyloid beta 40 ELISA Human	RIG013R	RUO
Amyloid beta 42 ELISA Human	RIG012R	RUO
Amyloid beta 42 Ultrasensitive Human ELISA	RIG017R	RUO
Amyloid Precursor Protein ELISA	RIG019R	RUO
Tau (pS199) Human ELISA	RIG015R	RUO
Tau (pS396) Human ELISA	RIG014R	RUO
Tau (pT181) Human ELISA	RIG020R	RUO
Tau (pT231) Human ELISA	RIG016R	RUO
Tau (Total) Human ELISA	RIG011R	RUO
Tau (Total) Mouse ELISA	RIG021R	RUO

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