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Cytokines in relation to heart failure

Účasť cytokínov pri srdcovom zlyhávaní

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Abstract. It is accepted that heart failure (HF) is largely influenced by inflammation. Since cytokines are the main "hormones" of the immune system, they have increasingly received attention in this context, especially due to the observation that blood levels of various cytokines correspond to the clinical outcomes of HF. The purpose of this review is to provide information about the cytokines involved in the HF syndrome, their connection to this disease and implementation in new treatment choices. Another aim is to elaborate on the complex relationship between the cytokines and the neurohumoral axis and involvement of inflammatory escalation in chronic HF.
The increased bioavailability of specific inflammatory molecules indicates that inflammation is an omnipresent factor during HF. It is responsible for its induction and progression, but also for its maintenance, including remodeling and regeneration; or even the reoccurrence of adverse cardiac events.
Nowadays, cytokines impose a conundrum: a problem that currently cannot be solved. Cytokines needed for the innate immune response are the same, which cause several diseases. Similarly, cytokines supposedly beneficial in the HF setting, can also be the cause of its clinical deterioration. This is the reason why we still do not possess adequate anti-cytokine therapy for HF treatment. Fig. 2, Ref. 73, on-line full text (Free, PDF) www.cardiologyletters.sk
Key words: cytokines – heart failure with preserved ejection fraction – heart failure with reduced ejection fraction – inflammation

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Abstrakt. V súčasnosti sa akceptuje, že srdcové zlyhávanie (SZ) je do značnej miery ovplyvnené zápalom. Keďže cytokíny sú hlavnými „hormónmi" imunitného systému, v tejto súvislosti sa im venuje čoraz väčšia pozornosť, najmä vďaka zisteniu, že hladiny rôznych cytokínov v krvi zodpovedajú klinickým výsledkom pri SZ. Cieľom tejto prehľadovej práce je sumarizovať poznatky o najdôležitejších cytokínoch, ktoré sa zúčastňujú pri rozvoji klinického syndrómu SZ, a tiež navrhnúť spôsob, akým by ovplyvnenie cytokínov bolo možné zahrnúť do nových možností jeho liečby. Ďalším cieľom je popísať komplexný vzťah medzi cytokínmi a neurohumorálnou osou, ako aj zapojenie eskalácie zápalovej reakcie v rozvoji chronického SZ.
Zvýšená biologická dostupnosť špecifických zápalových molekúl naznačuje, že zápal je všadeprítomným faktorom počas SZ. Zodpovedá za jeho nástup a progresiu, ale aj za jeho udržiavanie vrátane prestavby a regenerácie srdca, a tiež za výskyt nežiaducich srdcových príhod.
V súčasnosti cytokíny v kontexte SZ predstavujú „hlavolam", ktorý v súčasnosti nie je možné vyriešiť. Cytokíny zúčastňujúce sa v mechanizmoch nešpecifickej imunity sú rovnaké ako tie, ktoré spôsobujú ochorenia, vrátane SZ. Obdobne cytokíny, ktoré sa spájanú s klinickým zhoršením SZ, sú podľa viacerých prác v podmienkach SZ prospešné. To môže byť dôvodom, prečo stále nemáme k dispozícii adekvátnu anti-cytokínovú terapiu v liečbe SZ. Obr. 2, Lit. 73, on-line full text (Free, PDF) www.cardiologyletters.sk
Kľúčové slová: cytokíny – srdcové zlyhávanie so zachovanou ejekčnou frakciou – srdcové zlyhávanie s redukovanou ejekčnou frakciou – zápal

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Cytokines

Cells are constantly exchanging information among themselves. One way of molecular exchange of information involves the secretion of cytokines (Greek: "to set cells in motion"), which are small proteins with a wide array of regulating functions, including physiologic and pathologic processes (1). Cytokines are sometimes defined as "hormones" of the immune system because of their ubiquitous availability inside the human body, the ability to act on the secreting cells (autocrine action), neighbouring cells (paracrine action) or on distant cells (endocrine action) linked with their important role in the innate and acquired immune system. However, this statement does not consider the fact that cytokines are more potent than hormones as well as the redundancy and pleiotropy in function and secretion. Pleiotropy refers to the observation that cytokines exhibit more than one function while acting on different cell types and that one cytokine can be secreted by multiple cell types instead of one specific glandular cell. Even though these different cell types share a common cytokine receptor, the produced effect by the signaling molecule is cell-dependent and can be modified by cytokine action as a co-activator. On the other hand, the term redundancy is used because of the overlapping in cytokine function, most likely due to similar receptor subunits and intracellular signaling pathways (2, 3). Moreover, blood levels of hormones are less variable than those of cytokines (1).

Heart failure

Heart failure (HF) is considered as a clinical syndrome, a cluster of signs and symptoms comprising a variety of cardiovascular disorders, leading to an impairment in appropriate filling or ejection of the ventricle (4). For many years, it was the common belief that HF may exclusively result from haemodynamic disturbances (5). However, this alone could not explain the progredient character of this disease. Moreover, it was noted that the neurohormonal system seems to be chronically activated and the cause of disease progression is independent of changes in haemodynamics. This led to the creation of a new hypothesis, the so called neurohormonal hypothesis, stating that an overexpression and overactivation of the neurohormonal system eventually ensues in HF progression (6). Later on, it became more evident that cytokines are important mediators in the pathogenesis of HF as well; and the previous hypotheses were complemented with the newly formed cytokine hypothesis (7).

HF can be classified according to the degree of exercise intolerance (e.g. New York Heart Association or Canadian Cardiovascular Society Functional Classification), the current disease state and development (grade A through D), the heart function defined by ejection fraction (preserved and reduced EF) and by the underlying cause (4).

Heat Failure with Preserved Ejection Fraction (HFPEF)

Hallmarks

The diastolic dysfunction of HFPEF is characterized by a wide spectrum of hallmarks. These include impaired relaxation abilities, cardiomyocyte stiffening and altered filling properties. Furthermore, oxidative stress and a decrease in the nitric oxide (NO) pathway (both processes involved in and affected by inflammation) are main drivers behind HFPEF (8). All these features of HFPEF make it a separate entity of the traditional image of HF, denoted by a reduced ejection fraction, lowered contractility and LV dilation, which is nowadays known as HF with reduced ejection fraction (HFREF) (9).

The initial pathway

HF may result from direct cardiac damage or indirectly from the action of chronic systemic inflammatory processes elsewhere in the body (10). Comorbidities with a systemic inflammatory response show increased levels of various cytokines and pentraxin 5, along with VCAM-1 and E-selectin in endothelial failure, whereas the collagen-fiber-degrading action of matrix metalloproteinase (MMP) is impeded (11, 12). Chronic inflammatory states also come along with an increase of the reactive oxygen species (ROS) production, for example in cardiac endothelium. Peroxynitrite ($ONOO^-$), a member of the reactive nitrogen species is also formed, which leads to a direct decrease of the vasodilatory NO (11).

ROS and $ONOO^-$ are reactive molecules created during inflammatory processes (13). The production of ROS can be triggered in mitochondria by TNF-α activity (14) from a variety of cells, most notably endothelial cells. IL-1β increases ROS formation through NADPH oxidase stimulation, e.g. in macrophages. So does IFN-γ, although it can also utilize the mitochondrial pathway of ROS generation. Normally, the cell employs a wide array of defensive mechanisms against the harmful effect of highly reactive molecules (13). Nevertheless, long-lasting inflammation can deplete the intracytoplasmic iron stores, subsequently weakening the cellular ROS scavenging capabilities (15). Angiotensin II (Ang-II) is a potent inducer of mitochondrial abnormality due to its NADPH oxidase stimulating effect. Abnormal mitochondria tend to release more ROS than their normal counterparts. This can produce a vicious cycle of ROS generation (9).

ROS and $ONOO^-$ by their action limit the effect of the cardioprotective NO/sGC/cGMP/PKG pathway in cardiac endothelium (12, 16). As the name suggests, this pathway sets in motion NO-mediated activation of soluble guanylate cyclase (sGC). This enzyme in turn produces cGMP that

triggers protein kinase G (PKG) activity. PKG is an enzyme, which regulates intracytoplasmic pH and Ca^{2+} balance together with stopping of the mitochondrial apoptosis pathway giving it an important role in the prevention of ischemia/reperfusion injury, myocardial stiffness, myocardial remodeling including extracellular matrix (ECM) fibrosis and cardiomyocyte hypertrophy (12, 17, 18, 19, 20).

In the reperfusion injury, the re-established perfusion through the coronary vasculature induces the ATP-dependent uptake of calcium by the sarcoplasmatic reticulum. However, the rapid increase of calcium ions exceeds its capacity, eventually leading to a rapid Ca^{2+} outflow back into the cytosol. These Ca^{2+} oscillations are known to induce arrhythmias, abnormal contractures and rupture of the sarcolemma. Moreover, together with ROS, the abnormal high Ca^{2+} levels can induce mitochondrial permeabilization by the formation of transition pores, responsible for inducing programmed cell death (18). ROS and $ONOO^-$ directly decrease the NO amount, which leads to a subsequent diminution in the cGMP bioavailability and limitation of the PKG activity (21). Oxidative stress is also responsible for lowering of the sGC reactivity to NO. Endothelial dysfunction characterized by a chronically depleted NO amount can further exacerbate the HF condition (17).

Structural abnormalities

Figure 1 shows the abnormalities in structure, function and signaling in HFPEF conditions. Pathological hypertrophy involves a loss of cardiomyocytes through apoptosis and necrosis (22), compensated via an increase of cardiomyocyte size and inhomogeneous collagen deposition (23). Interestingly, different HF types were linked to the presence of certain hypertrophy phenotypes (22). Although it is not considered as the main disease mechanism for HFPEF, concentric hypertrophy was previously seen as highly implicated in HFPEF development (24). On the other hand, HFREF is mostly associated with eccentric hypertrophy (22).

In hypertrophy, the cardiomyocytes increase in size and exhibit changes confined to the cellular architecture and signaling due to pressure overload or neurohumoral stimuli. Appropriate ROS levels induce hypertrophy, although very high levels can trigger apoptosis. In hypertrophied cardiomyocytes, sarcomeres reassort to a serial or parallel arrangement, Ca^{2+} homeostasis is changed and the expression of hypertrophic genes (e.g. atrial and brain natriuretic peptides – ANP and BNP, or myosin heavy chain) is induced. Especially the renin-angiotensin-aldosterone system (RAAS) was proven to induce signaling pathways that promote proteosynthesis and the expression of fetal-like genes that have been linked to cardiac hypertrophy (25).

Fetal protein α skeletal actin (αSKA) is a marker of concentric hypertrophy (26). Nakayama et al. demonstrated that mice with a high expression of the type-2 IP3 receptor leading to higher Ca^{2+} levels in cardiomyocytes showed an increased expression of αSKA. They further hypothesize that the calcineurin-NFAT (nuclear factor of activated T-cell) cascade is responsive to the IP3 signaling (27). High Ca^{2+} levels therefore seem to cause the HFPEF typical concentric hypertrophy, suggesting again that the cytokine sensitive NO/sGC/cGMP/PKG pathway, which has an impact on the Ca^{2+} levels, is heavily involved in the process (25). On the other hand, eccentric hypertrophy can be induced by cytokines activating the glycoprotein receptor 130 and its downstream signaling in contrast to the concentric mostly employing G protein-coupled receptor. Another striking difference of both is the dissimilarity in ECM turnover. The concentric type exhibits more ECM deposition, but a far lower relative MMP-9 activity (26). In the context of HF, the disturbed Ca^{2+} metabolism (calcineurin, NFAT), the increase of ROS (direct interaction, apoptosis, NF-κB), inflammation (fibrosis) and mechanical stress (through MAPK activation) can lead to cardiac hypertrophy (23).

Functional abnormalities

The impedance of the NO/sGC/cGMP/PKG pathway also increases the Ca^{2+} levels in myocardial cells during diastole (12, 21). Low levels of Ca^{2+} during the relaxation phase are essential for ventricular filling (28). Due to the inhibition of PKG caused by the systemic inflammation, myocardial relaxation is impeded (14), possibly through a longer-lasting action potential, which leads to an incomplete or delayed relaxation (9).

The reduced phosphorylation of the elastic protein titin and ventricular remodeling through fibrosis are also responsible for ventricular rigidity (11). Normally, titin acts like a spring, which mediates recoil and resists against straightening during diastole. The titin compliance is dependent on post-translational alterations, e.g. phosphorylation established

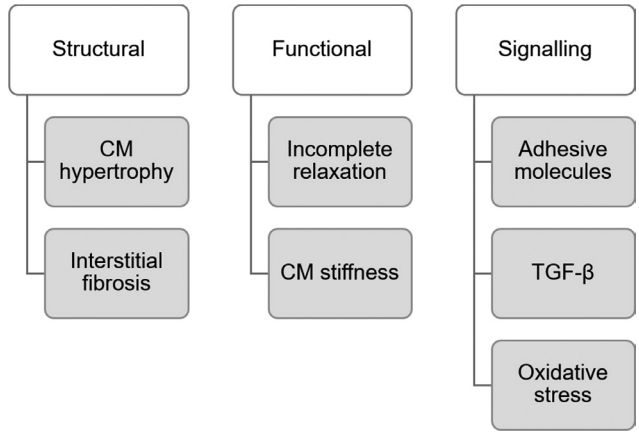

Figure 1 Structural, functional and signaling abnormalities in HFPEF (19
CM – cardiomyocyte, TGF-β – transforming growth factor-β

through PKG. Again, inflammation can reduce PKG activity and thereby inhibit titin activity through hypophosphorylation. ROS can also directly affect titin, causing its chemical modification and stiffness (9).

Signaling abnormalities

The ventricular remodeling results from TGF-β mediated attraction of fibroblasts, their differentiation into myofibroblasts and stimulation of collagen secretion (11, 29). TGF-β has been proposed as the main driving force for the cardiac fibrosis (30). The release of TGF- β in the subendothelial space by monocytes is assisted by VCAM-1 and E-selectin (11). Both molecules are reactively expressed by endothelial cells in response to the systemic inflammation mostly after IL-4 or IL-6 stimulation and are required for monocyte transmigration from the circulation (14). The main stimuli behind the ECM alterations are inflammation and RAAS activation. The role of the sympathetic nervous system (SNS) and natriuretic peptides is considered less relevant for HFPEF (21). The sequence of events culminating in HFPEF development is depicted in **Figure 2**.

Heart failure with reduced ejection fraction (HFREF)

Hallmarks

For HFREF, a progredient loss of cardiomyocyte mass is suspected to be the root of the LV remodeling. These can be the consequence of oxidative stress, although originating from intracardiac causes, e.g. ischemia or infection. Afterwards, patchy replacement fibrosis occurs with areas showing a significant loss of myofibril density. At HFREF beginning, the pressure overload leads to concentric hypertrophy, which after increasing of oxidative stress and cell death eventually becomes eccentric (19).

Similar to HFPEF, microvascular endothelial inflammation e.g. caused by increased levels of TNF-α and IL-6 may be present, but from the cardiac disease itself. Therefore, in advanced HFREF, the pro-inflammatory mediators released after ischemia, infection, etc. can cause endothelial dysfunction and thereby further exacerbate the disease (19).

Cellular response after direct myocardial damage

The time period following myocardial ischemic injury is divided into three phases regarding its cellular responses. The first phase is the acute inflammatory phase (0-4 days after onset) and is characterized by an infiltration of inflammatory cells recruited by chemokine CCL2. The monocytes can differentiate into M1 macrophages, which act pro-inflammatorily. They are potent releasers of IL-1 and TNF-α and recruit other M1 macrophages. M1 macrophages are also responsible for degrading the damaged tissue through phagocytosis and proteolysis (11, 31) and preparing the cardiac tissue for healing. The stimuli for the macrophage polarization into the M1 phenotype are IL-6, IL-12, IL-23, IFN-γ and NO (32). Therefore, Th1 cells, lymphocytes and NK cells participate in the M1 activation, whereas alternatively activated M2 require Th2 cells, mast cells and basophils (11, 31).

The second phase encompasses reparative processes: thus is called the healing phase (4-14 days). During this phase, recruited fibroblasts start to produce a collagen scar tissue and differentiate into myofibroblasts (11). The monocytes now attracted by CX3CR1 signaling differentiate into M2 (11, 31). This type of macrophages is induced by IL-4, IL-10, IL-13 (32) and TGF-β (33). They are known to release IL-10, TGF-β and arginase, which is a potent inhibitor of NO formation (32).

The third phase as the final phase involves chronic inflammation (>14 days). During this phase, inflammation may reoccur or still persist. Predominant cell types are T lymphocytes and the pro-inflammatory M1 macrophages. Lymphocytes, especially CD4+ T cells, have been proven as the most important cells leading to M1 phenotyping. In the setting of an extensive inflammatory response, HF is most likely to occur. This occurs as a consequence of either excessive recruitment of inflammatory mediators as in the case of a large ischemic area; or impaired clearance, suggesting an important role of the lymphatic system. Finally, abnormal cardiac remodeling and eventually a reduction of heart function follows (11, 34, 35).

CCL2 is a chemokine actively induced by IL-6 (14), similarly as the chemokine receptor CX3CR1 (36). Therefore,

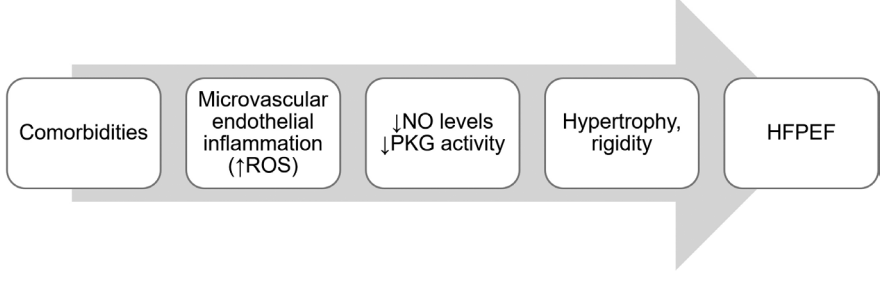

Figure 2 Sequence of events leading to HFPEF (19)

IL-6 plays a major role in the recruitment of the monocytes later maturating into M1 and M2 macrophages (37, 38).

Inflammatory escalation

The inflammatory response can become persistent or recurring (11). Especially in their persistency, T cells seem to play an important role, namely $CD4^+$ T-helper (Th), $Foxp3^+$ T-regulatory (Treg) and natural killer T (NKT)-cells. Heavily involved in their activation are antigen presenting cells such as dendritic cells (DC). An increase of the DC population corresponds to a rise of activated T-cells (39). Cytokines promote the formation of monocytes and other immune cells also in the periphery (40). Following the early remodeling after myocardial ischemia, the spleen and heart showed an enlargement of the pro-inflammatory macrophages and DC population, reflecting an enhanced pro-inflammatory gene expression. Mediastinal lymph nodes and the spleen screen for antigens and host memory T cells in a higher amount. Th cells then get activated against antigens of the cardiac tissue itself with the memory cells responsible for the immunologic memory. Unfortunately, the self-antigens that stimulate the T-cell response are yet to be identified. However, they are suspected to be the targets of the chronically activated inflammatory immune response in HF resulting in cardiac injury and remodeling. The problem is also the relative lack of Treg cells, which would dampen the systemic immune response (39). IL-2 is the main driver of Treg survival and development (14), giving it an essential place in initiating myocardial healing and reducing chronic activation of immune cells (39).

Nevertheless, not all Th subtypes are elevated in the HF setting. The Th1/Th2 ratio is altered in favor of the anti-inflammatory Th2 response leading to the secretion of IL-4, IL-5 and IL-13. Furthermore, in favor of Treg, the Th17 population is augmented (increased Th17/Treg ratio), which in contrast to Th2 accounts for a pro-inflammatory effect (39). After exposure of Th17 to e.g. IL-1β or IL-6, they increase the IL-17 secretion. Cells releasing the A-type of IL-17 are fibroblasts, B and T lymphocytes, bone marrow cells as well as those of endothelial and epithelial origin. A variety of tissues express the corresponding receptor and react to receptor stimulation with secretion of pro-inflammatory cytokines (e.g. IL-1), chemokines and MMP. The other IL-17 types demonstrate similar effects (14).

IL-4 after its release from Th2 behaves as a strong inhibitor of the pro-inflammatory cytokines IL-1, Il-6 and TNF-α. It is also supporting the Th2 differentiation. The other cytokines achieve negative control of the pro-inflammatory cytokines in a similar fashion (14). M2 type of macrophages is induced by IL-4, IL-10 and IL-13, which then release IL-10, TGF-β and arginase (32).

Essential for the injury-repair response are the damage-associated molecular patterns (DAMP) molecules. Cells undergoing necrosis, necroptosis or apoptosis release their contents into the extracellular space. These are sensed by DAMP molecules via the pattern recognition receptors, e.g. Toll-like receptors (TLR). Alternatively, pathogen-associated molecular patterns (PAMP) can sense non-self-molecules, including LPS or teichoic acids from bacteria or viral nucleic acids. DAMP and PAMP (via TLR4) initiate a pathway resembling the IL-1 signaling. This eventually culminates in the activation of the transcription factor NF-κB, which mediates the synthesis of pro-inflammatory cytokines. The expression of TLR4 for example is increased by IL-1β and was found to be rather high in the heart. TLR4 is a potent inducer of IL-1, IL-6 and IL-8, indicating that a potential upregulation can exacerbate HF (41).

The neurohumoral system

The neurohormonal system is responsible for adaptive responses aimed at maintaining cardiovascular homeostasis, i.e. cardiac output normalization. If chronically activated, it can trigger hemodynamic stress and worsen the heart pumping. Therefore, it is considered as a cardinal mechanism of HF progression (42), possibly also mediated through its impact on the immune system. It was proven that many immune cells express receptors for angiotensin (mostly AT1R), catecholamines and natriuretic peptides (21).

Renin-angiotensin-aldosterone system

AT1R as one of the main Ang-II receptors is also expressed on cardiac fibroblasts, where it initiates TGF-β release after ligand binding (43). Monocytes are also potent releasers of TGF-β (44). TGF-β acts on the fibroblasts and turns them into collagen-secreting myofibroblasts through the canonical/SMAD mediated pathway (45). Note that the connective tissue deposition is antagonized by sGC (46) and supported by IFN-γ, TNF-α and IL-1β (47).

The latter three support endothelial to mesenchymal differentiation (EndMT) (48), which is another TGF-β mediated mechanism (49). One third of all cardiac fibroblasts have endothelial progenitors, implicating EndMT to be an essential driver of cardiac fibrosis. TGF-β utilizes Smad-dependent and independent pathways, which proceed to positively increase the expression of signaling pathways leading to EndMT – Notch, Snail and Slug. The synergizing effect of IFN-γ, TNF-α and IL-1β stems from their assistance in expression of these three molecules (47). Many endothelial cells can undergo EndMT, albeit those in the coronary vasculature are more prone. As the name suggests, the cells undergo morphologic changes leading to endothelial cells acquiring mesenchymal phenotype with subsequent expression of respective surface

markers. This gives them the ability to invade and migrate through the ECM (50).

TNF-α and IL-1 also induce MMP expression aiming to degrade ECM components. JNK and NF-κB pathways seem to play a predominant role in MMP expression (51). Especially MMP-9 serves as a biomarker for cardiac remodeling. Its levels are usually increased directly following an ischemic injury allowing neutrophil and macrophage entry to the infarction site. Other cells able to release MMPs are e.g. fibroblasts, endothelial cells and cardiomyocytes. MMP-9 levels correlate directly with IL-6 or other reactant proteins and clinically with lower EF and sustained remodeling in chronic HFREF. Besides the aforementioned pro-inflammatory cytokines, $ONOO^-$ can also directly cause MMP release and activation (52).

Two distinguished pathologies typically make up the HF etiopathology: endothelial as well as myocardial dysfunction. Soluble GC acts beneficially to the patient with HFREF in both mechanisms. It reduces the cardiac afterload through vasodilation and therefore the strain on the cardiomyocytes, and it can slow the progression of cardiac fibrosis and hypertrophy (53). In animal models, sGC stimulators were demonstrated to reduce the transformation of cells to collagen-secreting myofibroblasts. There is also evidence that sGC stimulators can inhibit Smad-dependent fibrosis (45, 54, 55). The reason for the low sGC activity is reduced NO availability evoked by ROS and limited NO production due to eNOS dysfunction (54).

In this context, Ang-II acts as pro-fibrotic through inducing the M2 macrophage phenotype that recruits fibroblasts and releases TGF-β and arginase. The AT1R also has been linked to a higher ROS production activating NADPH oxidase (56, 57). Furthermore, Ang-II induces the production of the interleukins IL-1β, IL-6, IL-8, IL-17, TNF-α, among others (58). IL-1β and TNF-α increase the number of AT1R, resulting in the auto-stimulatory vicious cycle leading to an excessive amount of these pro-inflammatory cytokines in the setting of post myocardial infarction (MI). However, there is also a counter-regulatory mechanism inhibiting the IL-1β and TNF-α release, since AT1R restrains M1 macrophage phenotype formation, subsequently leading to a relative escalation of M2 macrophages. This counter-regulation also augments cellular proliferation and angiogenesis, besides promotion of fibrosis through TGF-β. Likewise, an exaggerated RAAS action leading to uncontrolled pro-inflammatory stimuli is normally limited by the suppressive effect of AT1R itself on hematopoietic cells, suggesting a very important feedback mechanism (21, 59).

In T cells, Ang-II augments the release of the Th1 cytokine IFN-γ, whereas the Th2 cytokine IL-4 sequestration is impeded. In addition, AT1R is responsible for altering the Th1/Th2 ratio in favor of the pro-inflammatory Th1 cells, subsequently also increasing the amount of IFN-γ releasing cells. Taken together, Ang-II is responsible for a variety of impacts on the immune system, although not all of them are considered harmful on other organ systems (21).

Sympathetic nervous system

The SNS is able to communicate to and guide the immune system directly through interaction with its components and indirectly through the adjustment of blood and lymphatic flow, besides controlling the distribution and formation of lymphocytes and their release of inflammatory mediators (60). Equally to the RAAS, the SNS can also under certain circumstances shift from a beneficial adaptive response into a pathological maladaptive one (21). The stress reaction in the human body is supposed to be a temporary one. In longer duration, it can exhibit adverse effects caused by increased catabolism, reduced growth or the immune system modulation (61). The adrenergic receptors are G protein-coupled receptors assigned to the α1, α2, β1, β2 and β3 receptor subtypes (62). All of them are found on the surface of various immunocompetent cells (63).

Norepinephrine (NE) is the cardinal neurotransmitter in the SNS. However, it shows higher affinity to α-AR than to β-AR. Consequently, in immune cells close to the NE source also β-AR are activated, whereas at greater distance, only α-AR. SNS can either trigger a dominant pro-inflammatory response via stimulating α-AR, e.g. increase of TNF-α, or anti-inflammatory by β-AR mediated release of IL-10 (64). IL-10 is an important anti-inflammatory mediator, e.g. through inhibiting the MHC II expression on monocyte-macrophage cells and the formation of cytokines and chemokines induced by TLR agonists (14). Thus, the response of the immune cells is influenced by the SNS depending on the distance to the neurotransmitter source. The cellular activity at the point of stimulation also seems to control its response to NE stimulation. Moreover, certain pro-inflammatory cytokines can alter the adrenergic response (64).

In an acute setting, Pongratz et al. reported both functions of the SNS, pro-inflammatory and anti-inflammatory, depending on the distance from the catecholamine source. They hypothesized that this establishes a local boundary for inflammation. However, chronic SNS stimulation acts pro-inflammatorily again, e.g. through enhancement of blood and lymphatic flow, assisting in antigen presentation and release of specific mediators (60). This indicates an important role of SNS in maintenance and transition between the three phases of inflammation, with an acute inflammatory phase being followed by the reparative phase and eventually the chronic phase at which an uncoupling from the central regulatory mechanisms occurs (11, 60).

The natriuretic peptides

ANP, like the other natriuretic peptides, is heavily involved in the regulation of the immune system, besides its natriuretic properties. Generally, it prevents the TNF-α release from macrophages and counteracts its effects. These

include minimized formation of VCAM (21) and ICAM (65), impeding of vascular permeability and inhibiting iNOS. Besides, it can influence the activity of various immune cells, e.g. stimulation of NK activity and respiratory burst in polymorphonuclears. In DC, ANP diminishes a potentially exaggerated Th1 response and promotes Th2 differentiation (21). They also respond to ANP stimulation with producing less IL-12 and TNF-α, whereas the production of the anti-inflammatory IL-10 is augmented, suggesting that ANP has an important function in generation of immune tolerance. ANP also reduces the production of IL-1β and the formation of NO in response to LPS in macrophages. The two pro-inflammatory transcription factors NF-κB and AP-1 are both less activated in the presence of ANP. The recruitment of monocytes that later differentiate into M1 pro-inflammatory macrophages is inhibited through the ANP mediated inhibition of CCL2 chemokines. Additionally, IL-8 formation is impeded (65), thereby inhibiting the attraction of immune cells to the damaged tissue site, e.g. T cells. IL-8 can also promote ECM degradation through MMP (14).

KO mice lacking the ANP receptors exhibited a much greater ventricular mass, indicating an anti-hypertrophic role of ANP. It was shown that ANP inhibits the Ang-II mediated hypertrophy of cardiomyocytes, through limiting of the ROS leakage through the sodium/hydrogen exchanger (NHE). Moreover, ANP acts as an anti-proliferative on cardiac fibroblasts and reduces TGF-β mediated fibrosis (65).

The decrease of ROS inhibits the TNF-α mediated pathway (66) and reduces the excessive loss of myocardial cells after myocardial ischemia. Cardiomyocyte hypertrophy is avoided through the inhibiting action of ANP on NHE (65). High cytosolic Ca^{2+} levels lead to or augment hypertrophy, excessive contraction and reperfusion injury (18). NHE compensates for the acidosis coming along with reperfusion by exchanging H^+ with sodium ions. Na^+ is swapped with Ca^{2+} leading to high cytosolic Ca^{2+} levels. In this context, ANP appears to be an emergency hormone, preventing the damaging effect of NHE (65).

BNP is a mediator with pro-inflammatory and anti-inflammatory properties. It induces the secretion of IL-10 from macrophages. This interleukin reduces the release of various cytokines, e.g. IFN-γ, TNF-α, IL-1β and IL-6 with the majority of them acting pro-inflammatorily (21). BNP also decreases the circulating IL-12 and TNF-α levels by acting on DC (67). Interestingly, TNF-α and IL-1β seem to selectively augment BNP production, especially in conditions involving significant inflammation (68). IL-12 is known to be a potent inducer of a Th1 response, since it acts in concordance to IL-18 in the release of IFN-γ (69).

Moreover, BNP reduces collagen formation, but increases the MMP synthesis. Generally, BNP levels and the MMP-9/metallopeptidase inhibitor-1 ratio is higher in HFREF, reflecting a strong degree of ventricular wall remodeling and ventricular dilation (70). On the other hand, the ECM formation appears more eminent in HFPEF (26). High BNP and MMP-9 levels were linked to worse clinical outcomes, indicating ongoing inflammation and remodeling due to persistent myocardial injury (70).

Treatment modalities

The treatment backbone of HF in general includes beta blockers (BB), angiotensin-converting enzyme inhibitors (ACE-I), AT1R receptor blockers (ARB) and hydralazine-isosorbide dinitrate. Additionally, there are new treatment options that are promising alternatives for the future, but almost all regimens exhibit immunomodulatory effects (71).

The effect of BB is not uniform and varies among the different members (21). Carvedilol is a non-selective BB with residual α-AR blockade (71). Besides reducing the SNS overstimulation, the drug in mice improved survival through reducing IL-12, IFN-γ and ROS production, apart from lowering of IL-6 levels in patients with cardiomyopathy (21). Metoprolol belongs to the β1-selective BB (71). This drug in particular appears to increase the EF through upregulating T-cells through an IL-2 dependent mechanism (21). Another BB demonstrating benefit in morbidity and mortality is bisoprolol, a selective β1-AR blocker (72).

ACE-I and ARB are both inhibitors of RAAS. Losartan is a member of the ARB class and together with the ACE-I perindopril presented with a reduced expression of pro-inflammatory genes and an augmentation of the anti-inflammatory IL-10 levels. Moreover, losartan and irbesartan reduce TGF-β mediated EndMT and consequent cardiac fibrosis. ACE-I also demonstrates a significant reduction in TGF-β. A potent combination is formed from sacubitril-valsartan. The combination reduces IL-1β and IL-6 secretion by macrophages with consequent inhibition of cardiac remodeling through e.g. MMP or ROS production (21).

Monoclonal antibodies designed to reduce inflammation are already widely used in rheumatic disorders, suggesting that HF patients might also benefit from them. Sadly, trials with the monoclonal antibody infliximab, which is directed against TNF-α, could not reproduce this positive effect in HF settings. A slight EF increase was observed in mild doses, but when administered in higher doses, the drug even increased morbidity and mortality (72). The suspected reason for the adverse effects of infliximab at higher doses is the drug binding to TNF-α secreting cardiomyocytes, where it subsequently leads to apoptosis. Moreover, the TNF-α levels in high doses of infliximab are reduced to sub-physiological levels. Another possible reason is that infliximab might abolish the beneficial role of TNF-α in acute ischemia (11). Likewise, the TNF-α decoy receptor etanercept showed no clinical benefit (72).

IL-6, TGF-β and IL-8 neutralizations also disappointed as well as unspecific immunosuppression, e.g. by corticosteroids,

intravenous immunoglobulin, methotrexate and cyclosporine. Animal studies with IL-1β as the main circulatory form of IL-1 have shown that anti-inflammatory targeting can indeed also improve the prognosis after MI. In humans, anakinra was the most promising agent in inhibiting IL-1β action (72). In HFPEF treatment, this translates into a significant reduction of the inflammatory response. Although being unable to alter the EF, this immunologic therapeutic drug was adequate to reduce the HF incidence. Another monoclonal antibody – canakinumab proves benefit by reducing IL-1 and IL-6 levels, reflected in a CRP decrease (73). The monoclonal antibodies proved to be more efficient in reducing of the risk of MI, yet increased the risk of fatal infections (72).

Prior to IL-1 release stands the inflammasome. It consists of three components; an adaptor protein, a sensor molecule that responds to DAMP and PAMP as for example NLRP3, and the pro-form of interleukin-1 converting caspase-1. After stimulation, the pro-caspase-1 cleaves pro-IL-1 into the mature IL-1β. The process of NLRP3 inflammasome aggregation is impeded by colchicine, which is successfully used in the treatment of gout. Moreover, it exhibits anti-fibrotic properties and seems to protect the endothelium (73).

In the setting of cardiac inflammatory disorders, the Treg response is quantitatively and qualitatively blunted, creating an imbalance between Th17 derived pro-inflammatory IL-17 and Treg. Since IL-17 is responsible for macrophage activation and CCL2 release, it plays an important role in recruitment and differentiation of pro-inflammatory macrophages, especially in long-standing inflammation of the cardiac tissue. This creates therapeutic options, which can either be through administration of artificially produced Treg cells directly or alternatively indirectly through IL-2 stimulation. Unfortunately, there are not enough data available to estimate its impact on clinical outcome in HF patients (73).

Heightened attention has also been received by the NO/sGC/cGMP/PKG pathway as a possible way of treatment. The administration of NO donors has been surpassed by sGC stimulators/ activators because NO itself is neutralized by ROS. Clinical test on their efficacy are still ongoing, but they seem to have mixed results as HFREF patients developed hypotension (72).

Relaxin is a pregnancy hormone, which can mediate a variety of beneficial responses in HF. It augments vascular endothelial growth factor levels, fibrosis reducing MMP, NO-mediated vasodilation and limits TGF-β-induced fibroblast-myofibroblast transition (72).

Conclusion

The increased bioavailability of specific inflammatory molecules indicates that inflammation is an omnipresent factor during HFREF and HFPEF. It is not just responsible for its induction and progression, but also for the maintenance, including remodeling and regeneration; or even the reoccurrence of adverse cardiac events. The inflammatory processes in HF are of beneficial as well as detrimental nature, depending on the size, duration and the localization. The development of HFPEF is especially affected by the action of inflammatory mediators. The exact role of inflammatory mediators has not been fully established yet and appears to be rather complex, resulting in rather disappointing therapeutic results of various immunomodulators. These observations further underline the importance of patient-specific treatment based on the character of the underlying inflammatory processes, which lead to the diverse picture of the disease.

Thus, it is fair to say that cytokines impose a conundrum: a problem that currently cannot be solved. The cytokines needed for the innate response of the immune system are the same which cause several immune diseases. Similarly, cytokines supposedly beneficial in the HF setting can also be the cause of its clinical deterioration. Vice versa, the excessive inhibition of cytokines believed to be detrimental to cardiac function cause a paradoxical worsening of the clinical condition.

Disclosure

No conflicts of interest are declared by the authors.

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