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TOPICAL REWIEV

C-reactive protein, cytokines and inflammation in cardiovascular diseases

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Abstract: Inflammation of vascular cell wall is the key problem and proinflammatory cytokines and chemokines play a great role in it. These molecules, togheter with C-reactive protein (CRP) can predict risk of coronary events. It is questionable to what extend are CRP and pro-inflammatory cytokines purely acute phase markers and to what extend are they active inflammatory participants. Besides inflammation, other prominent mechanism in the pathogenesis of atherosclerosis and atherothrombosis – underlying causes of coronary events, is genetics. Gene polymorphisms including polymorphisms of inflammatory markers are studied and one of them, polymorphism of monocyte chemoattractant protein (MCP-1/CCL2) and its receptor CCR2 (key components of atherosclerosis) belong to most studied one. MCP-1/CCL2 and CCR2 polymorphisms have been implicated as susceptibility factors for chronic stable angina pectoris and myocardial infarction by several independent investigators. It seems that CCL2/CCR2 axis plays an important role both in post-ischemic and post-reperfusion inflammation and could become a new therapeutic goal in selected cardiovascular diseases as well as in stroke in future. Inhibition of this axis disrupts ischemic-reperfusion injury by decreasing edema, leucocyte infiltration and expression of inflammatory mediators. One can suppose that identifying genes influencing inflammatory biomarkers might improve understanding of genetic determinants of cardiovascular disease our management and prevention *(Tab. 2, Fig. 1, Ref. 105).* Full Text (Free, PDF) *www.bmj.sk.*
Key words: atherosclerosis, C-reactive protein, cytokines, cytokine gene polymorphism, inflammation.

Inflammation is a complex of defensive mechanisms reacting to the entry of harmful agents to the organism or cells, in order to eliminate or at least to dilute the agent, repair damaged cells or tissue and restore homeostasis. From this definition it is clear, that inflammation does not accompany only infectious diseases (1) but also others, causing cell, tissue or organ injury (Tab. 1).

Inflammation plays an important role also in the etiology of ischemic heart disease (IHD), myocardial infarction (MI), angina pectoris (AP) and hypertension, however, its mechanism in various stages of pathological process is not well understood (2–7). If the cause of IHD is atherosclerosis or other, it is accompanied by inflammation. Various types of inflammatory cells, cytokines, chemokines and other soluble factors were confirmed (2, 8–11).

Tab. 1. Inflammation and its induction agents.

1. Infectious – bacteria, fungi, viruses
2. Mechanical – scratching, cutting
3. Physical – burning, radiation
4. Allergic
5. Autoimmune
6. Atherosclerosis and cardiovascular diseases
7. Cancer
8. Nutritional disorders – hypoxia, lack of proteins, vitamins, etc.
9. Other causes

Inflammation and atherosclerosis

Atherogenesis and atherotrombosis are accompanied by inflammation (12). At present, atherosclerosis is considered to be an inflammatory disease and atherosclerotic plaque inflammation the cause of intima erosion, rupture and subsequent ischemia (13, 14). The question is, whether the inflammation is the cause or the result of the atherosclerotic plaque rupture. The answer is, both. Even lipid deposition (LDL – low density lipoproteins) is accompanied by inflammation – macrophages and T-lymphocytes enter vessel wall and foam cells (macrophages filled with LDL particles) develop (15). Macrophages become activated, produce a large amount of cytokines and chemokines promoting inflammation and MMP-9 (matrix metaloproteinase), smooth muscle cells proliferate and intima becomes thickened. Later,

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fibrosis develops and calcification appears in vessel wall. The more intensive is the inflammation, the higher is the activation of macrophages and atherosclerotic plaque is more instable. A rupture may develop, causing activation of thrombocytes, thrombus formation and subsequent ischemia (14). The inflammation in the atherosclerotic plaque is promoted also by mastocytes, complement and C-reactive protein (CRP). Activated mastocytes support instability of the atherosclerotic plaque by histamine, chymase and tryptase production and support coronary spasm by the production of endogenous vasoconstrictors. The ability of macrophages to become activated is extremely important in the atherosclerotic plaque rupture. In subjects with a genetically higher ability of macrophage activation, the rupture and subsequent thrombosis and ischemia may develop in a smaller atherosclerotic plaque, even in a limited coronary atheroma (16). This means that two identical atheromas do not need to be identical and differ in their prognosis. The difference is determined by genetic polymorphisms, e.g. by different ability of macrophages to become activated.

The inflammation plays also an important role in the development of post-ischemic organ dysfunction in acute coronary syndromes, and in the healing process after myocardial infarction (17). These facts highlight the value of non-specific inflammatory markers in patients with cardiovascular diseases.

CRP – production, regulation of the production and plasma/serum levels of CRP

CRP, a part of an acute phase reaction, was previously considered to be a marker of underlying infection or tissue injury. Later, it was found that it is also a marker of chronic systemic inflammation and that there exist some associations between increased levels of CRP and clinical course of the acute MI and other acute coronary syndrome (18–21). It was confirmed that CRP is a better risk predictor of the cardiovascular events then LDL levels (22). Its increased levels are considered a risk factor for atherosclerosis progression and complications even in healthy individuals (23). Increased levels of hsCRP were found also in patients with chronic heart failure, diastolic heart failure and dilated cardiomyopathy (24, 25, 26).

CRP is nowadays added to so called soluble pattern recognition receptors (PRR), sensors of threatening, that recognize some evolutionary conserved substances both from external intruders on the surface of microorganisms and internal structures that originate from our damaged cells, organs or tissues. Some molecules of danger synthesized during threatening of our organism could also be recognized by CRP (27, 28). After binding to them, the human CRP activates different humoral factors (the complement system), cells (phagocytes) and transducing signals. That evokes the immune response against the intruders and mediates a potent proinflammatory pathophysiological effects, too.

Plasma CRP is produced mostly by hepatocytes and is under the regulation of cytokine IL-6. Normal values range round 1.3 $mg.l^{-1}$ in adults (29, 105). Median CRP levels are somehow higher in apparently healthy adults compared to blood donors and is characteristic for a given individual. CRP levels demonstrate neither seasonal nor diurnal variations and are not influenced by food intake (21, 30, 31). CRP levels have a tendency to increase with age, reflecting an increased incidence of subclinical pathologic processes (32, 33). After a stimulus, within 6 hours, plasma CRP levels increase above 5 $mg.l^{-1}$ and reach the maximum within 48 hours. After that, the level of CRP return to very low "reference values" in plasma with the same speed. Gene coding for CRP is localized on the chromosome 1 (1q2.12.5) and the main inductor of gene transcription is IL-6. IL-1 and complement act synergically (32, 34, 35). The expression of CRP is regulated mainly at transcription level. Post-transcription mechanisms play also an important regulatory role, e.g. during inflammation CRP stay in the endoplasmatic reticulum is shortened from 18 hours to 75 minutes, enabling a faster CRP production (35). The half-life of CRP in plasma is approximately 19 hours and is constant during various conditions in healthy and sick people. Therefore, the only factor determining the level of CRP is its production speed (36), which directly reflects the intensity of pathological process.

High sensitive CRP (hsCRP)

In the mid of 1990s, a new method ELISA – imunoassay was establish to evaluate the level of high sensitive CRP (hsCRP), which has much higher sensitivity then classic methods used previously. It has been proved that higher levels of hsCRP, previously considered to be within normal range, have a strong predictive value in the development of coronary events in the future. First studies concerned patients with stable, unstable and severe unstable angina. These studies showed the predictive value of hsCRP levels regarding future coronary events (37,38) and brought a lot of interest into the predictive values of hsCRP. Studies demonstrating the relationship between higher level of hsCRP and future atherothrombotic events, such as coronary events, stroke, peripheral artery disease, were initiated (39, 40, 41). A cardiovascular risk scale according to hsCRP levels was developed (42) (Tab. 2).

CRP, cytokines, chemokines and other nonspecific inflammatory markers

The level of CRP closely correlates with other non-specific inflammatory markers, which show similar although less significant predictive association with future coronary event (43, 44).

Tab. 2. HsCRP scale of cardiovascular risk – according to the American Heart Association (Pearson et al, 2003).

Cardiovascular risk	hsCRP level
Low	<1 mg x l^{-1}
Medium	1–2 mg x l^{-1}
High	2–3 mg x l^{-1}
Infection	3–10 mg x l^{-1}

Many studies have shown that increased levels of fibrinogen, CRP and IL-6 are associated with the risk of coronary heart disease, clinical course, prognosis and severity of atherosclerosis (45, 46). Similar association was found with the level of IL-8, where the risk of coronary heart diseases was higher in men compared to women and was independent from both traditional risk factors and CRP (47). Authors could not exclude the possibility that IL-8 reflected a pre-clinical atherosclerosis. Concentrations of complement components, mainly the C3 to C4 ratio and the level of BNP (brain natrium uretic peptide) could also predict the mortality and severity of cardiovascular disease (48, 49, 50).

Zouridakis et al showed 4 markers predicting rapid progression of coronary heart disease – CRP, sICAM (soluble intercellular adhesive molecules), neopterin and MMP-9 (51). Their levels are higher in „progressors" than in "non-progressors". According to their results the patients with CRP concentration in the medium quartile had 3-fold risk of coronary heart disease progression compared to the patients in the lowest quartile, and patients with sICAM levels higher than 271.4 $ng.ml^{-1}$ (average) had 4-fold increased risk compared to the patients in the lowest quartile. Individuals with neopterin level higher than 7.5 $nmol.l^{-1}$ (medium quartile) have 5-fold increased risk of coronary heart disease development and progression compared to individuals with neopterin levels in the lowest quartile. Patients with MMP-9 concentration higher than 47.9 $g.l^{-1}$ (median) have 3-fold increased risk of coronary heart disease progression compared to the patients in the lowest quartile. As the last two markers are indicators of the activity of macrophages that are key cells playing a causative role in the process of atherosclerosis, the evaluation of their activity seems to be useful in patients at risk. A systemic therapy might prevent development or progression of coronary heart disease (52).

CRP and proinflammatory cytokines – the cause and the result of the inflammatory process

It is questionable to what extend are pro-inflammatory cytokines and CRP purely acute phase markers and to what extend are they active inflammatory participants. Increased levels of IL-6 were found in patients with instable angina, where inflammatory reaction may promote conversion of a stable atherosclerotic plaque to an instable one (53). It was also found that plasma CRP level can predict future cardiovascular events or mortality due to coronary heart disease even in healthy individuals (20, 23, 54). CRP predicts cardiovascular risk even in Japanese, who generally have lover levels (55). These findings show the possibility that both the progression of atheroma and the plaque rupture might be predicted by a follow-up of CRP levels. The role of TNF-alfa and IL-6 in the atherogenesis and thrombosis was also shown. Pro-inflammatory cytokines TNF-alfa, IL-6, IL-1 and also CRP are in large amounts produced except of liver also by adipocytes. Production of IL-6 with obesity and approximately 30 % of IL-6 in healthy individuals come from adipocytes (7, 56). These cytokines inhibit insulin signalization and cause insulin resistance, and also enhance the development of endothelial dysfunction – they increase the expression of adhesive molecules, pro-thrombotic factors, acute phase proteins, which can increase a cardiovascular risk via a feed back mechanism (57, 58). It was found that, both CRP and pro-inflammatory cytokine levels correlate with blood pressure, dyslipidemia, HDL (high density lipoproteins) and level of triglycerides, smoking, diabetes, insulin resistance, markers of endothelial dysfunction and obesity (23, 32, 59–62). The correlation with BMI (body mass index) was also found, which could partially reflect the fact that the majority of basal CRP and IL-6 is produced in adipocytes (44). Weight decrease was associated with plasma CRP decrease even in healthy individuals (56).

In the pathogenesis of coronary heart disease and atherosclerosis, chemokines play an important role, too. The most important are: MCP-1 (monocyte chemotactic protein), MIP-1alfa (macrophage inflammatory protein), IP-10 (IFN-gama inducible protein, with Mr=10 000), RANTES (regulated on activation normal T-cell expressed and secreted) and eotaxin (63). MCP-1, called also CCL2 (MCP-1/CCL2) is chemotactic to monocytes, T-lymphocytes and NK cells and participates in the development and restoration of diseases characterized with infiltration of monocytes (64, 65). It modulates fibroblasts and endothelial cells function and has an important role in the pathogenesis of myocardial infarction, thrombotic occlusion, myocardial ischemia, and also in the reperfusion and healing process after myocardial infarction (17). Tarzami et al. showed that MCP-1/CCL2 had a dual role in myocardial ischemia – beside chemotactic activity protected myocardial cells against hypoxia induced cell death (66, 67).

Nonspecific inflammatory markers in cardiovascular diseases – what do they reflect?

Another question is what exactly these non-specific markers reflect. Four possibilities can be found and three of them directly or indirectly highlight the role of atherosclerosis: 1) Atherosclerosis, plaque development, its instability, rupture and resulting atherothrombosis are inflammatory events. The first assumption could be that increased levels of inflammatory markers reflects inflammation of the vessel wall. This is possible, but some contradictory results concerning the level of CRP and atherosclerotic plaque size exist. This discrepancy could be explained by genetic polymorphism causing a different ability of macrophages to be activated as it was mentioned above, which explains a possible rupture even in a small atherosclerotic plaque (16). 2) Chronic systemic non-vascular infection is also pro-atherogenic and acute systemic inflammatory episodes are markedly associated with atherosclerotic processes (68). CRP could reflect inflammation in some other part of organism, although the correlation of Chlamydia pneumonie and Helicobacter pylori antibodies with the development of coronary heart disease is not very clear (69). 3) Generally, the above mentioned associations of CRP and IL-6 levels with BMI and IHD risk factors increase the possibility that inflammatory markers associated with the risk of atherothrombosis could reflect a certain metabolic state, which is also pro-atherogenic and is a predisposition to atherothrombotic

events, that means it's also pro-inflammatory. In fact, CRP level predicts development of type 2 diabetes independently from traditional risk factors (70). In insulin-resistant obese individuals, increased CRP levels decrease in parallel with the improvement of insulin resistance related to weight loss (71). 4) The fourth possibility is the fact that individuals differ in their sensitivity to various stimuli leading to acute phase proteins production. Therefore, those who are "higher CRP responders", either due to genetic mechanism or other acquired mechanism (for example, BMI) are simple more sensible to atherosclerosis progression and complications.

Genetic background of inflammation in cardiovascular diseases

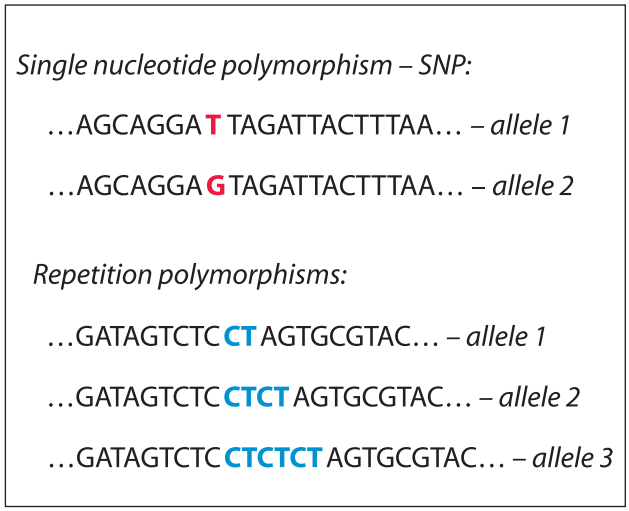
Main terms

People differ in the risk of development and death due to various diseases including cardiovascular disease, and differ also in inflammatory reactions (2, 72). Inter-individual genetic differences play an important role.

Human diseases are roughly divided into three categories according to genetic factors: 1) "monogenic diseases" (caused by one gene defect), 2) complex diseases (with multigenic or polygenic predisposition) and 3) diseases without genetic predisposition. The best results were achieved in "monogenic diseases". Recently, the attention is shifted to complex diseases caused by several genes, including majority of socially burden diseases.

Genetic polymorphism means that more than one allele (variant) of gene is present in population. It is referred to when the frequency of the most frequent gene allele in population is lower than 99 %, e.g. when the frequency of the rare gene allele is higher than 1 %. Single nucleotide polymorphism (SNP) belongs to the most common types of genetic polymorphism, including exchange, insertion or deletion, and repetitive polymorphisms (Fig. 1). The number of SNP polymorphisms in genome is estimated to 10 millions (73). Gene variants, influencing gene expression, are called functional gene polymorphisms. SNP polymorphism may be present in coding, regulatory and in non-coding gene regions (74).

Fig. 1. Diagram of the most frequent DNA polymorphisms.



While the polymorphisms in coding regions tend to change the structure of primary protein or result in protein defect, the polymorphisms in regulatory gene areas, influencing gene transcription, may affect gene expression. It is suggested that majority of polymorphisms playing a role in genetic predisposition of complex diseases, are found in regulatory gene areas (74).

Individual genetic susceptibility to complex diseases is present, when an inter-individual difference in disease risk exists, not determined by environmental factors. Regarding diseases, we distinguish susceptibility gene variants (allele), which predispose for disease development (they are more frequently found in patients compared to general population) and protective alleles, which on the contrary, are less common in patients than in healthy subjects.

Genetics, inflammation and cardiovascular diseases

The inheritance of cardiovascular diseases is polygenic, i.e. several genes can influence their development and clinical course. Except of polymorphisms of genes coding for homocystein, lipid metabolism, factors of coagulation, β2-adrenergic receptors, genes regulating blood pressure and others, inflammatory factors genes play an important role, too (75–78). These genes are numerous and gene polymorphisms related to different stages of inflammatory response have been found (79, 80, 81).

Genetics of CRP

The basal level of CRP both in patients and healthy controls are genetically determined. In repetitive measurements in healthy individuals it was found that concentrations of CRP were relatively stable. It means, that knowledge in CRP genetic component may contribute to the stratification of so far healthy individuals into groups with higher or lower risk for cardiovascular disease development (83, 84). In human, the CRP gene is located on chromosome 1q23, in a conserved genetic region, which codes for important proteins of immune system and also proteins important for cell-to-cell communication (82). Szalai et al sequenced 1156 nucleotides long promoter area of the CRP gene and identified two SNPs – one bi-allelic (-409A/G) and one three-allelic (-390C/T/A), which modulated basal CRP concentration in healthy individuals by influencing transcription factor binding (84). The results of "Framingham heart study" in 1640 unrelated participants revealed in 9 from 13 studied SNPs a relationship with CRP level (85). Knowing factors, which regulate plasma CRP levels, either basal or induced by infection or other inflammation, is very important also for the cardiovascular risk prediction. It was found that patients with homozygous +1444TT allele of CRP gene had significantly higher plasma CRP levels induced by inflammatory stimulus (86). This effect was independent from IL-6 concentration, IL-6 -174G/C SNP and conventional

cardiovascular risk factors. The production of CRP is except of CRP gene regulated also by IL-1 beta, IL-1Ra and IL-6 genes (87, 88, 89). In 160 patients with angiographically confirmed coronary heart disease, the association of higher plasma CRP level with the presence of IL-1B (+3954)T allele was found as well as a possible relationship between IL-1RN(VNTR)*2 allele and lower CRP concentrations (88).

Acute coronary syndrome is associated with the activation of endothelial cells and systemic inflammation. It was found that genetic variations in the IL-1 locus influenced inflammatory processes – the IL-1RN*2 and the -511 alleles, respectively, contributed to changes in the plasma level of soluble markers of endothelial inflammation such as von Willebrand factor (vWF) and E-selectin (90). A correlation between higher plasma CRP level and presence of CD14 260TT homozygous allele was also found, which could be associated with the higher ability of macrophages to become activated and produce pro-inflammatory cytokines (80, 81).

Gene polymorphisms of MCP-1/CCL2 and CCR2 and the risk of cardiovascular disease development

Pro-inflammatory cytokines and chemokines play an important role in the pathogenesis of heart diseases (63). Gene polymorphisms of chemokine MCP-1/CCL2, a molecule that plays an important role in atherosclerosis, and its receptor CCR2 belong to most studied one (2, 91, 92). MCP-1/CCL2 is a potent chemoattractant for monocytes, T cells and NK cells. MCP-1 induces the transmigration of $CCR2^+$ monocytes from the circulation, promotes their differentiation to lipid-laden macrophages (93, 94) and contributes to the proliferation of arterial smooth muscle cells (95) which, along the macrophages, constitute the key cellular components of atherosclerotic plaques. This chemokine plays a dual role in myocardial ischaemia. In addition to several negative roles in the process of atherosclerosis, thrombotic occlusion of a coronary artery and in the process of reperfusion, this chemokine protects myocytes from hypoxia-induced cell death and has also positive effect in myocardial infarct healing (17, 66, 67).

Polymorphism of MCP-1 and its receptor – CCR2 have been implicated as susceptibility factor for chronic stable angina pectoris (2) and myocardial infarction by several independent investigators (92, 96). An association of CCR2 polymorphisms with the number of closed coronary artery vessels in coronary artery disease was also found (97). Deletion of MCP-1/CCL2 or CCR2 resulted in a large (50–80 %) reduction in atherosclerotic plaque size (65, 98). However, the data on contribution of the MCP-1 polymorphisms to the pathogenesis of coronary atherosclerosis are not uniform. McDermott et al found that the presence of MCP-1 -2578G allele in homozygous form was significantly associated with both myocardial infarction occurrence and higher MCP-1 plasma level. Increased MCP-1 levels were associated with age, smoking, BMI and waist to hip ratio (99). In other study, the plasma MCP-1 level was independently associated with the prognosis of patients with acute coronary syndrome (100). Higher levels of MCP-1 were associated with higher age, Caucasian race, early onset of coronary heart disease, smoking, hypertension, hypercholesterolemia and higher hsCRP levels (100). Similar association was found in the group of patients with detected calcium in coronary arteries.

It was found that CCR2 -/- mice show smaller area of infarction after ischemic-reperfusion injury, what correlated with decreased oxidative stress of their leucocytes (101). So it seems that CCL2/CCR2 axis plays an important role in post-ischemic and post-reperfusion inflammation and could become a new therapeutic goal in selected cardiovascular diseases as well as in stroke in future. It is assumed that CCL2/CCR2 axis inhibition disrupts ischemic-reperfusion injury by decreasing edema, leucocyte infiltration and expression of inflammatory mediators (102). However, the studies of Tarzami et al showed that MCP-1/CCL2 played a dual role in myocardial ischemia – beside chemotaxis it also protected myocardial myocytes from hypoxia induced death (66, 67). Nevertheless, there is a difference in the role of inflammation in acute and later stages of pathological process (103).

Vascular inflammation plays a central role in atherosclerosis and inflammatory biomarkers, such as CRP, IL-6, MCP and sICAM predict risk of cardiovascular disease (104). Thus finding genes that influence systemic levels of inflammatory biomarkers may provide insight into genetic determinants of vascular inflammation and cardiovascular disease.

Conclusion

Biomarkers of vascular inflammation have both genetic, inflammatory and environmental determinants and identifying genes influencing inflammatory biomarkers might improve our understanding of genetic determinants of cardiovascular disease.

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