Two Forms of C-Reactive Protein and Their Implication for Atherogenesis: Focus on Monomeric Form

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Abstract

In this review, we discuss the role of C-reactive protein (CRP), a biomarker of inflammation, in the development of atherosclerosis. CRP exists in two forms: the native pentameric form (nCRP) and the non-native form (mCRP), which is formed during inflammatory processes. The article explores the functions of both forms in atherosclerosis. nCRP binds to molecules with uncoated phosphocholine groups and activates the classical complement pathway. It can bind to enzymatically-modified LDL (E-LDL) and ox-LDL, affecting their pro-inflammatory properties and reducing LDL oxidation. nCRP also interacts with endothelial cells and reduces the absorption of acetylated LDL by these cells. In contrast, mCRP, formed in the presence of pathological conditions, exhibits distinct pro-inflammatory characteristics. While the role of nCRP in atherosclerosis remains unclear in animal models, mCRP has been associated with inflammation and is found in atherosclerotic lesions. The study suggests that nCRP may have atheroprotective effects, while mCRP may contribute to the progression of atherosclerosis. Further research is needed to fully understand the complex mechanisms of CRP in atherosclerosis.

Keywords: Atherosclerosis; C-reactive protein; Inflammation; CRP; Cardiovascular disease

Significance | A review on inflammatory protein in atherosclerosis progression.

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1. Introduction

The C-reactive protein (CRP) was discovered by Tillet and Francis in 1930. It was found in the blood serum of patients who had an acute phase of pneumococcal infection. Initially, CRP was considered a pathogenic secretion, since its level was increased in various diseases. CRP got its name due to the ability to precipitate fraction C (somatic C-polysaccharide of pneumococci). But further studies of synthesis in the liver indicated the native origin of CRP, which became the starting point for future research (Nehring et al., 2021).

The concentration of the pentameric (native) form of CRP – nCRP is considered as a prototypical biomarker of inflammation. Due to the fact that it repeatedly increases with inflammatory stimuli, inflammatory changes can be diagnosed with high accuracy (Sproston and Ashworth, 2018). CRP also meets the requirements of biomarkers: independence from external factors, low cost of measurements, reproducibility of individual measurements, availability of reagents and ease of testing. nCRP has a long half-life, and its level does not depend on daily fluctuations (Musunuru et al., 2008). It is also worth noting that modern highly sensitive immunoassays to determine the level of

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CRP show the same result in frozen and fresh blood plasma. The development of highly sensitive CRP immunoassays (hsCRP) carried out in the 1990s revealed that even moderately elevated CRP values are an independent predictor of coronary changes in the future (Grufman et al., 2014).

The Center for Disease Control and Prevention of the American Heart Association has ranked CRP as an independent biomarker of cardiovascular risk (Nakamura et al., 2018). Appropriate measurements to establish the concentration of hsCRP were recommended for asymptomatic individuals with a moderate risk of developing cardiovascular diseases. According to the results of the conducted studies, persons with a concentration of hsCRP < 1 mg/l belong to the low–risk group, and persons with a concentration of hsCRP >3 mg/l belong to the high-risk group (Kamath et al., 2015).

Further clinical trials, including: "the Canakinumab Anti-Thrombosis Outcome Study" (CANTOS) (Aday and Ridker, 2018) and earlier "Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin" (JUPITER) (O'Keefe et al., 2009), strengthened the use of hsCRP as an independent biomarker of cardiovascular diseases.

Although there are more and more facts that speak about the effectiveness of assessing the risk of cardiovascular diseases using CRP, it has not yet been established whether CRP is involved in the development of vascular pathology and atherothrombosis. The data on the pathophysiological role of CRP, which are currently available, are contradictory. Although its causal role in the development of atherosclerosis has been proposed, numerous in vitro and in vivo studies have yielded contradictory results. The reason for this could be that there are two forms of CRP (Yousuf et al., 2013). Basically, CRP is excreted from hepatocytes into the bloodstream, where it circulates in a native (pentameric) form. In areas where local inflammation is present, nCRP binds to the membranes of activated cells and their microparticles. There, the protein undergoes irreversible dissociation into insoluble monomers (mCRP) on phosphate heads of phospholipids (mainly phosphocholine) (Melnikov et al., 2020). As a result of dissociation, the solubility of CRP is significantly reduced, and mCRP receives distinct pro-inflammatory characteristics, which can enhance and localize inflammation. That is why there is a discrepancy between the results of studies of the pathophysiological role of CRP. Part of this difference can be explained by the lack of the ability to recognize different forms of CRP (especially in early studies) (Boncler et al., 2019).

This review contains comprehensive analysis and synthesis of current research on the role of C-reactive protein (CRP) in atherosclerosis. By examining the functions of both native CRP and non-native pentameric CRP, the review sheds light on the intricate interactions between CRP and the development and progression of atherosclerosis. The review's findings not only contribute to a deeper understanding of CRP's impact on the disease but also have important implications for future research and clinical practice. With a clear focus on native and non-native CRP, this review provides valuable insights that could pave the way for novel therapeutic strategies and enhance risk assessment and management in patients with atherosclerosis. Recently, a valuable review on CRP in atherosclerosis was published (Kürsat Kirkgöz, 2023). Our review thoroughly examines the latest findings on the association between C-reactive protein (CRP) and atherosclerosis, integrating recent studies and advancements in the field. Secondly, we emphasize the clinical implications of CRP as a biomarker for cardiovascular disease, shedding light on its potential as a diagnostic tool and therapeutic target. Lastly, our review offers a comprehensive analysis of both the strengths and limitations of existing research, providing readers with a balanced perspective. Together, these aspects make our review a valuable addition to the current literature on CRP and atherosclerosis. Additionally, our review aims to address the existing gaps and open questions regarding the differential roles and mechanisms of nCRP versus mCRP in atherosclerosis, providing valuable insights into their respective contributions to the disease process.

2. CRP in atherosclerosis

Functions of CRP (native CRP) in Atherosclerosis

CRP in the pentameric (native) form, as well as in the presence of Ca2+, binds to molecules with uncoated phosphocholine (PCh) groups, such as membrane and platelet-activating factor. Each of the subunits in the pentamer has a PCh-binding site (Singh et al., 2009). Thanks to mutagenesis and the three-dimensional structure of the PCh-binding site, it turned out that Glu81, Phe66 and Thr76 determine creating the pocket on CRP to bind and accommodate PCh. At the moment when the CRP binds to the PCh (which contains the ligand), it activates the classical complement pathway (Gang et al., 2015).

Deposited LDL in the arteries can undergo various modifications. But in experiments to determine the role of CRP in the development of atherosclerosis, two LDLs obtained in vitro are used: enzymatically-modified LDL (E-LDL) and oxidized LDL (ox-LDL) (Linton et al., 2019). Due to the fact that the PCh groups that are present in LDL are exposed in E-LDL, CRP is able to bind to E-LDL in a Ca2+-dependent manner. At the same time, CRP usually does not bind to ox-LDL. But it is worth noting that CRP can bind to ox-LDL in the event that LDL is oxidized enough to expose part of the PCh. Binding of CRP to ox-LDL, regardless of the effect of PCh on LDL, is possible only in a pathological environment, while this environment can affect CRP structurally. It was found that CRP binds to complexes that consist of β2-glycoprotein I and ox-LDL. CRP also binds to cholesterol. And

with atherosclerotic changes, it is mainly localized in the necrotic nucleus, which is rich in cholesterol. CRP also binds to LOX-1, which is a receptor for ox-LDL (Singh et al., 2008).

It was previously revealed that CRP, E-LDL and ox-LDL are involved in interrelated pathophysiological processes (for example, in the formation of LDL-loaded foamy macrophage cells). At the same time, opinions differ on the effect of CRP on the formation of foam cells (Ridker, 2016). Since it was found that CRP is located intracellularly in foam cells, a number of researchers have suggested that CRP forms complexes with LDL, which enhances the binding of macrophages and LDL, thereby facilitating the absorption of LDL and CRP by cells (Wirtz and von Känel, 2017).

E-LDL associated with CRP is not able to form foam cells, this was found when using pure complexes of CRP and E-LDL for the treatment of macrophages. Also, this fact clearly indicated that CRP can prevent the formation of foam cells. In another study, it was confirmed that the CRP and LDL complexes are not able to penetrate macrophages (Ridker, 2016). In addition, in experiments with foam elements, where endothelial cells and acetylated LDL were used, it was found that mCRP reduces the absorption of acetylated LDL by endothelial cells. In another experiment in which endothelial cells were used as a model for the formation of foam cells, it was revealed that CRP increases LDL transcytosis through endothelial cells. mCRP has also been shown to reduce the uptake of ox-LDL by macrophages (Nadimon et al., 2018). Later, it was suggested that due to a decrease in the aggressive reaction of macrophages to ox-LDL, the interaction of mCRP with ox-LDL may contribute to slowing down the formation of foam cells. In addition, it has been suggested that mCRP can perform a protective role. It reduces the risk of LDL modification by facilitating the removal of residual native LDLs from the extracellular space (Nambiar et al., 2014). However, since each time CRP forms a complex with modified LDL (CRP-E-LDL and mCRP-acetylated LDL), the formation of foam cells is inhibited, another assumption was put forward. The development of atherosclerosis should be slowed down if every LDL molecule retained in the artery wall is associated with CRP.

Other effects of interaction between CRP and modified LDLs have also been reported. At the same time, it remained unclear whether the CRP could contain spontaneously formed mCRP (Agrawal et al., 2010). CRP, after binding to LDL, causes a modification of the LDL charge. If cells are treated with a combination of CRP and ox-LDL, the production of pro-inflammatory cytokines by macrophages decreases. Due to its properties, CRP suppresses the sensitivity of LDL to oxidation caused by copper. Accordingly, further oxidation is prevented at the moment when CRP binds to ox-LDL. CRP increases the time required for copper ions to oxidize LDL. CRP can prevent the binding of minimally modified LDL (mmLDL), with monocytes and weaken the mmLDL-induced adhesion and activation of monocytes, due to the isolation of mmLDL (Chang et al., 2012). When bound to lysophosphatidylcholine present in ox-LDL, CRP suppresses the proatherogenic effects of macrophages, and also inhibits the association of ox-LDL with macrophages. This effect may contribute to slowing the progression of atherosclerosis. This means that CRP not only can prevent the formation of foam cells, but also reduces the pro-inflammatory effects of modified foam cells and LDL (Chang et al., 2012).

To determine the effect of CRP on the development of atherosclerosis, human, mouse and rabbit CRPS were used. Three different mouse models of atherosclerosis were used for human CRP, ApoE−/−mice, LDLr−/−mice and ApoB100/100LDLr−/−mice, as well as a rabbit model of atherosclerosis (Teupser et al., 2011). CRP was administered passively or was transgenic. In most studies conducted with ApoE−/−mice, it was found that CRP is neither atheroprotective nor proatherogenic. This became clear due to the fact that both passively administered human CRP and transgenically expressed human CRP did not affect the development, progression or severity of atherosclerosis (Hirschfield et al., 2005). In two studies using ApoE−/−mice, CRP slightly worsened the course of the disease. Another study using ApoE−/−mice found that CRP contributed to early changes in atherosclerosis (Torzewski et al., 2008). This was because LDL transcytosis through endothelial cells increased and LDL retention in the vessel walls increased. At the same time, there was no effect of CRP on the development of atherosclerosis in mice with LDLr−/−. In another study, CRP slowed the development of atherosclerosis, when using ApoB100/100LDLr−/−mice that are rich in LDL develop hypercholesterolemia. This suggests a possible atheroprotective role of CRP (Kovacs et al., 2007). There was also no effect of transgenic human CRP on the formation of atherosclerotic aortic lesion, nor on coronary, when using the rabbit model (Koike et al., 2009). Mice with CRP deficiency were used to monitor any possible role of endogenous mouse CRP in the development of atherosclerosis. In both ApoE−/−CRP−/−mice and LDLr−/−CRP−/−mice, the size of atherosclerotic lesions was either equivalent or increased compared to that of ApoE−/− and LDLr−/−mice (Torzewski, et al., 2014). Based on this, it can be assumed that mouse CRP can mediate atheroprotective effects. The effect of rabbit CRP on the development of atherosclerosis in rabbits was studied using CRP antisense oligonucleotides. This led to a strong decrease in CRP, but the atherosclerotic lesions of the aorta and coronary arteries did not change significantly. Based on this, it can be assumed that inhibition of CRP in blood plasma does not affect the development of atherosclerosis in rabbits (Yu et al., 2014). With the available data, it can be concluded that the pentameric (native) CRP either does not have the ability, or only

partially can protect against atherosclerosis in animal models. In Figure 1, we summarized the selected major effects of mCRP unducing atherosclerosis.

Functions of Non-native Pentameric CRP (Non-native CRP) in Atherosclerosis

If a biological protein modifier is present, the structure of the CRP itself changes. This leads to the formation of a new CRP, which then generates mCRP. Accordingly, during the dissociation of CRP to mCRP, there is another intermediate stage - non-native CRP. It has also been proven that antibodies that are specific to mCRP react with non-native CRP (Braig et al., 2017). CRP is also modified in the presence of abundant damaged cell membranes. And CRP can change the structure and generate mCRP when CRP binds to activated platelets and apoptotic cells. The main types of CRP deposited in inflamed tissue are manifested when CRP binds to microvesicles of cellular origin, and passes through structural changes, without violating pentameric symmetry. It has also been recorded that mCRP is deposited on burn wounds with inflamed and necrotic tissues (McFadyen et al., 2018).

CRP can be modified with hypochlorous acid and hydrogen peroxide, since the acidic state of the pH changes the CRP. For example, the modification of CRP with hypochlorous acid occurs by chlorination and oxidation of amino acids (Boncler et al., 2018). Due to this, the protein unfolds, the surface becomes more hydrophobic and aggregates are formed. This means that when entering the inflammatory environment, the CRP is exposed to pathological conditions, as a result of which the structure of the CRP changes to a non-native pentameric conformation. This leads to the complete dissociation of CRP and the formation of mCRP.

The recognition functions of non-native CRP differ from the functions of CRP, except for binding to PCh. Binding to modified LDL, regardless of the presence of PCh and Ca2+, is one of the functions of CRP in its non-native pentameric conformation. In comparison with CRP, native CRP can easily bind to ox-LDL, regardless of the nature and degree of oxidative status (Bottazzi et al., 2019). At the same time, native CRP binds to E-LDL more avidly than CRP. Also, the new lysophosphatidylcholine- binding site, which is located on the opposite side of the known PCh-binding site, becomes functional in the absence of Ca2+ (Agrawal et al., 2014). A conformational rearrangement of CRP is also required for the binding and action of CRP on endothelial cells. The presence of non-native CRP in lesions is indicated by the deposition of CRP and its joint localization with LDL in atherosclerotic lesions. Other fragments of LDL molecules interacting with CRP also include cholesterol and apolipoprotein B. But it is worth noting that the fragment of the modified LDL with which the native CRP interacts is unknown (Pathak et al., 2020). The binding site with the native CRP for modified LDLs remains unclear. There is an assumption that the binding site includes amino acid residues that are involved in the formation of the interconnection contact area. This is possible due to the fact that the area of the interconnect contact is hidden in the CRP, but is available in a non-native CRP. It was also determined that a single sequence motif called the cholesterol binding sequence, which binds cholesterol from the amino acid residue from 35 to 47, is responsible for mediating the interactions of mCRP with various ligands. Apparently, the universality of the sequence that binds cholesterol is due to its internal disordered conformation (Jaipuria et al., 2017).

As mentioned earlier, despite the inconsistency of the results of the study to determine the effect of CRP on the development of atherosclerosis in animals, a study using mCRP on ApoE−/−mice showed that mCRP can have an atheroprotective effect (Schwedler et al., 2005). Also, based on the data obtained in in vitro experiments, it can be assumed that native CRP may be more atheroprotective than CRP, given the difference between the recognition functions of CRP binding LDL and native CRP. Earlier it was reported about the CRP mutant, which was created through the use of site-directed mutagenesis. This CPR mutant is able to bind to ox-LDL without the need for any further structural changes (Bian et al., 2014). Thanks to the use of the LDLr−/−mouse atherosclerosis model, it was possible to evaluate the effect of such a CRP mutant on the development of atherosclerosis. In mice that received mutant CRP (with a non-native pentameric structure), the development of atherosclerotic lesions in the entire aorta was reduced. Summing up, we can say that CRP is an atheroprotective molecule (Singh et al., 2019).

3. mCRP in Vascular Pathology and Thrombogenesis

mCRP in Angiogenesis

The development of unstable atherosclerotic plaques is directly promoted by neoangiogenesis. In turn, mCRP has proangiogenic effects (for example, migration of endothelial cells and the formation of tubes in the culture of endothelial cells of human arteries and aorta) (Camaré et al., 2017). The increase in Notch-1 expression and stimulation of angiogenesis occurred due to 12-hour incubation of endothelial cell culture with CRP. In vivo, Notch-1 participates in the formation of germination and branching patterns during angiogenesis stimulated by vascular endothelial growth factor (VEGF) (Boras et al., 2014). Also, due to mCRP, increased regulation of endothelial Notch-3 was induced. In their study, Boras et al indicates that mCRP in combination with Notch-3 promotes angiogenesis and stabilizes new vessels. This reduces the risk of bleeding from immature vessels. Incubation of endothelial cell culture with mCRP contributed to an increase in the thickness of vascular sprouts in the collagen gel

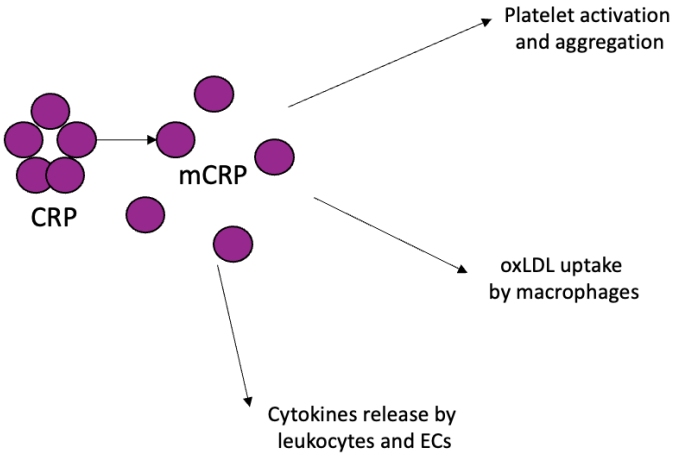

Figure 1. Selected effects of mCRP towards atherosclerosis development.

(Akil et al., 2021). It also led to a decrease in the expression of vascular endothelial cadherin and increased regulation of N-cadherin expression. CRP inhibited cell proliferation, induced endothelial cell dysfunction, and stimulated apoptosis and decreased expression of endothelial nitric oxide synthase (eNOS) by inhibiting the PI3K/Akt pathway. It was found that the interaction of CRP with the PI3K/Akt pathway can inhibit the migration of endothelial cells. And it was shown that blocking the PI3K/Akt pathway with a LY294002 inhibitor prevented the proangiogenic effects of mCRP (Karar and Maity, 2011).

mCRP in Endothelial Dysfunction

Interaction with microdoses of endothelial lipid raft is one of the ways in which mCRP can activate the endothelium. With proteins of the inner membrane, which may be inaccessible from the surface, mCRP can interact by embedding into the lipid raft. Many important components of intracellular signaling cascades are contained in lipid rafts (Head et al., 2014). mCRP induced gene transcription of monocyte chemoattractant protein-1 (MCP-1), inter-cellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), E-selectin, and IL-8 within 4 hours of incubation with human coronary artery endothelial cells (HCAEC). At the same time, incubation with nCRP induced gene transcription after 6-12 hours in the same study (Thiele et al., 2015). The maximum was reached after 24 hours, this corresponds to the known kinetics of CRP dissociation. Different gene expression profiles and different effects on the functionality of endothelial progenitor cells (EPC) derived from umbilical cord blood were induced by mCRP and nCRP at concentrations of 1-5 mg/ml, which correspond to serum CRP levels in patients with cardiovascular diseases. At the same time, mCRP stimulation of EPC led to the release of interferon-α, which in turn led to pro-inflammatory activation of cells. A similar functional response was observed in EPA in patients with systemic lupus erythematosus (Yu and Su, 2013). This was also caused by interferon-α, and possibly accelerated the development of atherosclerosis. mCRP interacted with the luminal but not basolateral surface of intact HCAEC monolayers. This led to increased expression of MCP-1, IL-8 and IL-6, induction of phospholipase C signaling pathways, nuclear factor (NF)-kB and p38 mitogen-activated protein kinase (MAPK) (Futosi et al., 2013).

mCRP in Leukocyte Activation

Due to adhesion, stimulation of leukocyte coagulation and transmigration into the vessel wall, mCRP is able to enhance the inflammatory response. This contributes to the development of atherosclerosis and its complications, and also aggravates the local inflammatory reaction (Badimon et al., 2012). A significant increase in IL-8 synthesis is observed 4 hours after incubation of whole blood and isolated neutrophils with mCRP. Already after 8 hours, the effect of nCRP stimulation on the synthesis of IL-8 by neutrophils is manifested. The maximum production of IL-8 occurs after 24 hours. This also coincides with the kinetics of dissociation. mCRP contributes to the widespread activation of neutrophils, which is observed in patients with coronary heart disease, by increasing the production of chemokines (in particular IL-8 and MCP-1). Also, the interaction of neutrophils with the endothelium is facilitated by the fact that mCRP induces the expression of ICAM-1 and E-selectin (Marchini et al., 2021). mCRP noticeably enhances the adhesion of neurotrophils to activated mCRP HCAEC. This indicates the importance of the synthesis of chemokines induced by mCRP. Neutrophils mainly adhered to activated mCRP HCAEC only in the presence of mCRP. This means that mCRP is involved in stimulating the interaction of neutrophils and endothelium. Incubation of nCRP neutrophils for 24 hours promoted the release of IL-8, which was mainly mediated by CD16. The production of IL-8 and NO by neutrophils was significantly reduced by inhibition of CD16 by a monoclonal antibody (Heemskerk and van Egmond, 2018). It is worth noting that nCRP does not bind to CD16, unlike mCRP. Due to this, it can be assumed that during 24 hours of incubation, nCRP dissociated into mCRP. mCRP then bound to CD16 and stimulated neutrophils (Heuertz et al., 2005). At the same time, mCRP, due to the increased regulation of CD11b/CD18 on neutrophils, mCRP promoted the adhesion of neutrophils to the activated lipopolysaccharide (LPS) HCAEC (Khreiss et al., 2004).

mCRP induced polarization of T cells and maturation of monocytes into macrophages of the proinflammatory M1 phenotype. Thus, with the help of cytokine signaling pathways, T-cells can stimulate the polarization of monocytes (Zha et al., 2021). When stimulated by proinflammatory cytokines derived from Th1 cells, macrophages acquire the proinflammatory M1 phenotype. And IL-13, which was obtained from Th2 cells, induces polarization into the anti-inflammatory phenotype M2 (Muraille et al., 2014). Conversely, macrophages polarized in the early immune response can affect the polarization of T cells. That is why these two types of cells are usually coordinated in their immune actions. mCRP induced the polarization of T cells into the Th1 phenotype, as well as the maturation of macrophages into the M1 phenotype (Ahmed and Ismail, 2020). Based on these data, it can be assumed that mCRP affects both the innate and acquired immune response. mCRP can also stimulate the uptake of LDL by macrophages. Which in turn mediates the formation of foam cells (Orekhov et al., 2020).

mCRP in LDL Opsonization and Macrophage Uptake

One of the most important characteristics that links CRP to atherosclerosis is the interaction of CRP with LDL. It was also

found that nCRP does not interact with intact LDL particles. But at the same time, nCRP interacted with enzymatically modified LDL in the presence of calcium (Taskinen et al., 2005). Enzymatic processing can expose lipid ligands for nCRP. And in the presence of calcium, this can lead to dissociation. mCRP binds to oxidized and enzymatically modified LDL particles, as well as intact LDL. And calcium enhances this binding. It has also been found that in the presence of calcium, mCRP can bind to LDL lipids by injection into the membrane (Boncler et al., 2019). mCRP binding reduced the uptake of oxidized LDL by macrophages and increased the uptake of native LDL. Due to the activation of the leukocyte integrin Mac-1 (CD11b/CD18), poorly modified LDLs have a strong proinflammatory effect on monocytes (Wu et al., 2017). This is the reason for the activation and adhesion of monocytes. mCRP has demonstrated significant affinity with poorly modified LDL, as well as increased binding of these LDLs to monocytes. Aggregated CRP opsonized LDL and mediated LDL uptake by macrophages.

mCRP in Atherosclerotic Lesions and Myocardial Infarction

Basically, mCRP is considered as a tissue-bound form of CRP. It was found in potentially vulnerable carotid plaques. In these plaques, CRP prevailed in areas that were rich in newly formed blood vessels, inflammatory cells, and smooth muscle cells. The high level of expression of other proinflammatory molecules (such as IL-6, cyclooxygenase-2 (COX-2), MCP-1) in these regions potentially reflects self-sustaining proinflammatory activity (Che Man et al., 2020). During this activity, tissue-bound CRP participates together with other pro-inflammatory molecules. Which suggests that tissue-bound CRP has the ability to maintain local inflammation and plaque destruction. CRP also increases the production of matrix metalloproteinase-1 (MMP-1) by monocytes. Based on this, it can be assumed that CRP can increase the vulnerability of plaques by contributing to the degradation of the extracellular matrix (Lin et al., 2021). In patients who underwent directional coronary atherectomy, tissue deposits of CRP were found in the samples of coronary plaques. Increased concentration was observed in thrombotic tissues and necrotic nucleus (Meuwissen et al., 2006; Keshavarz-Motamed et al., 2014). In addition, tissue deposits of CRP were found in samples of atherosclerotic plaques of the femur, which were obtained from patients with peripheral artery diseases. CRP deposits and mRNA CRP expression were found in the carotid artery plaques of patients who underwent carotid endarterectomy. CRP mRNA expression and CRP deposits were also found in samples of affected venous coronary bypass grafts (Di et al., 2021). For the most part, they were in the inflammatory active areas of the plaques. There, the deposits of CRP were colonized by smooth muscle cells. mRNA CRP expression and CRP deposits were absent in the fibrous membrane of plaques, areas of severe calcification and desobliteraterations obtained as a result of chronic coronary occlusions (Bennett et al., 2016).

CRP is deposited in samples of atherosclerotic plaques of the aorta and carotid arteries of a person in a monomeric, but not pentameric form. This was demonstrated in a study by Eisenhardt et al (Eisenhardt et al., 2009). mCRP is colocalized with macrophages in the intima of atherosclerotic lesions. In the same study, it was revealed that activated platelets with a "trigger" of membrane lipids that adhere to atherosclerotic lesions can cause dissociation of nCRP to mCRP (Sproston and Ashworth, 2018). In atherosclerotic lesions, circulating nCRP can serve as a potential source of mCRP. Dissociated mCRP can stimulate the expression of P-selectin on the plates. This can contribute to adhesion, rolling of monocytes and penetration into atherosclerotic lesions (Eisenhardt et al., 2012).

mCRP was detected in the blood plasma of patients with acute myocardial infarction less than 6 hours after the onset of symptoms. At the same time, peak values are reached after 24 hours, and after that they decrease. In patients with a negative troponin T test and with elevated hsCRP levels, the level of CRP in plasma was below the limit sufficient for detection (Patibandla et al., 2021). Based on this, it can be assumed that there is a correlation between the generation of mCRP and ischemic myocardial injury. mCRP can dissociate and then spread in the bloodstream on circulating microparticles. This may explain the presence of predominantly tissue-bound mCRP in the circulating blood. In their study, Zha et al. described the expression of mCRP by macrophages that infiltrate tissues after ischemic injury (Zha et al., 2021).

mCRP in Thrombogenesis

mCRP induced P-selectin expression, CD63, and GPIIb/IIIa conformation by platelets in a dose-dependent manner. Previously, it was found that both forms of CRP bind resting and activated platelets. But only activated platelets can dissociate nCRP to mCRP (Boncler et al., 2011). This, in turn, contributes to the further recruitment of platelets. Thanks to optical aggregometry, it was found that mCRP caused platelet aggregation and activation in platelet-rich plasma (PRP) of completely healthy volunteers. Despite the fact that nCRP treatment did not give any effect even at high concentrations. None of the forms of CRP affected blood clotting after 15 minutes of incubation. As assessed by rotational thromboelastometry and immunoassay, the properties of the clot, the level of the thrombin-antithrombin complex and procoagulant activity were not affected by CRP (Fernández-Bello et al., 2013). In study by Bisoendial et al. on healthy volunteers, it was found that blood clotting factors increase 4 hours after CRP infusion (Bisoendial et al., 2005). They observed a significant increase in

the level of prothrombin, D-dimer and plasminogen activator inhibitor type 1. In another study that was conducted by Molins et al. a significant increase in the thrombus area in the PRP of healthy volunteers incubated with mCRP was observed (Molins et al., 2008). Three-dimensional topographic visualization showed that mCRP affects the growth of a blood clot in a dose-dependent manner. Confocal microscopy also showed that mCRP was colocalized with adhered platelets in the entire volume of the thrombus (Slevin et al., 2018.)

Atherothrombosis resulting from plaque rupture is a severe complication of atherosclerosis. This complication can lead to myocardial infarction and death (Saha et al., 2015). Vulnerable, rupture-prone atherosclerotic plaques are characterized by a large necrotic nucleus with a high content of lipids and apoptotic cells, dense infiltration by macrophages, phenotypic switching of smooth muscle cells and migration into intima and a thin fibrous membrane. mCRP can maintain an active inflammatory state in plaque tissues, activate platelets and probably influence the formation of blood clots in other ways, thereby contributing to the development of a vulnerable plaque and a prothrombotic condition (Harman and Jørgensen, 2019).

4. Conclusion

In conclusion, this review provides a comprehensive analysis of the role of C-reactive protein (CRP) in atherosclerosis and highlights the functions of both native CRP and non-native pentameric CRP (non-native CRP) in the development and progression of the disease. The findings synthesized from the examined studies contribute to a deeper understanding of the complex interactions between CRP and atherosclerosis, while also revealing some important implications for future research and clinical practice.

The review underscores the dual nature of CRP, with native CRP demonstrating potential atheroprotective effects, while non-native CRP appears to have distinct functions that contribute to the pathological processes in atherosclerosis. Native CRP, in its pentameric form, interacts with molecules such as oxidized LDL (ox-LDL) and endothelial cells, exhibiting anti-inflammatory properties and inhibiting the formation of foam cells. This suggests a potential protective role of native CRP in impeding the progression of atherosclerosis.

On the other hand, the formation of non-native CRP, triggered by structural changes in the protein, leads to the release of monomeric CRP (mCRP), which exhibits pro-inflammatory characteristics. Non-native CRP demonstrates binding capabilities to modified LDL and lysophosphatidylcholine, contributing to the inflammatory response and potentially exacerbating atherosclerotic processes.

While studies utilizing animal models have provided valuable insights into the effects of CRP on atherosclerosis, there is still a need for further research to fully elucidate the role of CRP in human pathophysiology. Enhanced understanding of the mechanisms underlying the transition between native and non-native forms of CRP could potentially open new avenues for therapeutic interventions and the development of targeted treatments for atherosclerosis.

The implications of this review's findings for clinical practice are noteworthy. The assessment of CRP levels, particularly highly sensitive CRP (hsCRP), could serve as a valuable biomarker for evaluating cardiovascular risk in asymptomatic individuals. The inclusion of CRP measurements in risk assessment strategies has been supported by several clinical trials, and it has been recognized as an independent biomarker for cardiovascular diseases by prominent organizations such as the American Heart Association. However, a comprehensive understanding of both native and non-native CRP is crucial in interpreting CRP levels accurately and optimizing their clinical utility.

Author contribution

A.V.P. wrote, drafted; V.N.S., V.A.K., N.K.S., A.Y.P., A.N.O. wrote, reviewed and edited the paper.

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