NEW INSIGHTS INTO CHRONIC INFLAMMATION AND ATHEROSCLEROSIS

Cristian Silaghi1, Victor Cristea2
1Department of Biochemistry, 2Department of Immunopathology,
University of Medicine and Pharmacy „I. Hațieganu”, Cluj-Napoca

Abstract

The central role of chronic inflammation in atherogenesis is now well documented and there is increasing evidence of immune and inflammatory pathways involved in the pathogenesis of atherosclerosis. The review summarizes the involvement of cytokines in all stages of plaque development, the modulation of innate and adaptive immunity in atherosclerosis, the evaluation of cardiovascular diseases from the point of view of cytokines and some novel therapeutic implications.

Key words: Atherosclerosis, immunity, inflammation, cytokines

I. INTRODUCTION

1. Inflammatory theory in atherosclerosis

Atherosclerosis, reaching an epidemic proportion, is a progressive disease characterized by the formation of a plaque consisting of cholesterol, other lipids, connective-tissue elements and debris of dead cells, in the innermost layer of the artery (the intima) of the medium muscular and large elastic arteries (1, 2).

The oxidized LDL and its key role in atherogenesis, together with recombination techniques for obtaining knockout mice with ApoEnull or lacking the LDL receptor were the discoveries of the end of 1990s (3), making possible to clarify the importance of the inflammatory mechanism that will be first revealed by Russel Ross. He first proposed a theory which claims that atheroma formation is a „response to injury“, based on numerous pathophysiological observations in murines and humans, in which endothelial denudation was the initial step in the pathogenesis of atherosclerosis (4). The hypothesis was that a local injury in the endothelium will be succeeded by the adhesion and gathering of platelets which will secrete PDGF (platelet-derived growth factor) promoting in this way the proliferation of SMCs (smooth muscle cells). Now we know that the SMCs proliferation is not so impressive in the atheroma and it is believed to contribute to plaque stabilization (5). Michael Gibrone was the first to launch the concept of endothelial dysfunction and Ross embraced this new idea: „the endothelial dysfunction… induces the endothelium to have procoagulant instead of anticoagulant properties and to form vasoactive molecules, cytokines and growth factors“ (6).

Atherosclerosis is a dynamic process and the artery wall is an active site of affection implicated in chronic inflammation, innate and adaptive immunity and production of cytokines, chemokines and growth factors (7).

2. Structure and development of atherosclerotic lesions

Classically 3 types of atherosclerotic lesions are described: fatty streak, fibrous plaque and complicated lesion (2).
a. The fatty streak is an accumulation beneath the endothelium of foam cells derived from macrophages, T-lymphocytes and a small number of SMCs. The prevalence of the fatty streak is directly correlated with the LDL level in plasma and indirectly with HDL levels (2). The activated endothelium – for example by oxidation of LDL in intima – shows an increased adhesiveness...
because of a high expression of adhesion proteins [ICAM-1 (intercellular adhesion molecule), VCAM-1 (vascular cell adhesion molecule), E-selectine and P-selectine] present on the EC surface and the secretion of chemokines (1). The recruitment of monocytes and T-lymphocytes and their migration into the intima is modulated by these chemokines and adhesion molecules. Expression of VCAM-1 is induced by oxidized LDL as well as pro-inflammatory cytokines such as IL-1β or TNF-α (8). The process of monocytes differentiation to macrophages is characterized by the elevated expression of several scavengers’ receptors expressed on the surface of macrophages. The uptake of modified LDL particles by these receptors leads to the formation of lipid peroxides and facilitates gathering of cholesterol esters as cytosolic droplets [6]. This way foam cells are born and, once activated, these cells release cytokines, chemokines, hydrolytic enzymes and growth factors, which enhance inflammation.

b. The fibrous plaque. As the result of the accumulation of LDL in the intima and further foam cells formation, increased T cell and mast cell infiltration, SMCs proliferation and connective-tissue production, the fatty streak evolves to a fibrous lesion. Unlike scavenger receptors, the toll-like receptors can originate a signal cascade that will activate macrophages and generate inflammatory cytokines, oxygen, nitrogen radicals and proteases (9), so the inflammation can be maintained.

c. The complicated lesion is the last stage of atherosclerosis. An advanced lesion contains a dense fibrous plaque which protrudes into the vessel lumen and covers a core of SMCs, macrophages, extracellular matrix, T cells and debris, resulting in a lipid-rich plaque with minimally active SMCs (1, 7). This kind of plaque may become unstable leading to thrombus formation, clinically expressed by acute coronary syndromes (2). When the fibrous cap is straitened and eroded at some sites, there is an abundance of activated immune cells. These cells generate inflammatory cytokines and proteolytic enzymes, coagulation factors, radicals that may destabilize the plaque, weaken the cap by attacking the collagen and finally initiate the thrombus formation. Some representatives of matrix metalloproteinases (MMP) and cystein proteases may concur in the plaque rupture by a possible degradation of the matrix collagen within the plaque (10). The expression of MMP genes is induced by inflammatory cytokines [CD40 ligand and IL-1 produced by T-lymphocytes promote the production of MMPs by macrophages; mast cells of the plaque release TNF-α which is an inducer of MMPs and also serine proteinases tryptase and chymase, which activate MMP proenzymes (11)].

Cells that are implicated in atherosclerosis development include vascular cells (ECs and SMCs), immune cells (monocytes/macrophage, lymphocytes – T, B, and NK), platelets and mast cells. Each of them secretes or is upregulated by cytokines. In the atherosclerotic plaque cytokines are expressed at high levels (i.e. IL-1, IL-4, IL-6, IL-10, IFN-γ, TNF-α, MCP-1, TGF-β) (3, 7, 8).

II. CYTOKINES AND IMMUNE MECHANISMS IN ATHEROGENESIS AS A CHRONIC INFLAMMATION

1. Generalities

Cytokines are known as mediators and controllers of innate or adaptive immunity, inflammation, proliferation and differentiation of cell lineage (12), grouped in classes: interleukins (nowadays 33 of them are known), interferons (IFNs), tumour necrosis factors (TNFs), chemokines, colony stimulating factors (CSFs) and transforming grows factors (TGF).

There are several types of cytokine classifications:

Based on structural homology of their receptors are categorized in (3):

Class I and II cytokines in which the majority of interleukins (without IL-1, IL-18, IL-28, IL-32, IL33), IFNs [type I: IFN-α, IFN-β, IFN-λ1 (IL-28A), IFN-λ2 (IL-28B), IFN-λ3 (IL-29) and type II: IFN-λ] and CSFs [G-CSF (granulocyte-colony stimulating factor), M-CSF (macrophage-colony stimulating factor) and GM-CSF (mix colony stimulating factor)] are included and their effects are mediated through the Janus kinase-signal transducers and activators of transcription (JAK-STAT) pathway.

TNF superfamily (TNF-α, Lymphotoxina, FasL, CD40L, (CD154), RANKL-ligand for receptor activator of NF-κB) which activates the nuclear factor-κB (NF-κB) and mitogen activated protein (MAP) kinase signalling pathway.

IL-1 family (IL-1a, IL-1β, IL-1ra and IL-18, IL-32, IL-33) which activates the same nuclear factor-κB (NF-κB) and mitogen activated protein (MAP) kinase signalling pathway.
TGF superfamily – over 35 members in vertebrates and invertebrates organisms – (TGF-β with three isoforms β1, β2, β3 identified in mammals; Activin A and others) activate signalling proteins of the Smad family (Smad2/Smad3).

Based on pro- or anti-inflammatory activities (3, 12): pro-inflammatory cytokines: IL-1, IL-6, IL-12, IL-18, IFN-γ, TNF-a and anti-inflammatory cytokines: IL-4, IL-10, IL-13, TGF-β.

Based on type of cells who secrete them: immune cells [macrophages and lymphocytes (T, B, NK)], endothelial cells (ECs) and SMCs, platelets, mast cells or other cells (3, 12).

Based on categorizing T helper cells and pattern of cytokines they can secrete (3): Th1-cell-mediated immune response associated with IL-2, IL-17 and IFN-γ secretion and Th2- humoral immune response linked with IL-4, IL-5, IL-6, IL-10 and IL-13 secretion.

Chemokines are divided in subfamily a (CXC) and subfamily β (CC). Using their structure according to the position of N-terminal cysteines makes this classification, CC means that the 2 cysteines are adjacent and X designates an aminoacid who separates them. CXC subfamily includes IL-8/NAP-1 (neutrophil activating protein), MGS/GRO/NAP-3 (melanoma growth stimulating factor), IP-10 (IFN-inducible protein 10), platelets bases proteins (PBP, tromboglobulin β), PF-4 (platelet factor), ENA-78 (epithelial cell-derived neutrophil-activating peptide), fractalkine and CC subfamily includes MCP-1 (monocytes chemoattractant protein-1), 2, 3, 4, MIP-1α, 1β 3α, 3β (monocytes inflammatory protein), RANTES (regulated upon activation, normal T expressed and presumably secreted), eotaxin etc (3, 12).

2. Triggers of cytokines output; effects of cytokines on vessels wall and atherosclerosis plaque

Besides the classical risk factors for atherosclerosis development: age, gender, smoking, hypertension, sedentaryism, genetic predisposition, hypercholesterolemia (referring to LDL-cholesterol), other novel risk factors involved in pathogenesis of chronic inflammation in atherosclerosis were discovered [i.e. metabolic syndrome, infectious agents (Herpesvirus, Chlamydia pneumoniae), homocystein, angiotensin II, advanced glycation end-products (AGEs)].

LDL is considered the atherogenic fraction and modified LDL (minimally modified -mm- and oxidized-ox-) is the most likely triggering factor for cytokine production. Table 1 will totalise the initially and secondary triggers (3) (which preserve and amplify cytokine secretion).

Systemic and local effects of cytokines in atherosclerosis are (3): modulation of lipid metabolism; oxidation of LDL (induction cell oxidant stress); induction of chemokine release; effects on endothelial permeability (alter the permeability); modulation of endothelial procoagulant activity; induction of SMC migration/

| Table 1 |
|---|---|---|
| **Triggers of cytokines output in the formation and developing of atherosclerotic process** |
| **Initially triggers** | **Secondary triggers** |
| | | |
| 1. Modified lipids as bioactive mediators | OxLDLs | Other lipids oxidation products contained in modified LDL |
| | | Leukotrienes |
| 2. Platelet activating factor (PAF) | a) Lyso phosphatidylcholine (lysoPC) | b) Oxidized 1-palmitoyl-2-arachidonyl-sn-glycero-3-phosphorylcholine (OxPAPC) |
| **1. Infectious agents** | Chlamydia pneumoniae and herpes viruses (HV) (i.e. HSV, CMV) |
| **2. Heat shock proteins (HSP)** | HSP60, HSP70, HSP90 and gp96 |
| **3. Immune complexes** | OxLDL is an autoantigen, and rouse formation of immune complexes (ICs) |
| **4. MMPs** | MMPs -2,-3,-9,-14. |
| **5. Inflammosome** | |
| **6. ROS** | O2-, H2O2 and .OH |
| **7. Defectuos apoptotic cell clearance** | Z |
| **8. Angiotensin II (ANG II)** | |
| **9. Advance glycation end products (AGEs)** | |
| **10. Proinflammatory cytokines** | Cytokines ca act in an autocrine mode (for example IL-1 induce IL-1 production or TNF-a induces TNF-a), but in a signaling cascade too |
| **11. TLRs endogenous ligands** | |
| **12. Mechanical factors** | Hypertension and shear stress |
| **13. Adipokines** | Leptin, resistin and adiponectin |
| **14. Coagulation and platelet products** | Thrombin |
| **15. Others** | Serotonin nad mast cells tryptase |
proliferation; modulation of endothelial of SMC progenitor differentiation; upregulation/downregulation of endothelial-dependent vasorelaxation (through actions of IL-10 and IFN-? on nitric-oxide synthase-3); activation of adhesion molecule expression; modulation of scavenger receptor expression; stimulation of MMP expression; stimulation of microparticle release; modulation of extracellular matrix expression; regulation of neovessel formation; promotion of intraplaque neovascularization; modulation of fibrinolysis (PAI-1); induction of apoptosis (but protection of ECs from apoptosis – supported by a study in which IL-11 shows protection from T cell mediated EC injury); regulation of immune response (Th1/Th2/Treg); conversion of CD4+ naive T cells into CD4+ regulatory T cells.

3. Cytokines implications in the innate and adaptive immunity of the atherosclerotic process

A. Innate immunity in atherosclerosis has to be seen through the mechanisms of leukocytes chemotraction, adherence, diapedesis and metamorphosis of monocytes in resident macrophages localised in vessels intima, events that are ruled by cytokines, chemokines or growth factors.

- Chemotraction of circulating monocytes. Chemokines are capable to recruit leukocytes into arterial intima through mediation of their G protein-coupled receptors, emerging to integrins [7]. For example MCP-1 is induced by hypercholesterolemia (13) and lymphocytes recruitment is also promoted by IFN-? induced CXC chemokines (i.e. IP-10).
- Adherence and diapedesis of monocytes. Some of the triggers activity leads to cytokine secretion in the injured endothelium, which will amplify expression of genes encoding ECs adhesion molecules. Overexpression of ICAM-1, VCAM-1, E- and P-selectin on ECs, SMCs or macrophages is induced by IL-1? and ?, TNF-? and IL-18; endothelium adhesion of monocytes is also induced by IL-8 (14) and modified LDL particles. The involvement of VCAM-1 in the adhesion of monocytes also generates signalling that modulates a modification in endothelial shape, which will facilitate leukocytes emigration.
- Metamorphosis of monocytes in macrophages and foam cells. Monocytes will undergo a number of changes and will acquire the phenotype of macrophages, which will replicate in the intima of the arteries, finally leading to formation of foam cells, the prototype cell of atherosclerotic plaque. M-CSF is the stimulator of this metamorphosis, which enhances scavenger receptors expression at the surface of monocytes and increase cytokines and growth factors production by these monocytes. Once foam cells formed, they will produce cytokines, chemokines, growth factors, CSFs and proteolytic enzymes (IL-1?β, IL-6, IL-12, IL-18, TNF-?, TNF-β, IFN-γ, MCP-1, IL-8, PDGF, TGF-?, M-CSF, GM-CSF and MMPs) (15), which will augment inflammation in atherosclerosis leading to an unstable plaque.

B. Adaptive immunity. When antigens are recognised by TCR (T cell receptors) or BCR (B cell receptors), adaptive immunity develops.
- A T lymphocytes infiltrate in atherosclerotic plaque is always present and are predominantly CD4+ T cells. T cells respond or interact with oxLDL, MCP-1, ICAM-1, VCAM-1 and they adhere to activated endothelium as well as monocytes [16]. Other cells than CD4+ T Lymphocytes like CD8+, NKT, B cells, dendritic cells or mast cells have important regulatory properties in atherosclerosis.
- CD8+ T cells restricted by MHC class I antigens are present in atherosclerotic plaque. They recognise viral antigens present in the atheroma plaque, cause death of vascular cells and accelerate atherosclerosis development by producing TNF-?, lymphotoxin and IFN-?; these roles were studied in ApoE null mice (17).
- NKT, a minor subpopulation of T cells prevalent in fatty streaks, recognises lipid antigens presented by CD-1 molecules. When they are activated, atherosclerotic plaque has showed an increase in dimensions; IFN-γ and IL-4 production also increase in ApoE knockout mice (18).
- Dendritic cells (DC) can activate naive and memory T cells in local lymph nodes, but DCs differentiation to mature APCs requires the presence of cytokines or growth factors (CD40 and IL-1R activate DCs) (19). Signals coming from pro/anti-inflammatory cytokines (TNF, IL-1, IL-6, IL10 and TGF-?) affect their maturation (20). Also DCs play an important role in Th cells differentiation: IL-12 induce Th 1 differentiation; IL-6, IL-13 and TNFSF4 (cell surface molecule belonging to TNF superfamily) may have an implication in DC-induced Th2
differentiation (21). IL-10 and TGF-β (anti-inflammatory cytokines) convert DCs from cell inducing Th1 to cell inducing Th2 or regulatory T cell, as we will see in discussion about Th1/Th2 regulation.

- Antibody-producing B cells contribute to anti-atherosclerotic activity, although they are not so numerous in lesions. The confirmation of the protective effect of B cells is sustained by a study in which immunization with oxLDL of ApoE knockout mice lead to production of high levels of anti-oxLDL IgM antibodies and reduces size of atherosclerotic lesions (22).

- Mast cells accumulation at sites of plaque rupture may play an important role in coronary artery disease, as a result of production of numerous proteases that have roles in degradation of plaque fibrin and matrix (i.e. tryptase and chymase which can activate MMPs or transform angiotensin I in its active form angiotensin II) (11).

The predominant immune cells involved in development and progression of atherosclerotic plaque are T cells. Most of the CD4+ T cells are T helper cell type 1 (Th1 subtype) and secret proinflammatory cytokines IL-2, IFN-γ, and TNF-α and β, but a small amount of Th2 secreted cytokines can be found in atheroma plaque, such as IL-4, IL-5, IL-10 and IL-13 (23), providing help for antibody production of B cells. Production of IL-5 by Th2 cells is important for protective antibody production by B lymphocytes in response to immunization with oxLDL. IL-4 and IL-10 reduce ICAM-1 and VCAM-1 expression contributing to their antiatherogenic actions (24). Proinflammatory cytokines produced by Th1 cells induce innate immune responses by stimulating macrophages and vascular cells. IL-12 and IL-18 are the stimulatory cytokines for Th1 cells (25), whereas IL-4 is essential for induction of antigen-specific Th2 cells. IL-18 enhances directly Th1 polarization by increasing IFN-γ production and decreasing levels of Th2 cytokine IL-10 (23). IFN-γ is secreted by Th cells and macrophages, lowering their threshold for TLR-dependent activation. T cells also produce TNF-α, with potent NF-κB-activating properties. IL-1, TNF-α, and IFN-γ can enhance expression of CD40 ligand (CD40L) and CD40, cell-associated members of the TNF/TNF receptor (TNFR) family.

Activated T cells express CD40L (CD154), which binds to its CD40 receptor on macrophages, B cells, ECs, SMCs, and dendritic cells (DCs) (26). This, in turn, induces expression of tissue factor (TF), MMP, and adhesion molecules (27). IFN-γ influence on atherogenesis continues to be controversial. Regions of atheroma, abundant in T cells that secrete IFN-γ, show reduction in SR expression with a concomitant decrease in the number of foam cells (28). IFN-γ has recently been shown to improve the efficiency of antigen presentation and to enhance synthesis of TNF-α and IL-1 (29).

Th1 and Th2 have an important role in modulation of immune response and their balance is maintained by crossregulation. One model of this modulation is very popular in which Th2 responses were proposed to antagonize proatherogenic Th1 effects and thereby confer atheroprotection. This concept is excessively neatness, therefore a theory that shows an imbalance between pathogenic T cells (Th1/Th2) and a so-called „regulatory T cells“ (Treg cells) which response to altered self-antigens emerging in a reciprocal amplification of innate and adaptive immunity in atherosclerosis, is more reliable. Treg cells actively suppress immune activation and maintain immune homeostasis (3). IL-2 produced by nonregulatory conventional T cells modulates a feedback control mechanism.
between pathogenic and Treg cells. Besides IL-2 produced by peripheral T cells, which is crucial for Treg cells expending, IL-10 and TGF-β have been pointed to mediate Treg cells function. Atheroprotection properties of Treg cells are associated both with enhanced IL-10 production by CD4+ T cells and TGF-β-dependent Treg suppressive function.

Secretion of pro-inflammatory cytokines (IL-1, IFN-γ and TNF-α) by activated immune cell in atherosclerotic plaque will induce the production of IL-6. IL-6 stimulates the production of a large amount of acute-phase reactants in the liver (C-reactive protein, serum amyloid A and fibrinogen). Even if cytokines have an important role in all steps, their amplification at each step of the cascade makes the measurement of downstream mediators (i.e. CRP) useful for clinical diagnosis.

III. CLINICAL OVERVIEW OF CYTOKINES IN THE EVALUATION OF CARDIOVASCULAR DISEASE (CVD) RISK

The number of studies of cytokines involved in atherosclerosis and its complications [coronary artery/heart disease (CAD/CHD) or/and acute coronary syndrome (ACS): stable/unstable angina pectoris (SAP/UAP), myocardial infarction (MI); and congestive heart failure (CHF)] are overwhelming.

Besides cytokines, CRP is the most extensively studied inflammatory marker. The PROVE-IT-TIMI 22 study established that the risk of recurrent MI or death from coronary causes among patients with (ACS) is best predicted by the combination of LDL cholesterol and CRP levels (30). CRP is a stronger predictor than LDL level and that is added to the information provided by the Framingham risk score.

### Table 2

Cytokines with proven actions on vascular and immune cells in atherogenesis

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Source</th>
<th>Targeted cell</th>
<th>Actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1α and β</td>
<td>ECs, SMCs, macrophages, lymphocytes</td>
<td>Different cell types</td>
<td>Pro-inflamatory activity, stimulates endothelial and SMCs activation</td>
</tr>
<tr>
<td>IL-2</td>
<td>Activated T cells</td>
<td>Macrophages, T and B cells, NK cells</td>
<td>T-cell growth factor, stimulates NK activity, stimulates Treg cells</td>
</tr>
<tr>
<td>IL-4</td>
<td>Th2 cells, mast cells</td>
<td>T and B cells, mast cells, macrophages, hematopoietic progenitors</td>
<td>Proliferation and differentiation of B cells and Th2 cells (anti-inflammatory by inhibiting Th1 immune responses), stimulates VCAM-1</td>
</tr>
<tr>
<td>IL-5</td>
<td>ECs, T cells, mast cells</td>
<td>B cells</td>
<td>Stimulates growth and differentiation of B cells</td>
</tr>
<tr>
<td>IL-6</td>
<td>ECs, SMCs, macrophages, T cells</td>
<td>ECs, SMCs, T and B cells, neutrophils, macrophages</td>
<td>Induction of acute phase proteins (CRP, fibrinogen), differentiation of myeloid cells, SMCs proliferation</td>
</tr>
<tr>
<td>IL-8</td>
<td>ECs, mono-cytes, T cells</td>
<td>Neutrophils, T cells, monocytes</td>
<td>Pro-inflamatory activity, promotes leukocyte arrest</td>
</tr>
<tr>
<td>IL-9</td>
<td>Th2 cells</td>
<td>T cells, B cells, mast cells, eosinophils, neutrophils, and epithelial cells</td>
<td>Promotes proliferation and differentiation of mast cells, stimulates IgE production, inhibits monocyte activation, stimulates TGF-β in monocytes</td>
</tr>
<tr>
<td>IL-10</td>
<td>Macrophages, Th2, Treg and B cells, mast cells</td>
<td>Macrophages, T cells</td>
<td>Anti-inflammatory, inhibits Th1 responses, promotes proliferation and differentiation of regulatory T cells</td>
</tr>
<tr>
<td>IL-12</td>
<td>Th1 cells</td>
<td>T cells, macrophages</td>
<td>Pro-inflammatory activity, promotes NK and cytotoxic lymphocyte activity, induces IFN-γ</td>
</tr>
<tr>
<td>IL-18</td>
<td>Macrophages</td>
<td>T cells; NK cells; most of cell lineages</td>
<td>Pro-inflammatory activity, induces IFN-γ and other Th1 cytokines, promotes Th1 development and NK activity</td>
</tr>
<tr>
<td>M-CSF</td>
<td>ECs, macrophages, lymphocytes</td>
<td>Hematopoietic stem cells, neutrophils, macrophages</td>
<td>Growth and differentiation of macrophages</td>
</tr>
<tr>
<td>TNF-α</td>
<td>SMCs, macrophages, lymphocytes</td>
<td>Different cell types</td>
<td>Pro-inflammatory activity, tumor necrosis, neutrophil activation, fever, bone resorption, anti-coagulant</td>
</tr>
<tr>
<td>TGF-β</td>
<td>SMCs, platelets, macrophages, Treg and B cells</td>
<td>Different cell types</td>
<td>Anti-inflammatory; profibrotic; promotes wound healing, angiogenesis; suppresses Th1 and Th2 immune responses</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Th1 cells, NK cells, CD8+ cells, SMCs?</td>
<td>ECs, SMCs, macrophages, lymphocytes, NK cells</td>
<td>Pro-inflammatory activity, promotes Th1 immune responses/secretion of Th1-associated cytokines, inhibits extracellular matrix synthesis by SMCs</td>
</tr>
<tr>
<td>CD40L</td>
<td>ECs, SMCs, platelets, T cells, NK cells</td>
<td>ECs, SMCs, macrophages, lymphocytes, NK cells</td>
<td>Pro-inflammatory activity, stimulates MMPs secretion, promotes Th1 immune responses/secretion of Th1-associated cytokines</td>
</tr>
</tbody>
</table>
Association and Centers for Disease Control and Prevention have issued a statement recommending that CRP be used as a risk marker for CVD in individuals with a Framingham risk score between 10% and 20%. In their recommendations, CRP levels <1 mg/L were considered low-risk, 1 to 3 mg/L as average risk, and > 3 mg/L as high-risk for CVD (31). Elevation of plasma CRP levels were positively correlated with other pro-inflammatory cytokines (i.e. IL-6, IL-12, IL-18, TNF-α) or negatively correlated with anti-inflammatory cytokines (for example IL-10) in UAP and CHD (32, 33). Numerous in vitro experiments have showed the effects of CRP in atherosclerosis (i.e. CRP enhances expression of adhesion molecules, MCP-1), but we must be precautious because effects of recombinant CRP previously reported in the literature were artifactual due to the presence of sodium azide or contamination by bacterial products in the commercial CRP (34).

Among pro-inflammatory cytokines, IL-1β levels were measured in serum of patients with ACS together with sIL-2R, IL-6, TNF-α and IFN-γ. Serum IL-1β together with sIL-2R, IL-6, TNF-α were significantly higher in MI group or UAP group compare with controls (35). So IL-1β may have diagnostic value for ACS and can be useful marker reflecting disease stability.

In UAP studies a T cell passing burst has been observed and a link between T cell activation and CAD was tried to find through measuring IL-2 plasma levels. IL-2 and sIL-2R high levels were discovered in patients with SAP but not with UAP (36), in contrast with IL-1β.

Increased levels IL-6, the major stimulant for hepatic production of CPR, was positively correlated with elevated levels of CRP, IL-12, IL-18, TNF-α in UAP and CHD as we mentioned before. IL-6 can be predictive for future CAD based on studies in which IL-6 was found elevated in patients with UAP comparing with SAP patients (37). Moreover IL-6 was pointed to be an independent predictor of mortality in patients with ACS, even if in this FRISC II trial levels of hsCRP were measured too (38). We can suggest that IL-6 can be a marker for risk stratification in CAD patients, in an independent way or correlated with other pro-inflammatory biomarkers.

IL-7 plasma levels, produced by activated platelets, were found in high concentrations in UAP patients and also IL-7 augments expression of inflammatory cytokines in circulating mononuclear cells (39). This leads to a conclusion that IL-7 has an important role in promotion of instability in CAD. IL-8 also shows to be a predictive marker of future CAD. This is sustained by an EPIC-Norfolk population study where elevated levels of IL-8 were correlated with high risk for CAD and, like IL-6, this correlation appear to be independent of other biomarkers of cardiovascular risk, even if CRP levels were measured too (40).

IL-12 p40 mRNA and IL-12 p70 protein are abundant in atherosclerotic plaque. The balance between IL-12/IL-10 has a cross-regulatory role and contributes to the level of immune-mediated tissue injury in atherosclerosis (41). Clinical studies have showed elevated plasma levels of IL-12 in UAP and CHD along with CRP, IL-6, IL-18, TNF-α or IL-1, IL-12p70 and RANTES plasma concentrations were significantly elevated in UAP compared to SAP and MI as we mentioned before.

Regarding IL-18, clinical studies have revealed high concentration in UAP, MI and heart failure patients (42). IL-18 is considered an independent predictor of death in patients with CAD which had diagnosed with UAP, but, unlike IL-7 and IL-8 levels, with SAP too (43). All these observations lead to consider IL-18 an important biomarker of plaque instability.

Among anti-inflammatory cytokines, IL-10 has a strong anti-atherogenic role and it is confirmed by clinical studies. IL-10 serum concentrations were significantly lower in SAP and UAP patients group and the same study has showed a negatively correlation between hsCRP or IL-12 and IL-10 levels (33). High levels of IL-10 were strongly correlated with improved outcome of patients with ACS (44). So, decreased serum IL-10 level is a marker of plaque instability.

As we mentioned before, serum TNF-α together with IL-1β, sIL-2R and IL-6 were significantly higher in MI group or UAP group compare with controls and became significantly lower 4 months later in the follow-up patients (35). The soluble circulatory receptors of TNF-α (sTNF-R1 and sTNF-R2) can be measured with greater sensitivity and reliability than TNF-α (45). In a prospective cohort investigation, elevated levels of inflammatory markers measured in plasma of patients (sTNF-R1 and sTNF-R2, IL-6 and particularly CRP) are correlated with increased risk of CHD (32). This study concludes that high levels of sTNF-R1 and sTNF-R2 may be also associated with an increased risk and deserve further exploration in other populations.

Increased levels of CD40L in apparently health women have an higher risk of MI or stroke, but it is
necessary an adjustment with classic cardiovascular risk factors (26). Circulating levels of sCD40L can determine patients with recurrent ischemic events (46). So levels of sCD40L can reflect a predictive value in ACS patients.

**TGF-β** have anti-atherogenic effects and limits atherosclerosis by modulating the accumulation of lipids in the vessel wall and the inflammatory response. Serum concentration of active TGF-β1 is found in regions of the human aorta that has a high probability of developing atherosclerotic lesions.

Elevated M-CSF levels were found in patients with ACS compared with SAP patients, resulting that M-CSF is a powerful risk factor for adverse outcomes in SAP patients (48).

Even if IFN-γ has a pro-inflammatory activity and promotes Th1 immune responses, serum levels of IFN-γ showed no significant difference between MI or UAP group and controls, also showed no significant change when measured in follow-up patients (35).

**IV. THERAPEUTIC IMPLICATIONS**

Atherosclerosis, viewed as a chronic inflammatory disease, opens new windows in the prevention and treatment of cardiovascular complications.

As it is known, lipid-lowering statins have general anti-inflammatory properties and inhibit the formation of mevalonic acid by inhibition of HMG-CoA reductase. Their actions are proved in clinical trials by reduction in serum cholesterol levels (49).

Novel therapeutic strategies include targeting of inflammatory mediators. We can use anticytokines such as IL-1ra, IL-18BP or sTNF-α receptors as natural endogenous inhibitors of IL-1, IL-18 and TNF-α and, in this way, we can neutralize their pro-inflammatory effects in atherosclerosis. Promising results are obtained with Enancept, a soluble TNFR fusion protein, which is effective and safe in rheumatoid arthritis (50) and which can be used in atherosclerotic patients. Another novel strategy is targeting downstream inflammasome. Inflammasome activates caspase-1 and 5 (inflammatory molecules), so inhibitors of caspases will be a promising treatment of autoimmune inflammatory diseases by decreasing IL-1β and IL-18 production, which have a critical role in atherosclerosis too (inhibitor of caspase-1, Pralnacasan, is in clinical trials) (51). It is a strong immunosuppressant with selectivity for JAK3 and doesn’t induce sever contra effects such as suppression of red and white cells lineage. Activation of natural anti-inflammatory intracellular pathways (SOCS) can suppress signals from inflammatory cytokines which use JAK/STAT pathways. SOCS1 is proved to be benificial in controlling inflammation mediated by several cytokines in atherosclerosis and SOCS3 reduces the inflammation in inflammatory arthritis in murines (52) and it is a negative regulator of inflammations in ulcerative colitis and Cohn’s disease (53). So SOCS3 may have the same anti-inflammatory effects in atherosclerosis too.

Targeting the proximal triggers is the most promising strategy for interruption of inflammation in atherogenesis. Immunization using oxLDL can significantly reduce atherosclerosis, so, for long-term, prevention by vaccine therapy with disease related antigen will be attractive (54). Protective immunity induced by vaccination with oxidized epitopes of oxLDL may be related with stimulation of Treg cells and may represent a new therapeutic onset for prevention and treatment of atherosclerosis (3). Another way to preserve immune homeostasis in atherosclerosis is vaccination using peptides, because long term immunization, in this case, can transform T cells in CD4+CD25+ Treg cells which stimulate specific immunological tolerance upon encounter with antigen (55). Also vaccination with bacteria containing modified phospholipids or HSP60 shows promising results (56).

**V. CONCLUDING REMARKS**

Pro-inflammatory and anti-inflammatory cytokines are ubiquitous in the atherosclerotic plaque and their release produce an autoamplification cascade, therefore it is arduous to point the direct action of a single cytokine, even if it’s usually known that their production by inflammation or vascular cells is transient and the liberated cytokine act in an autocrine or paracrine mode. Cytokines inflammatory effects are superposable with their atherogenic effects so, we can conclude that proatherogenic cytokines are: IL-1, IL-2, IL-4, IL-6, IL-12, IL-18, TNF-α, CD40L, IFN-γ, M-CSF, MCP-1, RANTES, MIF and anti-atherogenic cytokines are: natural endogenous antagonist of IL-1 and IL-18 (i.e. IL-1ra and IL-18BP), IL-6, IL-9, IL-10 and TGF-β.

Clinical implications of inflammatory mechanisms in atherosclerosis lead us to use inflammatory markers for stratification risk and prognosis of cardiovascular
complications. Some of cytokines are independent predictors for CVD such as CRP or IL-18, others are independent risk markers for CAD (IL-6 or IL-8) and others are plaque instability markers (elevated levels of IL-1β, IL-6, IL-7, IL-12 and IL-18 or decreased level of IL-10).

Generally, the value of inflammatory markers as independent predictors is controversial; therefore we have to see their elevations or decreases corroborated. Besides cytokines, chemokines, growth factors and CRP, other biomarkers are extensively studied: Lp-PLA2 (lipoprotein – associated phospholipase A2); myeloperoxidase (MPO); pregnancy-associated plasma protein A (PAPP-A); B-type natriuretic peptide (BNP); MGP (matrix Gla protein) and other biomarkers.

Similarities between atherosclerosis and other chronic inflammation diseases (cirrhosis, rheumatoid arthritis, glomerulosclerosis, pulmonary fibrosis and chronic pancreatitis) were observed (6), so inflammatory response has almost the same pattern in arteries and other tissues.

We know that cytokines, once secreted, are bind by high specific receptors of nearby cells. Can we tell that circulating cytokines levels reflect their true activity? (for example TNF-a has a limited half-life and it’s hard to be measured in large-scale studies) (45). There are a lot of questions to raise such as: which is the cytokine more specific for atherosclerosis? Does the marker independently predict risk beyond conventional tools? Is atherosclerosis a local or a systemic disease? Can we put an equal sign between findings in animal and human atherosclerosis? Are all experimental studies accurate? (we saw before an example for CRP) Do therapies that decrease plasma levels of inflammatory markers also reduce CV risk? etc.

Therefore, in future we have to pay attention to implementations of novel therapies and to have biochemical assays widely available and reliable.

REFERENCES


