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Inflammation and hypoxia in atherosclerosis, coronary artery disease, and heart failure

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ABSTRACT

Inflammation is a process that occurs after tissue damage or counteract infection. It is a complex reaction involving various cells, proteins (chemokines, acute-phase proteins) and other factors. The precise understanding of the mechanisms affecting the distribution of inflammatory cells and their modulators in areas of inflammation may have a crucial role in the development of strategies blocking inflammatory processes. Hypoxia leads to deprivation of oxygen and when it develops, various mechanisms come into action to alleviate any consequences that it might cause. Hypoxia inducible factor (HIF) is one of the most important factors involved in the response to hypoxia. Recent studies provide a better understanding of both, inflammation and hypoxia, in the development of atherosclerosis and various diseases in the spectrum of cardiology.

Key words: inflammatory mediators, hypoxia, atherosclerosis

Introduction

Most of the major breakthroughs in understanding inflammatory processes took place in the nineteenth and twentieth century. The term "inflammation" originates from ancient Egypt, but the first written definition of this process and the description of its symptoms was put forward by Roman writer Celsus in the first century of our era. Celsus described four cardinal features of inflammation such as warming, pain, swelling and redness. The last element, loss of function, was added in the nineteenth century by Rudolf Virchow. In 1793, John Hunter, the eminent Scottish surgeon, argued that inflammation should not be considered a disease but a process which is beneficial to the host. This discovery resulted in the subsequent intensive worldwide research projects which led to the conclusion that inflammation should be perceived as one of the most complicated processes but extremely important for the survival of organisms [1].

Inflammation is a complex reaction to harmful agents, tissue necrosis or direct injury. The aim of this process is to remove or destroy the cause of cell damage as well as eliminate any cells or necrotic debris which formed as a result of the damage. In a set of complex reactions between various cell types and organs, damaged tissue is either repaired, regenerated or replaced by the scar tissue [2]. It should be emphasized that repair mechanisms are activated at the moment when damaging factor appears. Some of the inflammatory cells are recruited not only to eliminate the necrotic tissue but also to stimulate the formation of a new extracellular matrix mediated by cytokines. The repair process begins within 24 hours after damage by the inflow and then the proliferation of fibroblasts and endothelial cells. Most elements of these processes are controlled by the mediators which have the ability to interact with the extracellular matrix.

Based on the recognized morphological and clinical differences, inflammation has been divided into acute and chronic. Acute inflammation (AI) is an immediate systemic reaction which aims to transfer leukocytes to the site of the injury and to eliminate the cause. AI is characterized by short duration, presence of exudate and neutrophil infiltration. Depending on nature and intensity of the damage, acute inflammation can: resolve completely (with normalization of vascular permeability and inhibition of leukocyte infiltration, and tissue pattern return to its state before the injury), lead to fibrosis and formation of scar tissue (in tissues unable to regenerate
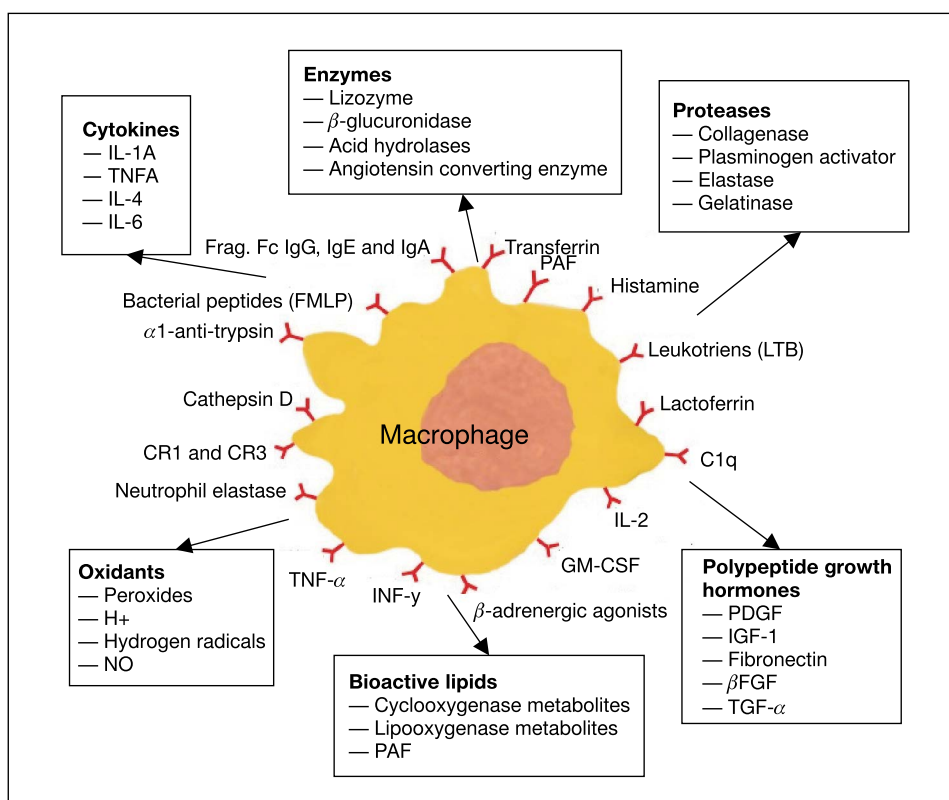
Figure 1. Receptors for external signals recognized by macrophages and macrophage secretions

or when excessive damage occurs), or it can progress to chronic inflammation (CI) in which mononuclear cells infiltration occurs along with vascular proliferation and scarring [2]. The key cells in chronic inflammation and immune responses are macrophages, sometimes called administrators or masters of chronic inflammation. The most important functional features of the macrophages are mobility, phagocytosis, the ability to recognize signals as well as production and secretion of mediators (Fig. 1).

Hypoxia, in general, is an inadequate oxygen supply, which can span from a single cell to the whole body. The process of hypoxia involves various compensation mechanisms which are initiated to restore oxygen homeostasis. Prolonged hypoxia is likely to directly cause cell injury and death [2]. One of the most important mediators in the adaptation to hypoxia is hypoxia inducible factor (HIF) which is a heterodimer transcriptional factor built of subunits α and β. Subunits α (HIF-1α, HIF-2α, HIF-3α) are sensitive to, e.g. oxygen supply and growth factors, but subunit β is constitutively expressed. Along with adaptation to hypoxia, HIF is seen as an important factor in embryogenic development and immune response [3, 4]. However, there is still no consensus on the exact mechanism of the cellular response to hypoxia. Numerous pathway models of hypoxia signalling have been proposed. Although all of these models present some clues, no clear conclusions can be drawn yet. However, they provide us with better understanding of what might become a framework for future experiments [5].

The number of patients suffering from heart failure constantly rises across the world. The treatment of this disease puts an ever-rising strain on healthcare. Immense amount of research has already been done on this subject, but relatively few new drugs and treatment options have been provided thus far [6]. Recently, new diagnostic aids have become available (e.g. C-reactive protein (CRP) measurements). From a practical point of view, while they are helpful, they also tend to be overused especially by the inexperienced doctors [7]. It has been widely agreed that both inflammation and hypoxia are integral parts in the development of a wide array of diseases in the spectrum of cardiology. HIF and inflammation mediators have been identified to be expressed in the course of atherosclerosis development. Moreover, their plasma levels change in various clinical scenarios, and altering their expression may also lead to different outcomes in particular diseases.

Acute-phase proteins

Acute and chronic inflammations, besides local changes, are frequently accompanied by various changes in the whole body and these may occasionally occur in location distant from the site of inflammation. This phenomenon has been defined as an acute-phase response (APR) [8]. Changes in APR can be divided into the following: changes in the concentration of plasma proteins, known as acute phase proteins, and various changes in the physiology and biochemistry of the whole body. A particular plasma protein is considered an acute phase protein if its concentration changes by at least 25% within hours after the onset of the inflammatory response. Proteins which display tendency to increase their concentration in acute-phase response are known as positive acute-phase proteins, while proteins with reduced concentrations are called negative acute-phase proteins. The concentration of proteins depends mainly on the severity of synthesis in hepatocytes. Factors known to have the ability to initiate the increase of acute phase proteins are: infection, trauma, surgical intervention, tissue infarction and advanced cancer. Over the past few years, acute phase proteins have become a diagnostic tool for diagnosis and monitoring of inflammation. According to the statistical analysis from the numerous recent studies, measuring plasma concentrations of these proteins can also help in predicting the risk of development, diagnosis and outcome of many diseases [9].

Meta-analysis undertaken by Kaptoge et al. revealed that adding plasma/serum CRP measurements could improve unfavourable inflammation course risk prediction. For patients with high cardiovascular event risk (10–20% risk of coronary heart disease or stroke over 10 years [10]), additional 1 in 440 events could be successfully predicted. In the same study, authors found that additional assessment factor such as fibrinogen, that also belongs to the acute phase proteins, could help to predict 1 in 490 events in the same risk group [11]. In a different study, plasma CRP levels were reported to be useful in assessing the risk of acute coronary syndrome occurrence, and more precise than predictions based on apolipoproteins (A-I, apoB) or cholesterol fraction levels (total cholesterol, LDL, HDL) [12]. CRP level is also a potent screening tool for coronary artery disease in patients presenting with chest pain [13]. Higher plasma/serum levels of CRP are found in patients suffering from acute coronary syndromes (myocardial infarction (MI), unstable angina pectoris) compared to those suffering from stable angina pectoris and patients not suffering from coronary artery disease (CAD) [14]. In a different study, CRP levels measured at rest were found to correlate positively with the presence of CAD. Nevertheless, the correlation was weaker than conventional risk factors

including: high age, diabetes or male sex [15]. Moreover, higher ceruloplasmin (also an acute phase protein) levels are associated with significant risk of death, MI or stroke in patients selected for coronary angiography [16]. High CRP concentration can be a predictor of left ventricle remodelling (LVR). In patients suffering their first ST segment elevation myocardial infarction (STEMI) treated with primary percutaneous coronary intervention (pPCI), CRP levels measured 24h after admission have a positive predictive value for LVR.

In addition, CRP levels correlated with future magnitude of LVR [17]. In a different study, CRP levels measured 2 days, one week as well as 2 months after STEMI occurrence correlated positively with the mass of infracted heart muscle in patients suffering from the first episode of STEMI [18]. Elevated CRP is a potent predictor of death and non-fatal recurrent MI in patients diagnosed with non-ST segment elevation MI or unstable angina [19]. Higher CRP levels measured 12–24 h after admission can be a valuable tool in predicting heart failure and death of patients who suffered MI [20]. Lower CRP levels were associated with lower risk of death in patients with congestive heart failure. A positive predictive value was especially strong for patients with ischaemic cardiomyopathy. The measurements of this protein levels also proved useful for risk stratification of those patients [21]. CRP concentration measured with high sensitivity method (hsCRP), below 3 mg/L before treatment, indicated possible successful outcome of cardiac resynchronization therapy and lower probability of death in patients with congestive heart failure [22] mean age 65.0 ± 11.8 years, NYHA class III/IV. High CRP plasma concentration on its own correlated with higher mortality in patients with acute coronary syndromes. The addition of CRP measurements can slightly improve accuracy of risk scoring systems for cardiac patients [23] but current risk score systems do not consider this factor. We studied the incremental predictive value of adding C-reactive protein to the Global Registry of Acute Coronary Events (GRACE. Plasma/serum CRP increase (but not baseline CRP levels) have also been found to indicate a late recurrence of atrial fibrillation after catheter ablation treatment [24]. Bouloukaki et al. suggest in their review that there is a connection between obstructive sleep apnea syndrome (OSAS), rising plasma/serum CRP levels and cardiovascular disease. Nevertheless, while CRP is strongly associated with cardiovascular disease, its association with OSAS is not definitively proven, leaving much room for speculation [25]. Authors cited above [12, 17, 18] suggest that CRP could be more than just a marker of LVR, of acute coronary syndrome occurrence risk or the size of myocardial damage when we consider cardiovascular disease. It has been observed that high expression of CRP can promote cardiac muscle remodelling after

MI in mice [26]. CRP plasma/serum levels have also been observed to correlate with the magnitude of atherosclerotic process. Moreover, CRP deposits are present in arterial intima at all stages of development of atherosclerosis, which suggests that it might have a potentially significant role in the pathogenesis of this disease [27]the origin and pathological significance of CRP in these lesions are not completely understood. In this study, we measured CRP levels in the plasma of hypercholesterolemic rabbits and investigated CRP expression at both the mRNA and protein levels using rabbit and human atherosclerotic specimens. CRP levels were significantly elevated in both cholesterol-fed and Watanabe heritable hyperlipidemic rabbits, and CRP levels were clearly correlated with aortic athero-sclerotic lesion size. Immunohistochemical staining coupled with Western blotting analysis revealed that CRP-immunoreactive proteins were found at all stages of atherosclerosis from the early to advanced lesions. CRP was present extracellularly and co-localized with apolipoprotein B but was rarely associated with the cytoplasm of macrophages and foam cells. Real-time reverse transcriptase-polymerase chain reaction analysis revealed that CRP mRNA in atherosclerotic lesions was barely detectable, and isolated macrophages did not express CRP mRNA, suggesting that CRP proteins found in the lesions were essentially derived from the circulation rather than synthesized de novo by vascular cells. These results suggest that there is a link between plasma CRP and the degree of atherosclerosis and that inhibition of plasma CRP may represent a therapeutic modality for the treatment of cardiovascular disease [27]. It has been indicated that hsCRP measurement in males ≥ 50 years and females ≥ 30 years with LDL cholesterol < 130 mg/dL can be helpful in determining patients suitable for statin therapy. Newer inflammatory markers such as lipoprotein-associated phospholipase A2 or myeloperoxidase, or growth differentiation factor-15 have also been investigated in relation to CAD. Nevertheless, their relevance to CAD is not yet clear [28]. Various trials which aim at reducing CRP levels using non-specific anti-inflammatory drugs have been undertaken, and their outcome is still uncertain. In spite of this fact, numerous more precise strategies of reducing CRP levels have been put forward including: CRP cross-linking, inhibition of CRP synthesis, blocking of CRP receptors, antisense strategies or blocking CRP-mediated complement activation. Potentially beneficial CRP-specific drugs require further research [29]. It has not been determined whether inappropriate levels of CRP promote disease [30]. However, it has been put forward by some researchers that randomized trial using CRP inhibitor (1,6-bis-phosphocholine) would make it clear whether CRP plays an active role in the development of CAD [31].

The role of chemokines and chemokine receptors in inflammation

Chemokines can be described as small proteins responsible for attracting leukocytes to areas of inflammation or damage [32], and, along with their receptors, they play a key role in directing the migration of mononuclear cells in the human body. Their role in inflammation and immunity has been studied extensively in recent years, and they have been already linked to various diseases. Human organism produces about 50 different chemotactic cytokines (chemokines), which can be divided into four groups, depending on their structure and function.

The first and largest group consists of CC chemokines (the CC name comes from 2 of the four cysteine residues lying beside each other in the molecule), known as β-chemokines, which are responsible for directing mononuclear cells to the areas of chronic inflammation (Tab. 1). The second group are the CXC chemokines (in this case single amino acid is located in between cysteine residues), also called α-chemokines, which are divided further into two more subgroups. In the first subgroup, chemokines contain the amino acid sequence: Glu-Leu-Arg (ELR motif) and have been identified to be chemoattractants of neutrophils. They contribute to wound repair, promote angiogenesis and promote the formation of granulation tissue by inducing fibroblast differentiation. Chemokines in the second subgroup (without the ELR motif) are the inhibitors of angiogenesis. The third group of chemokines is called CX3C (three amino acids separating first two cysteine residues), and it contains only one protein CX3CL1 fractakline (FKN) which acts as a cell adhesion receptor (when connected to the cell membrane) or as a chemoattractant (after being cut from the cell membrane by tumour necrosis factor alpha). Lymphotactin (XCL1 — only contains 1 cysteine residue) is the only member of the fourth group of chemokines. It attracts T lymphocytes and natural killer cells [32, 33].

Rising levels of chemokines coupled with chronic inflammation are confirmed factors in development of many vascular diseases including: atherosclerosis, restenosis, and transplant vasculopathy [34]. Athero-sclerosis is associated with the formation of athero-sclerotic plaques caused by hypercholesterolemia, turbulent blood flow and hypertension. CCL2 chemokine is present in all macrophages forming atherosclerotic plaque (Fig. 2). Increased levels of low density lipoprotein (LDL) have been identified to induce the production of CCL2 in the endothelium and smooth muscle cells at the site of atheroma formation. CCL2 has a significant impact on the interaction of oxidized lipoproteins and recruitment of the foam cells in the walls of blood vessels [33, 35, 36]. Various studies suggest

Table 1. CC, CXC, CX3C and XC Groups of Chemokines and Chemokines Receptors. Summarized from [33]

Receptor	Chemokine	Relationship with disease
CCR1	CCL3, CCL5, CCL7, CCL14	Rheumatoid arthritis, multiple sclerosis
CCR2	CCL2, CCL8, CCL7, CCL13, CCL16	Atherosclerosis, rheumatoid arthritis, multiple sclerosis, type 2 diabetes mellitus
CCR3	CCL11, CCL13, CCL7, CCL5, CCL8, CCL13	Allergic asthma, rhinitis
CCR4	CCL17, CCL22	Parasitic infection, graft rejection
CCR5	CCL3, CCL4, CCL5, CCL11, CCL14, CCL16	HIV-1 co-receptor, transplant rejection
CCR6	CCL20	Allergic asthma
CCR7	CCL19, CCL21	
CCR8	CCL1	Granuloma formation
CCR9	CCL25	Inflammatory bowel disease
CCR10	CCL27, CCL28	
CXCR1	CXCL8 (interleukin-8), CXCL6	Inflammatory lung disease, chronic obstructive pulmonary disease
CXCR2	CXCL8, CXCL1, CXCL2, CXCL3, CXCL5, CXCL6	Inflammatory lung disease, chronic obstructive pulmonary disease, angiogenic for tumour growth
CXCR3-A	CXCL9, CXCL10, CXCL11	Inflammatory skin disease, multiple sclerosis, transplant rejection
CXCR3-B	CXCL4, CXCL9, CXCL10, CXCL11	Angiostatic for tumour growth
CXCR4	CXCL12	HIV-1 co-receptor, tumour metastases
CXCR5	CXCL13	
CXCR6	CXCL16	Inflammatory liver disease, atherosclerosis
CX3CR1	CXCL1 (fractalkine)	Atherosclerosis
XCR1	XCL1 (lymphotactin) XCL2	Rheumatoid arthritis, IgA nephropathy, tumour response

that CX3CL1 is a key factor in cellular adhesion [37]. Studies in mice have shown that CX3CR1 receptor is present on cellular membrane of monocytes and is responsible for their adherence to the vessel wall and plaque creation [38]. CXCR2 and CX3CR1 chemokines have also been observed to have an impact at the early stages of atherosclerotic plaque formation [39, 40]. In addition, polymorphism in the promoter of CCL2 has been associated with an increased transcription of the CCL2 gene, and patients who were homozygous for the polymorphism were found at higher risk for CAD than patients who were heterozygous [41]. Further analysis showed that certain allele variants of receptor for the CX3CL1 and CC chemokine receptor 2 have a protective effect against the calcified atherosclerotic lesions [39, 40]. CX3CL1 chemokine has been identified as an important factor in the development of type 2 diabetes mellitus, which is strongly associated with atherosclerosis development. Although certain pathways have been identified, the significance of these findings is not yet clear [42]. Interestingly, statin treatment reduces expression of CX3CL1 in adult patients not suffering from CAD [43].

Activated platelets produce chemokines (CCL5 and CXCL4) with a tendency to accumulate on the sur-face of endothelium and monocytes, which, in turn, promotes migration of monocytes into inflammed vessel wall, thus, possibly promoting the development of atherosclerosis [44, 45]. Platelet factor 4 (PF4 or CXCL4) can also inhibit apoptosis of monocytes and support monocyte arrest [44, 46]. Von Hundelshausen et al. suggest that when atherosclerosis is considered, there is a room for the development of novel anti-platelet drugs concentrating on controlling the pro-inflammatory properties of platelets rather than their haemostatic function [47]. Blocking the CXCR4 chemokine receptor in mice promotes atherosclerotic plaque progression and promotes its instability. This finding was accompanied by the increased presence of neutrophils in atherosclerotic plaque, suggesting their possible role in the development of atherosclerosis. Similarly, Bot et al. report a positive correlation between CXCR4 expression and the level of the development of atherosclerotic plaques in samples from human carotid arteries [48].

Another chemokine, namely CXCL16, normally responsible for the stimulation of cell proliferation and adhesion as well as the migration of T lymphocytes, has also been identified as an atherogenic chemokine and its receptor — CXCR6 is present on the surface of macrophages, dendritic cells, natural killer T cells and
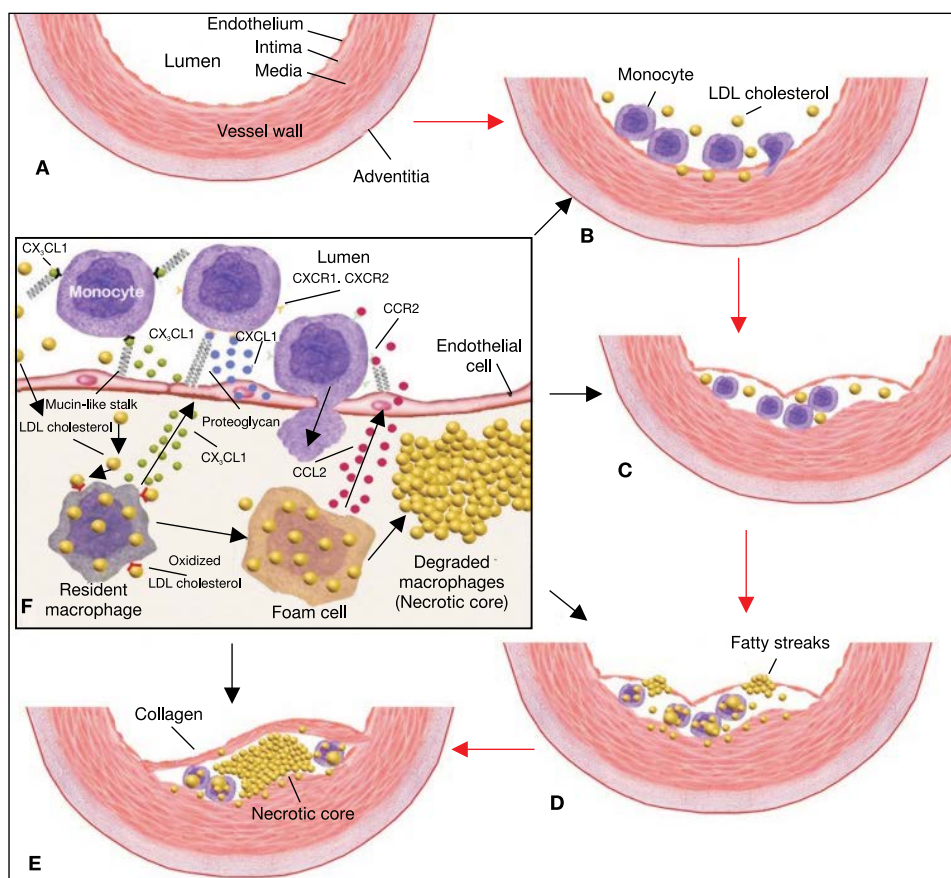
Figure 2. Evolution of arterial wall changes with recruitment of monocytes. **A.**, **B.** Endothelial injury (hyperlipidemia, hypertension, smoking, toxins, etc.), which is the result of dysfunction, increased permeability and leukocyte adhesion. Migration of leukocytes to the intima. **C.** Entering LDL lipoprotein to the vessel wall and then their oxidation. Movement of smooth muscle cells from media to intima. Transformation of monocytes to macrophages and their activation. **D.** Absorption of lipids by macrophages (foam cells) and muscle cells in the intima. **E.** Developed atherosclerotic plaque with proliferation of smooth muscle cells, deposition of collagen, proteoglycans and formation of the lipid core. **F.** Monocytes circulating in the blood bind to the vascular endothelium. Trapping monocytes and their binding takes place through different mechanisms. Specific chemokines (e.g., CX3CL1 and CCL2) secreted by macrophages and foam cells play the key role. Binding of the appropriate chemokine takes place through a specific receptor on monocyte. Capturing monocytes could be done through the presentation of chemokines such as CXCL1 by endothelial-cell. Modified from [33]

lymphocytes T and B. This receptor (also known as scavenger receptor), allows macrophages to bind oxidised lipids and may be important in atheroma formation [49].

Certain chemokines including: CCL2, CCL5 CXCL8, CXCL10 and CX3CL1 along with chemokine receptors: CCR2, CCR5, CXCR1, CXCR2, CXCR3 and CX3CR1 have been identified to be involved in the pathogenesis of hypertension. Exact nature of this process is not yet fully understood and requires further research [50].

New discoveries provide better understanding of physiological and pathogenic role of chemokines. Research data acquired thus far suggest that chemokines play an important part in the atherosclerosis development. Targeting chemokines or their receptors for pharmacotherapy might be beneficial for patients.

Nevertheless, a successful chemokine-focused therapy is probably still far away from daily practical use. However, intensive research as well as many successful model studies might open new possibilities in the future [51, 52].

The role of HIF-1α factor in the pathogenesis and treatment of cardiovascular diseases

HIF-1α is a heterodimeric transcriptional factor induced by hypoxia, which belongs to PerARNT-Sim (PAS) protein family. HIF-1α is a key factor associated with adaptation of cells to hypoxia [4]. The activation of HIF-1α leads to an enhanced production of various

proteins including: angiogenetic factors such as delta like ligand 4 (DLL-4), platelet derived growth factor beta (PDGF-β), vascular endothelial growth factor (VEGF) and other [53–56]. In recent years, there has been an increasing interest in HIF protein and many researchers tried to connect this factor and its effects associated with disease development.

According to the data from some recent reports, there is a clear indication that HIF-1α is involved in the pathogenesis of atherosclerosis, but its exact role still remains unclear. In experiments conducted on the cultures of smooth muscle cells from coronary arteries in humans, the stability of HIF-1α increased during hypoxia. This activity is associated with higher expression levels of VEGF which can also lead to an increased proliferation of those cells [56]. Furthermore, hypoxia causes HIF-1α dependent increase in macrophage migration inhibitory factor (MIF) expression. MIF causes the escalation of migration and the increased proliferation of vascular smooth muscle cells, both of which are important in the progression of atherosclerosis [57]. Na et al. suggest that there is a positive feedback circuit between HIF-1α and liver X receptor α which leads to the formation of foam cells [58]. Hypoxia and HIF-1α up regulation also stimulates expression of low density lipoprotein receptor-related protein 1 (LRP1) which is associated with cholesterol independent progression of atherosclerosis [59]. Sluimer et al. suggest that hypoxia, increased HIF-1α and angiogenesis are an integral part in the development of atherosclerosis [60]. HIF-1α can also be an important factor in the deep vein thrombosis. Evans et al. revealed that HIF-1α stimulates vein recanalization and thrombus resolution. However, the presence of thrombus does not increase HIF-1α expression [61].

Various studies suggest that the enhanced stability of HIF-1α during hypoxia promotes beneficiary effects when it comes to the outcome of myocardial infarction. In experiments on mice, smaller infarct size along with less apoptotic cells were observed when HIF-1α stability had been increased [55, 62, 63]. A possible explanation for a down regulation of apoptosis in such circumstances could be an up regulation of Cardiotrophin-1 (member of interleukin-6 family) by HIF-1α in the hypoxic environment [64]. Moreover, the enhanced expression of HIF-1α may lead to the impaired cardiac muscle contractility due to the reduced calcium ions uptake by cardiomyocytes along with certain amount of dilation in muscle structure. Rising HIF-1α level may also lead to a better chance for cardiomyocytes to survive hypoxia episodes (by preserving ATP). Effects observed in this study showed capability for the reverse potential of changes. Bekeredjian et al. hypothesised that HIF-1α is indeed responsible for the impaired contractility following ischemia. It should be mentioned that this study

was conducted in normoxic condition [65]. Constant activation of HIF-1α gene resulted in an increased number of capillaries in adult mice myocardium along with larger peak volume of perfusion for the whole heart. Angiogenesis was less pronounced in older muscles, as transport of HIF-1α to nucleus in the endothelial cells decreases with age [53, 66]. Moreover, up regulation of HIF-1α was associated with better response of aging myocytes to a positive inotropic stimulation [67]. In studies on cultured endothelial and myocardial cells, HIF-1α was reported as an important factor in pharmacologically induced and NO-mediated protection of cardiomyocytes in ischemia-reperfusion simulation [68]. In experiments on murine model of myocardial infraction, Huang et al. have proven that mesenchymal cell transplant combined with adenoviral mediated increase in HIF-1α expression caused higher levels of angiogenesis and lower levels of apoptosis than each of these methods on its own [69]. In cardiac hypertrophy model, HIF-1α partly restored cardiac muscle response to NO and natriuretic peptides [70]. Enhancing HIF-1α expression in diabetic mice resulted in the attenuation of negative effects of diabetes on myocardium. Expression of glucose transporters and glycolytic enzymes, along with myocyte ATP production, increased where HIF-1α levels were higher [54].

In the light of current knowledge on HIF function and effects, many researchers have applied these findings in a form of therapy, based on increasing expression of this protein. A promising phase I clinical study has been conducted by Kilian et al. In those studies they used adenoviral vectors to deliver HIF-1α DNA sequence into cardiac muscle via injection. Patients in this model have been suffering from the advanced multivessel CAD and had already undergone coronary artery bypass procedure after which revascularisation was not complete. Injections containing viruses were administered directly into the cardiac muscle during the bypass procedure. Success rate for this therapy cannnot be measured yet, mainly because the test group was too small (n = 13). Nevertheless, this study revealed that there were no complications caused by the administration of adenoviral vectors. Future studies are needed to reveal just how successful this form of therapy could become [71].

Similar studies have already been conducted on patients suffering from critical limb ischemia [72]. In a murine model of atherosclerosis, Christoph et al. have proven that the local inhibition of HIF activity (via adenoviral mediated siRNA silencing approach [73]) following arterial injury reduces formation of neointima, thus reducing restenosis after the injury [74]. Nevertheless, such therapy is not without potential negative consequences, as inadequate timing of HIF inhibiting therapy could promote atherosclerotic plaque instability [75]. Target therapies against hydroxylases (prolyl hydroxy-

lase domain proteins) leading to the increased HIF stability have also been proposed and successfully undertaken on animal models [76].

Conclusions

Recently, researchers have investigated various possible interactions between inflammation and hypoxia, and their influence on the development of atherosclerosis and various diseases. The aforementioned studies provide better understanding of the development and the pathomechanisms of diseases. Nevertheless, several questions still remain unanswered. Hypoxia and inflammation have been identified as taking part in pathogenesis of a disease. Recent discoveries suggest new possible forms of therapy and possibly an alternation of current guidelines for the disease treatment. However, these data must be interpreted with caution because most of the cited studies were undertaken either on animals or cultured cells. There is no guarantee that we can expect similar results in real life situations in humans. However, recent discoveries have indicated new paths for research and provided new diagnostic tools and better understanding of the disease.

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