REVIEW ARTICLE

Interleukin-10 and Coronary Disease

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Understanding of the pathophysiology of atherosclerosis has changed markedly over the past few decades. It is now widely accepted that inflammation plays a fundamental role in the genesis and development of atherosclerosis. Inflammatory mechanisms also appear to determine clinical presentation and disease outcome. Atherosclerotic lesions have high concentrations of inflammatory cells (T lymphocytes and activated macrophages) as well as an abundance of pro-inflammatory cytokines [interleukin (IL)-1, IL-6, IL-8, interferon-γ, tumor necrosis factor-α, etc.] that modulate local inflammatory responses. These may also alter plaque stability and facilitate the development of acute cardiovascular events. The role of anti-inflammatory cytokines in this context remains to be studied. IL-10 is an anti-inflammatory cytokine synthesised by T-lymphocytes and macrophages and has other anti-inflammatory effects. IL-10 expression within human atherosclerotic plaques has been demonstrated and animal experiments have shown that low levels of IL-10 lead to the development of extensive and unstable atherosclerotic lesions. Currently available evidence suggests a potential protective role for IL-10 in atherosclerosis. This new perspective on coronary disease as a chronic inflammatory process may open new avenues for the management of ischemic heart disease.

Key words: *Inflammation. Atherosclerosis. Interleukin-10.*

Interleucina-10 y enfermedad coronaria

Los conocimientos actuales de la fisiopatología de la aterosclerosis divergen marcadamente de los de las últimas décadas. Hoy día se acepta de manera generalizada que la inflamación desempeña un papel fundamental en el desarrollo y progresión de las lesiones ateroscleróticas, condicionando la aparición de manifestaciones clínicas en la evolución. El estudio histopatológico de las lesiones ateroscleróticas revela la presencia de células inflamatorias (linfocitos T y macrófagos activados), así como de abundantes citocinas proinflamatorias (IL-1, IL-6, IL-8, TNF-α, INF-γ, etc.), que modulan la respuesta inflamatoria local, alterando la estabilidad de la placa y favoreciendo el desarrollo de acontecimientos cardiovasculares agudos. Sin embargo, el papel de las citocinas antiinflamatorias no ha sido tan bien estudiado. La IL-10 es una citocina antiinflamatoria capaz de inhibir la síntesis de citocinas proinflamatorias por los linfocitos T y los macrófagos, así como otras funciones inflamatorias de estas células. Su presencia ha sido demostrada en las placas ateroscleróticas humanas y se ha objetivado en estudios de experimentación animal que los bajos valores de IL-10 condicionan el desarrollo de lesiones ateroscleróticas más extensas y morfológicamente más inestables. Las evidencias disponibles en la actualidad sugieren un potencial papel protector de la IL-10 en el desarrollo de la aterosclerosis. Este nuevo enfoque de la enfermedad coronaria como una enfermedad inflamatoria crónica puede abrir en el futuro nuevos caminos para la investigación en el campo de la cardiopatía isquémica.

Palabras clave: *Inflamación. Aterosclerosis. Interleucina-10.*

INTRODUCTION

Coronary artery disease (CAD) is highly prevalent and one of the most important causes of morbidity and mortality in industrialized societies.[1,2] The underlying process in CAD is atherosclerosis, and it is currently considered a chronic inflammatory disease of the arterial wall.[3-5] The most severe clinical presentation of this process is an acute coronary syndrome (unstable angina and infarcts [AMI),[6-8] which occurs secondary to the occlusion of the diseased arteries.

Histological study of the atherosclerotic plaques reveals the presence of progressive infiltration and accumulation of lipids, inflammatory cells (monocytes/macrophages, T-lymphocytes), smooth muscle cells (SMC), and an extracellular matrix in the arterial wall.[1,2]

The identification of the inflammatory cells in the atherosclerotic lesions, as well as the complementary fac-

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ABBREVIATIONS

DIC: disseminated intravascular coagulation.
SMC: smooth muscle cell.
CMV: cytomegalovirus.
COX-2: cyclooxigenase-2.
APC: antigen presenting cells.
ED: endothelial dysfunction.
DNA: deoxyribonucleic acid.
CAD: coronary artery disease.
TF: tissular factor.
G-CSF: granulocyte colony stimulant factor.
GM-CSF: granulocyte and monocyte colony stimulant factor.
HSV: herpes virus simplex.
AMI: acute myocardial infarction.
ICAM-1: intracellular adhesion molecule-1.
IL: interleukine.
INF-γ: interferon γ.
iNOS: nitric oxide synthetase.
LDL: low density lipoproteins.
MM-LDL: minimally modified LDL.
ox-LDL: oxidized LDL.
LPS: lipopolysacharide.
MHC-II: major histocompatability complex class II molecules.
MCP-1: monocyte chemoattractant protein-1.
M-CSF: macrophage colony-stimulating factor.
ECM: extracellular matrix.
MMP: metalloproteinase.
NF-κβ: nuclear factor κβ.
NO: nitric oxide.
RPC: reactive protein-C.
PDGF: plaque-derived growth factor.
PGI2: prostaglandin I2.
mRNA: messenger ribonucleic acid.
MPTI: metalloproteinase tissular inhibitor.
Th1: type 1 T-helper cells.
TNF-α: tumor necrosis factor-α.
VCAM-1: vascular cell adhesion molecule.
VEGF: vascular endothelial growth factor.

tors, immunoglobulins, cytokines,[3,9] and others, implicates the involvement of the immunological system in atherogenesis. During this inflammatory reaction, a great quantity of cytokines is produced by macrophages and activated T-cells present in the plaque,[3] charged with modulating inflammatory response. This process may alter plaque stability and favor the development of acute events.[3] Nevertheless, the manner in which this local or systemic, or both, immunological response is initiated and propagated to produce or favor the development of atherosclerotic lesions is still not completely clear.

Numerous studies of experimental animals fed with diets rich in cholesterol have shown that immunosuppression causes the development of more extensive and more severe atherosclerotic lesions as compared to controls.[10-12]

In recent years, multiple scientific studies have emphasized the role of the immunological system cells (monocytes, lymphocytes, etc.) and pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, INF-γ, etc.)[3,13-17] in the development of atherosclerosis. Nevertheless, there is little evidence available on the potential role of anti-inflammatory cytokines in this process.

The aim of this article is to review the knowledge currently available concerning the potential protective role of anti-inflammatory cytokines, specifically interleukine 10 (IL-10), in the pathogenesis and development of atherosclerotic lesions.

PHYSIOPATHOLOGY OF ATHEROSCLEROSIS

The development of atherosclerotic lesions is a process that begins in the second or third decades in the life of an individual, and can be divided into various stages through which the composition of the atherosclerotic plaque changes progressively until it acquires the morphology of a mature plaque.

Endothelial dysfunction

The first event in the development of atherosclerosis is the appearance of endothelial dysfunction (ED).[3,18-21] The endothelium plays an important part in maintaining the equilibrium of vascular bed function. It has a regulatory role in vasomotor tone by the production of vasodilator substances, such as nitric oxide (NO) and prostacycline (PGI2), and also of vasoconstrictor substances such as endothelin 1 and angiotensin II.[22] It also possesses anti-atherogenic (antiaggregate, anti-adhesive, anti-proliferative and antioxidant) and anti-inflammatory properties, segregating chemoattractant substances from monocytes and lymphocytes, as well as modulators of vascular growth.[23-26]

There are multiple causes of ED that favor the development of atherosclerosis including the presence of elevated modified LDL values (ox-LFL, MM-LDL); free radicals; immunoregulatory substances (TNF-α, IL-1β, LPS); infectious microorganisms (HSV, *Chlamydia*, CMV, etc.); genetic alterations; elevated serum homocysteine values, and classic risk factors (hypertension, diabetes, smoking).[27,28]

Endothelial dysfunction leads to a loss of the homeostatic functions of the endothelium, resulting in the adhesion of plaques and inflammatory cells (monocytes and lymphocytes to the vascular wall;[27] an increase in endothelial permeability that allows the deposit of modified LDL at the intimal level;[29] a liberation of cytokines and growth factors that produ-

ces the proliferation of smooth muscle cells and the attraction of more inflammatory cells to the altered arterial wall.[27] As a consequence, it also causes perturbation of the thrombolitic-thrombotic equilibrium in the endothelial bed that promotes the development of thrombotic phenomena, such as the abnormal regulation of vasomotor tone secondary to decreased bioavailability of NO, with the subsequent tendency to arterial vasoconstriction.[30,31]

The role of low-density lipoproteins (LDL). The formation of fat striae

The first histopathological change detectable in the early phases of atherosclerosis is the accumulation of LDL particles in the subintimal space.[32] These LDL undergo an oxidation process that activates the endothelium, favoring the development of atherosclerotic plaque.[33,34] The modified (oxidated) LDL cause the expression of adhesion molecules (ICAM-1, VCAM-1)[35] and the synthesis of monocyte chemoattractant protein-1 (MCP-1) by the endothelial cells. This favors the union of the circulating monocytes and lymphocytes to the dysfunctional endothelium and later the migration of these cells to the sub endothelial space, promoting, at the same time, the differentiation of monocytes to macrophages.[3,36] The oxidated LDL also change the production of free radicals and NO, favoring oxidative stress in the arterial wall,[3,36,37] increasing the apoptosis of the endothelial cells.[38]

The monocytes that are attracted to the dysfunctional endothelium by liberated chemoattractants adhere to the wall via the adhesion molecules (ICAM-1, VCAM-1) expressed by the damaged endothelial cells, internalize in the sub endothelial space and mature into macrophages, which capture the ox-LDL to transform themselves into frothy cells, initiating in this way the formation of fat striae.[3]

These cells loaded with lipids produce free radicals for the specific oxidation of LDL and free new cytokines for the attraction of more monocytes and lymphocytes to the dysfunctional endothelium and for the migration and proliferation of SMC in the intima.[34,39-41] These processes auto perpetuate the mechanism that favors the development and progression of atherosclerotic plaque.

Vulnerability of atherosclerotic plaque

Atheromas are dynamic structures where equilibrium exists between the destructive influence of the inflammatory cells and the stabilizing effect of SMC.[6,42] The latter are responsible for synthesizing extracellular matrix (ECM) proteins, the principal component of the fibrous cover of atherosclerotic plaques that gives the lesion stability.[36] In atherosclerotic plaques there is a balance between the processes of synthesis and collagen degradation that are narrowly controlled by the inflammation mediators and regulate the contents of same in atherosclerotic lesions.[43]

The vulnerable plaque (that have a tendency to rupture) are characterized by a highly lipidic nucleus, an elevated infiltration of inflammatory cells (macrophages and T-lymphocytes), few SMC, and a thin fibrous cover.[4,14,44] The plaque-activated T-lymphocytes produce INF-?, which inhibits the proliferation of SMC and their ability to synthesize collagen.[45] The activated macrophages produce metalloproteinases (gelatinase, stromelysine, and interstitial collagenase) that degrade the ECM proteins, favoring the disruption of plaque,[46] and synthesizing the tissular factor (TF),[47] 1 of the principal activators of the coagulation cascade, which promotes thrombosis of the plaque. These macrophages also induce the apoptosis of the SMC, with a consequent decrease in collagen synthesis and weakening of the fibrous cap, destabilizing the plaque.[36]

In addition to the monocytes, the T-lymphocytes are equally attracted to the dysfunctional arterial wall by chemoattractant substances, and they are activated at the wall, initiating the production of more cytokines such as INF-γ, TNF-α; interleukins (IL-1, IL-2, IL-6, IL-8) and growth factors such as GM-CSF that activate the monocytes present in the plaques and favor their proliferation, enabling a local inflammatory response.[3,39,40,48]

The result of the interaction of these factors is a progression of the atherosclerotic lesion from its initial state of fat striae to complex atherosclerotic plaque.[49] The rupture or ulceration of the unstable plaque results in the exposure of the procoagulant and prothrombotic surfaces to the blood, causing the activation of platelets and the formation of thrombi, which can trigger clinical complications by occluding the vessel lumen or producing asymptomatic plaque growth.[6,50]

Therefore, in all the different developmental studies of atherosclerotic plaques, signs of chronic inflammation can be noted, and various physiopathological mechanisms that influence the development, progression, and instability of atherosclerotic lesions have been described.[3]

INFLAMMATION AND ATHEROSCLEROTIS

Traditionally, atherosclerosis has been considered an illness caused by the accumulation of lipids, where the vulnerable plaques are those with greater lipidic nuclei and a thin fibrous cap, whose rupture would respond to the forces of mechanical stress. Nevertheless, today there is scientific evidence that confirms the role the inflammatory response, whether local or systemic, plays in the development of the atherosclerotic process and in triggering acute cardiovascular events (Figure 1).[51] Patients with unstable angina have elevated reac-

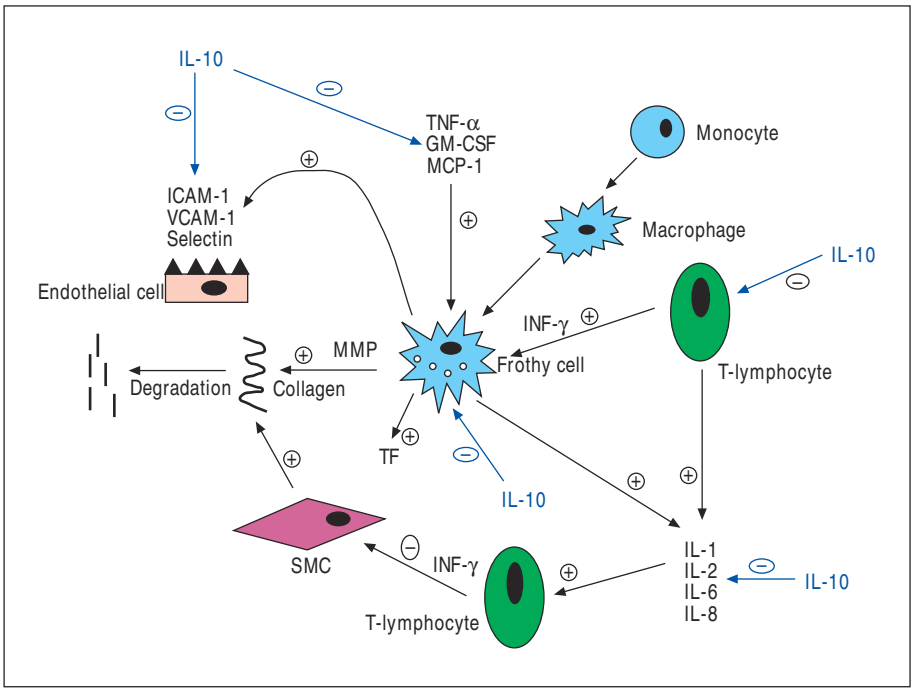

Fig. 1. Inflammatory processes in atherosclerosis. Activated T-lymphocytes and macrophages are present in the unstable plaque that segregate proinflammatory cytokines (INF-γ, TNF-α, IL-1, IL-2, IL-6, IL-8), metalloproteinases that degrade the fibrous cap and chemoattractant factors of the inflammatory cells that promote the expression of adhesion molecules. IL-10 has potent anti-inflammatory properties and acts by limiting the local inflammatory response, which provides stability to the atherosclerotic lesion.

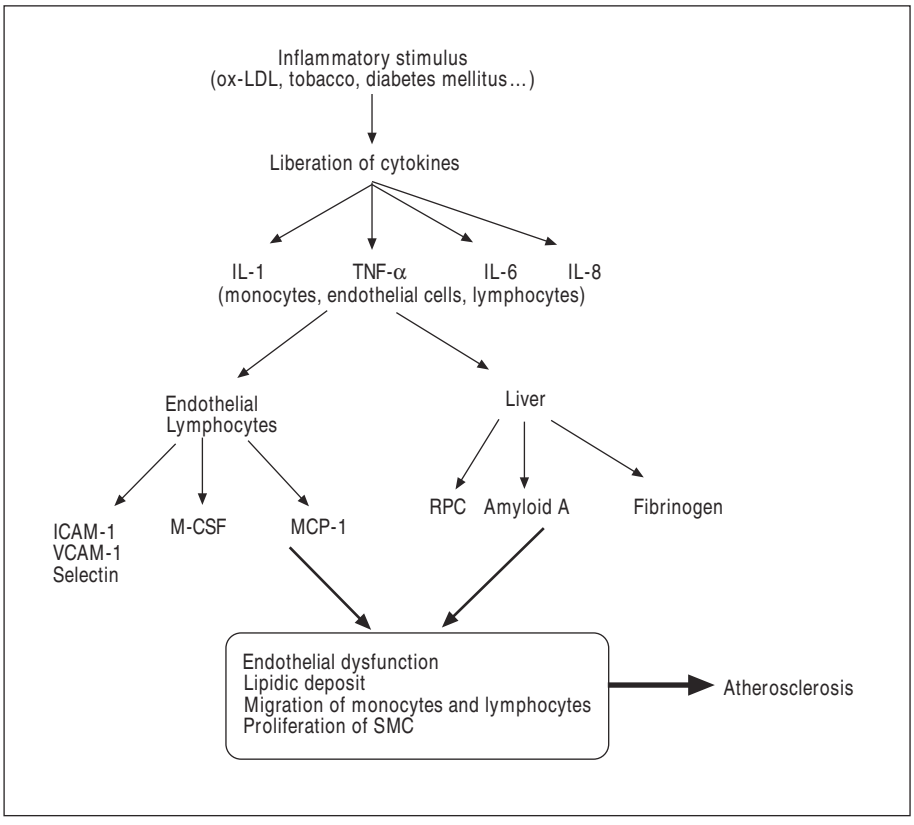

Fig. 2. The local or systemic inflammation leads to the liberation of cytokines, which promotes the synthesis of inflammatory mediators that favor the development of atherosclerosis.

tant values in the acute stage (reactive protein-C [RPC]), serum amyloidal A, fibrinogen) and proinflammatory cytokines (IL-1, IL-6, IL-8)[52] (Figure 2). The elevated levels of these inflammation inhibitors (RPC, fibrinogen, amyloid A, IL-6) are sensitive markers for inflammation and correlate with the development of coronary artery disease and its severity,[53,54] as well as the presence of acute coronary events.[55,56] The RPC, in addition to being considered an independent predictor of the development of cardiovascular com-

plications, appears to play a role in the pathogenesis of atherosclerosis, as it has been associated with an increase in the risk of thrombosis as it has been associated with an increased risk of cardiovascular complications by promoting the expression of tissular factor by the monocytes[57] and activating the complement cascade.[51] It has also been observed that it promotes the expression of adhesion molecules by the endothelial cells and increases cLDL capture by the macrophages in the plaque by opsonization.[58]

IL-6 is a cytokine with potent pro-inflammatory properties that induces the expression of reactants in the acute phase (the major inducer of hepatic production of RPC) and the migration and differentiation of the activated macrophages.[59] It also contributes to triggering acute coronary syndromes by enabling the synthesis of metalloproteinases and the expression of LDL receptors in the macrophages, as well as an increase in cLDL capture and the secretion of chemoattractant substances, such as MCP-1, by the same.[59] Finally, it regulates the expression of adhesion molecules and cytokines, such as IL-1β, and TNF-α, which increases the inflammatory reaction.[59] At the same time, the liberation of IL-6 is stimulated by IL-1, both acting together and with TNF-α, increasing the synthesis of IL-8 and reactants in the acute phase.[52]

IL-1 also induces the expression of genes for the synthesis of activating factors of the coagulation system and fibrinolysis inhibitors and the migration of neutrophils to the sub endothelial space, mediated by an increase in the expression of adhesion molecules in the endothelial cells and in the production of GM-CSF (granulocyte and monocyte colony stimulant factor).[52]

IL-8 ins a pro-inflammatory cytokine produced by various types of cells, including monocytes, macrophages, and T-lymphocytes, and its presence has been detected in frothy cells in human atheroma plaques. It has associated prothrombotic properties by increasing the procoagulant activity of the monocytes, by increasing the synthesis and expression of the tissular factor on the surface of these cells, and pro-atherogenic properties by decreasing MPTI-1 (metalloproteinase tissular inhibitor 1) properties, which favors the predominance of the degradation of the fibrous cap of the plaque over synthesis.[60]

The synthesis of pro-inflammatory cytokines is mediated in large part by the nuclear transcription factor NF-κβ. This is associated with the induction of protein codifying genes, which are vital for the inflammatory processes related to the rupture of atherosclerotic plaques. This factor is activated by diverse stimuli, such as cytokines, viruses, mitogenes, pathogenic microorganisms, modified LDL, oxidative stress, etc.[61] The activation of this factor has been detected in macrophages, endothelial cells, and SMC of atherosclerotic plaques,[62,63] and there are experimental studies that demonstrated direct correlation between NF-κβ activity and the severity of the coronary lesions.[51] NF-κβ is found in the form of an inactive heterodimer in the cytoplasm bound to protein inhibitors that are generically known as I κβ.[36] This heterdimer consists of 2 sub-units, p50 y p65. When the cell is activated by 1 of the previously mentioned agents, I κβ phosphorilates and undergoes ubiquitination, which acts as a signal for its protelitic degradation. The p50/65 dimer relocates to the nucleus and activates the transcription of target genes that induce the expression of cytokines (TNF-α), interleukins (IL-1, IL-2, IL-6, IL-8), growth factors (MCSF, GM-CSF, G-CSF), chemoattractant substances (MCP-1), adhesion molecules (ICAM-1, VCAM-1, E-selectin), and enzymes (MMP, iNOS, COX-2),[64,65] that enable the local inflammatory response and destabilize the atherosclerotic plaque.

The inflammatory cells present in the atheromatous plaque express the immune mediator CD40 and its ligand CD40L.[66] The existence of positive T-cells for accumulated CD40L in the plaques, principally in plaque rapid growth areas and with a greater tendency to complication, suggests that this ligand intervenes in the pathology of the process. The interaction of CD40 with its ligand promotes a humeral and cell response cellular.[67] The interruption of this union via the administration of anti-CD40L antibodies limits, experimentally, the development of certain autoimmune illnesses such as lupus nephritis, multiple sclerosis, thyroiditis, implant rejection disease, and others.[68-72] It has also been shown *in vitro* that the interaction of CD40 and CD40L activates functions related to atherogenesis, including the production of pro-inflammatory cytokines,[71] metalloprteinases,[73,74] and the expression of adhesion molecules[75] and tissular factor.[74]

THE ROLE OF IL-10 IN ATHEROSCLEROSIS

Among the anti-inflammatory cytokines, IL-10 is considered the anti-inflammatory interleukine *par excelance*.[76,77] It was originally identified as the inhibitory factor in cytokine synthesis (CSIF),[78] as it inhibits the production of cytokines by T-lymphocytes, particularly IFN-γ, by Th1 cells in the murine systems.[79] Nevertheless, this inhibition is only seen when the macrophages act as antigen presenting cells (APC).[80]

Later studies revealed that IL-10 is in fact a cytokine with pleiotropic properties that acts on different types of cells, including thymocytes,[81] cytotoxic T-cells,[82] mastocytes,[83] B84 cells, and monocytes-macrophages.[80]

IL-10 is principally produced by a lymphocyte subtype CD4+ (Th2) and also in large quantities by macrophages. It is a cytokine with potent anti-inflammatory properties that is capable of inhibiting important functions of these 2 types of cells.[76,79] Therefore, it has been described as inhibiting the production of pro-inflammatory cytokines by macrophages and T85

cells, activated by various stimuli.

IL-10 has been identified in early and advances atherosclerotic lesions,[86,87] principally located in the cytoplasm of the macrophages, although it is also located in SMC and the extracellular matrix.[87]

Inflammatory properties of IL-10: action mechanisms

One of the first properties attributed to IL-10 was its capacity to inhibit the synthesis of cytokines. There are many published studies supporting this affirmation. Waal Malefyt et al[79] showed that both human IL-10 or its viral recombinant form added to monocyte cultures activated by INF-γ or LPS, or both, as is produced endogenously in response to these stimuli, was capable of inhibiting the production of inflammatory cytokines, including IL-1α, IL-1β, IL-6, IL-8, TNF-α, INF-γ, GM-CSF and G-CSF by the monocytes. Added to these effects, endogenous IL-10 has an auto regulatory effect on it own production, reducing the synthesis of mRNA IL-10 by the activated monocytes. Wang et al[85] showed that it could also act on T-cells, inhibiting the production of IL-2, TNF-β, INF-γ and GM-CSF. It also decreased the expression of major histocompatability complex class II molecules (MCH II) by the monocytes/macrophages, and the capacity of these to act as antigen presenting cells, ultimately limiting the specific proliferative antigen response of T-lymphocytes[79,88] and, therefore, the inflammatory response.

Various mechanisms have been proposed for the manner in which IL-10 inhibits the synthesis of pro-inflammatory cytokines. One of the most studied is the inhibition of the nuclear transcription factor NF-κβ by IL-10 in monocytes and T-cells by the intervention of secondary oxygen free radical messengers.[79] This results in a reduction in the synthesis of pro-inflammatory interleukins, adhesion molecules, growth factors, and chemoattractants of immunological system cells that limit the local inflammatory response in the plaque. This IL-10 action mechanism differs from that of IL-4, another anti-inflammatory interleukine that also inhibits the synthesis of pro-inflammatory factors, but by a process that does not involve NF-κβ but secondary to an increase in mRNA degradation of said molecule.[85]

O´Farrel et al proposed as another possible mechanism to explain the anti-inflammatory effects of IL-10 the inhibition of interferon production via the activation of STAT group transcription factors.[89] In a parallel manner, it has been demonstrated that IL-10 can inhibit the expression of pro-inflammatory genes that present areas rich in AU (ARE),[90] such as TNF-α, IL-1α, IL-1β, βGM-CSF, IL-8, and others, destabilizing its mRNA by acting on these ARE mofits.[91]

In addition, it has been shown that IL-10 is capable of inhibiting monocyte response mediated by CD40-CD40L interaction, which appears play relevant role in atherosclerosis.[67] Mach et al demonstrated that the *in vivo* block of this interaction by antibodies in mice subjected to an atherogenic diet limited the size of the atherosclerotic plaques, reduced it lipidic content, T-cells and macrophages, and decreased the expression of adhesion molecules (VCAM-1).[67] Therefore, this experimentsl animal model demonstrates another mechanism by which IL-10 appears to play a protective role by limiting the development of atherosclerotic lesions.[92,93]

IL-10 modulates the immune cell response

We have seen that T-cells participate in the immunological response via the liberation of cytokines. Within the helper T-cells there are 2 types that may mediate different immunological responses. Th1 cells principally produce IL-2 and INF-γ, which is associated with the activation of macrophages and other T-cell subtypes. On the other hand, Th2 cells basically synthesize IL-4 and IL-5, which increase the humeral response and inhibit the Th1 cell response, which predominates in unstable atherosclerotic plaques.[94]

IL-10, together with IL-12, plays an important part in the regulation of these 2 types of immunological response. IL-12 is an important T-cell growth factor[95] that is principally produced by activated monocytes[96] and selectively induces a Th197 pattern of immunological response. Uyemura et al[81] showed the presence of IL-12 in atherosclerotic plaques. This enables the chronic inflammatory response of the plaque T-cells and macrophages, leading to the destabilization and rupture of the plaque by various mechanisms. Among these is the liberation of INF-γ[48] by Th1 cells, stimulating the synthesis of MMP by the macrophages and producing a decrease in the expression of genes for collagen synthesis by SMC and an increase in the apoptosis in said cells, finally destabilizing the plaque by weakening its fibrous cover. In addition to role of regulating the immune cell response, INF-γ is capable of potentiating VCAM-1, MHC II, and LDL receptors in vascular cells.[48]

An experimental study on apoloprotein E deficient mice showed that the MRNA of IL-12 is detected early in atherosclerotic plaques that the mRNA of IL-10, and that the daily administration of IL-12 accelerated the development of atherosclerosis in the mice.[98]

The Uyemura group[81] proposed that the endogenous production of IL-10 by T-cells and activated human monocytes, in response to modified LDL stimulation, inhibits IL-12 production and, therefore, facilitates the Th2 immune response decreasing the pro-inflammatory response. These findings suggest that a cross-regulation exists in the production of IL-10 and IL-12 that modulates the local inflammatory response.

The presence of IL-10 in atherosclerotic plaques

IL-10 has been identified in early and advances stages of atherosclerotic lesions, principally located in the macrophage cytoplasm, although it is also in SMC and the extracellular matrix (Figure 1).[86,87]

Mallat et al[87] showed, in humans, not only the presence of IL-10 in atherosclerotic plaques but also that a strong association exists between the high IL-10 values in the lesions and a reduction in iNOS (nitric oxide sythetase) expression and cell death in the plaques.[99,100] This suggests that IL-10 plays an important role in limiting the local inflammatory response, preventing excessive cell death in the plaque and promoting, consequently, its stability.

It is known that NO is produced under normal conditions by endothelial cells and that it has important vasodilator and antiatherogenic properties, by inhibiting plaque aggregation, the activation of adhesion molecules, and the proliferation and migration of SMC.[101] In initial studies of atherosclerosis, before the lesions visible on angiography appeared, endothelial dysfunction was already present, which caused a reduced NO bioavailability, whether by a decrease in its synthesis,[102] its liberation,[103]

or by an increase in its inactivation.[104] On the other hand, it promotes the synthesis of iNOS, allowing it to bind to super oxidized anions. As a consequence, it produces an increase in the oxidative stress of the atherosclerotic lesion and with it a tendency to vasoconstriction, greater LDL intravascular oxidation, and the activation of NF-kβ, promoting the expression of genes causing the inflammatory response and stabilizing the plaque.[105]

Toshiyuki et al[100] showed in *in vitro* studies that endogenous IL-10 plays an essential role in protecting macrophages infected by salmonella from cell death by preventing the excessive production of TNF-α by destroying the bacteria. TNF-α has been shown to be capable of inducing cell death by apoptosis in various types of cells, in addition to being and important mediator of inflammatory effects.[106,107]

Cohen et al[99] later showed that *in vitro* IL-10 could inhibit the apoptosis of T-cells, and that this was mediated in part by the over-expression of Bcl-2 protein, preserving in the lymphocytes rescure from apoptosis their capacity to proliferate upon IL-2 stimulation.

Interleukin-10 and the vulnerability of atherosclerotic plaque

It is known that one of the principal conditions for the destabilization of atherosclerotic plaques is the degradation of the extraxcellular matrix (ECM) and the fibrous collagen cover. The macrophages present in the plaques are in charge of modulating the exchange of the ECM, synthesizing and segregating degrading ECM enzymes, called metallproteinases (MMP), as well as its corresponding inhibitors (MPTI). The principal MMP studied are interstitial collagenase, stromelysine, and gelatinase 92 Kid and 72Kd. These are initially synthesized inactively and are later activated by various stimuli such as oxidative stress, and pro-inflammatory cytokines.[108] There are also 2 types of MMP inhibitors: MPTI-1 and MPTI-2.[109] MPTI-1 interacts with active forms of collagenase and stromelysine, as well as with the precursor and active form of gelatinase 92Kd. MPTI-2 specifically inhibits the proenzyme and active form of gelatinase 72Kd.[108]

Lacraz et al[108] showed in *in vitro* experiments that IL-10 has a specific regulatory effect on macrophag4es and monocytes, overriding MMP synthesis and stimulating instead the synthesis of its inhibitor, MPTI-1. This suggests that IL-10 has powerful anti-inflammatory effects, counteracting the degradation of the macrophages and changing the protease/anti-protease balance, favoring the preservation of EMV and the fibrous cover, giving the plaque stability.

Regulation of prothrombotic events: the role of IL-10

One of the important factors in the triggering of an acute coronary syndrome is arterial occlusion by a thrombus superimposed on a complicated plaque.[3,39,49,110,111] Among the triggers of intravascular thrombosis is the expression by endothelial cells and monocytes of the tissular factor (TF).[112]

This is 1 of the principal initiators of the coagulation cascade *in vivo*, by binding to factor VII and favoring its activation. The complex formed by TF/FVIIa later activates factors X and IX from the common coagulation pathway.

TF is not expressed in the cells under normal conditions, but as a response to various stimuli, endotoxine (LPS) being the most effective, but also to pro-inflammatory cytokines (IL-1, MCP-1, growth factor from PDGF platelets, and others).[113]

In 1993, Pradier et al[114] showed in *in vitro* experiments on isolated monocytes that IL-10 has and inhibitory effect on the expression of TF by the cells in response to the previously mentioned stimuli, that is transcriptional inhibition (mRNA).[115]

These findings were later confirmed by Landmark et al,[116] who observed that IL-10 maintained its inhibitory effect *in vivo* over TF expression by the monocytes when induced by LPS, also acting on mRNA. In another recent study on humans in whom endotoxemia was experimentally induced, IL-10 was shown to be capable of inhibiting the action of the coagulation system as well as attenuating fibrinolysis.[117]

All the preceding suggests that IL-10 could be very

useful in the treatment of some pathologies that present an increased risk of thrombosis due to monocyte procoagulant activity, such as DIC or ischemic heart disease.[116]

Experimental evidence of the protective role of IL-10 in atherosclerosis

IL-10 is cytokine that has an important regulatory effect on the immune response. Its capacity to inhibit the synthesis of cytokines and various cell functions of macrophages and T-lymphocytes converts IL-10 into a powerful anti-inflammatory agent. If we extrapolate this concept to atherogenesis, understood to be a chronic inflammatory disease of the vascular wall, we can hypothesize that this molecule could play a protective role in the pathogenesis of atherosclerosis.

Multiple experimental *in vivo* and *in vitro* animal studies have been performed, and the results support the theory of the protective role of IL-10, in the formation as well as in the stabilization of atherosclerotic plaque. Mallat et al[118] demonstrated that C573L/6J mice deficient in IL-10 (IL 10-/-) had increased susceptibility to the development of atherosclerotic lesions compared to wild mice (producers of IL-10, IL 10+/+). Also, on studying the composition of atherosclerotic plaques, it was observed that in the IL10-/-, mice there was a greater infiltration of inflammatory cells, increased IFN-γ (characteristic of the Th1 response) and a lower collagen content with respect the plaques of wild mice, findings that suggest that these plaques are more vulnerable or unstable with a higher tendency to rupture.[3] Later, on studying the effect of DNA transfer of IL-10 to the IL-10-/- mice fed an atherogenic diet they achieved a 60% reduction in the size of the atherosclerotic lesions. On the other hand, they proved that in IL-10-/- mice in an environment free of pathogens, the total atherosclerotic lesion surface was 4 to 5 times less than the IL-10-/- mice subjected to normal conditions, in spite of not finding differences in their lipidic profile. This data supports the theory of the intervention of pathogenic microorganisms in the development of atherosclerosis.[119]

In summary, this study demonstrates that IL-10 has a profound impact both on the development and the composition of atherosclerotic lesions, as well as a protective effect against environmental pathogen. These findings were later corroborated by Pinderski et al,[120] who demonstrated *in vivo* that transgenic IL-10 mice over-expressing IL-10 in T-cells, upon being fed an atherogenic diet showed a significant reduction in the development of atherosclerotic lesions as compared to wild mice or IL-10 deficient mice under the same conditions (Figure 3). It was also observed that the latter also developed larger lesions, with more inflammatory infiltrate, lipidic infiltrate, and a nearly imperceptible fibrous cover. In a parallel manner, the experiments *in vitro* of this group showed that pretreatment with IL-10 in these mice could inhibit the interaction of LDL-activated monocytes with the endothelium and their adhesion to same. This may be explained in part by the capability of IL-10 to inhibit the expression of adhesion molecules (VCAM-1, ICAM-1) by endothelial cells.

Similar results have been obtained in a recent study performed in humans that supports the hypothesis of the protective role of IL-10 in atherogenesis by contributing to the maintenance of plaque stability, avoiding acute events. Smith et al[121] demonstrated that patients with unstable angina had significantly lower amounts of IL-10 in their blood than those with chronic stable angina, suggesting that low IL-10 values are associated with greater clinical stability (Figure 4).

On the other hand, a significant liberation of IL-10 has been shown in multiple studies of myocardial ischemia-reperfusion and pulmonary bypass in humans.[122-124] Yang et al[125] observed in an experimental model of ischemia-reperfusion in IL 10-/- mice that they had an exaggerated inflammatory response in the reperfused tissues as compared to wild mice. This was revealed as an increase in neutrophil infiltration in the reperfused tissues and an increase in production of TNF-α, ICAM-1 and products of NO degradation, ultimately leading to an increase in the size of the AMI and myocardial necrosis as well as the mortality rate in the immundepressed mice.

These findings provide evidence that the endogenous production of IL-10 has protective effects against myocardial infarction and reperfusion damage by inhibiting the production of TNF-α, iNOS, the expression of adhesion molecules, and recruitment of neutrophils. There are currently many studies underway that suggest the potential use of IL-10 in various case of reperfusion damage.[126-131]

Other properties of IL-10: a new therapeutic agent?

Recently, anti-tumor properties have been associated with IL-10 related to its capability of decreasing the synthesis of VEGF (vascular endothelial growth factor), TNF-α and MMP-9 (92Kd gelatinase 92Kd), as well as in preventing the angiogenesis associated with tumor growth.[132,133] The main stimulus for angiogenesis is ischemia and inflammation, and both conditions are found narrowly related to ischemic illness.[134,135]

Given the anti-inflammatory properties attributed to IL-10, it is being studied as possible therapy for a great number of chronic diseases, including rheumatoid arthritis, inflammatory intestinal disease, multiple sclerosis, eosinophilic allergy, Wegener granulomatosis, cardiac transplant rejection, and others.[136] For example, the pathogenesis of inflammatory intestinal disease (Crohn´s disease and ulcerative colitis) is cha-

racterized by an unbalance in the activity of Th1 and Th2 lymphocytes, with a predominantly Th1 immune response, that cause a massive inflammatory response in the intestinal mucous. IL-10 is an anti-inflammatory cytokine that regulates the production of pro-inflammatory cytokines derived from Th1 lymphocytes, promoting a Th2 immune response, which is essential in the fight against inflammation.[137] The topical, intravenous, and subcutaneous use of recombinant human IL-10 (rhuIL-10) has been studied in humans with inflammatory intestinal disease.[138] While the results obtained by Fedorak et al[138] promoted the use of IL-10 by finding a clinical and endoscopic improvement in illness after the subcutaneous administration of rhuIL-10 in patients with Crohn´s disease, Colombel et al[139] were not able to demonstrate that the treatment prevented the endoscopic recurrence of the disease in post-operative patients with Crohn´s disease. The discrepancies in these results may be due to the difficulty in selecting a subgroup of patients who would potentially benefit from the biological therapy, as well as identifying the most appropriate way to administer the required dose.[140]

It was observed in experimental animal studies that IL-10 deficient mice spontaneously develop a severe form of enterocolitis.[141] Nevertheless, the transfer of CD4+ T-cells from transgenic IL-10 mice (which over express IL-10) is capable of overcoming, in IL10-/- mice, the development of colitis[142] in situations in which it would normally occur.

The results of the studies mentioned suggest that IL-10 should be considered a new therapeutic tool in the field of atherosclerosis treatment. Nevertheless, the administration of IL-10 as long-term therapy, with all its immunosupressor actions, may present unexpected consequences as it is able to potentially produce an antigen-specific energy. Different experimental studies have shown that IL-10 increases susceptibility to certain infections, principally those involving intracellular pathogens such as *Chlamydia* and *Listeria monocytogenes.*[136] The recombinant BALB/c mice stimulated with *Chlamydia* produced higher IL-10 values than the C57BL/6J wild mice. The BALB/c mice presented, as a consequence, a less aggressive inflammatory response against the *Chlamydia* infection, succumbing in a greater number than the C57BL/6J143 mice.

On the other hand, the IL 10-/- mice, deficient in IL-10, upon being infected with subtle doses of *Chlamydia*, developed less granulomatous lesions than wild mice.[144]

CONCLUSIONS

The current knowledge about the physiopathology of atherosclerosis has changed radically from that of the last decades. Today it is generally accepted that inflammation plays a fundamental role in the development and progression of atherosclerotic lesions, leading in the long or short term to the appearance of clinical signs. Nevertheless, the intrinsic mechanism by which this inflammatory response is triggered and develops continues to be clearly understood. The best knowledge of the physiopathological phenomenon underlying the process of atherogenesis will allow new investigative paths to be opened to combat the disease.

From this point of view, various studies performed to study the relevance of IL-10 in atherosclerosis suggest that it has a protective role limiting the local in-

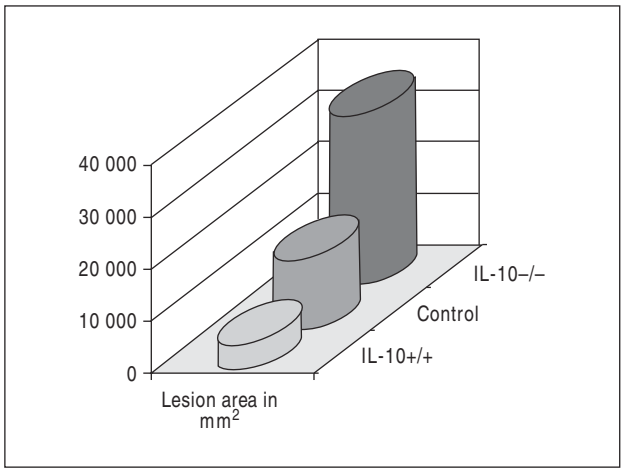

Fig. 3. Surface of the atherosclerotic lesion in the aortic root according to serum IL-10 values in wild mice (control), transgenic mice (IL10+/+), and IL-10-deficient mice (IL-10-/-). Observe the smaller surface of the lesion in the transgenic mice vs the controls (5.433±4.008 vs 13.574±4.212 mm^2; *P<.05). Note the marked increase in the lesion size of the IL-10-/- deficient mice vs the IL-10+/+ mice (33.250±9.117 mm^2; **P*<.0001) (modified from Pinderski O, et al[120]).

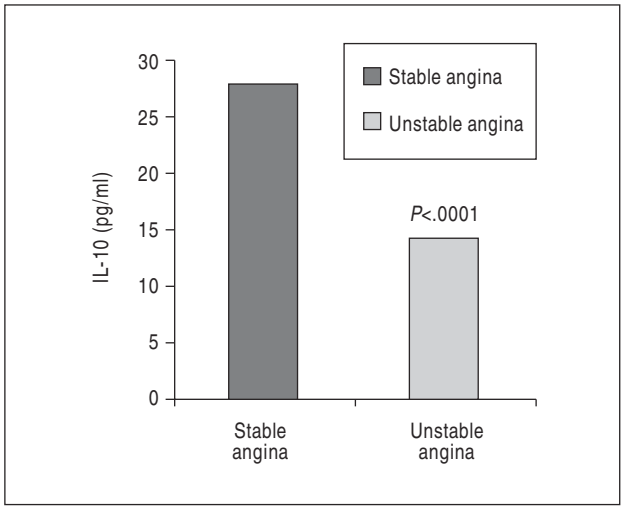

Fig. 4. Mean serum concentrations of IL-10 (pg/ml) in patients with unstable and chronic unstable angina (14±10.1 vs 28.43+12.1; *P*<.0001) (modified from Smith D, et al,[121]).

flammatory response, which favors the progression and instability of the atherosclerotic plaque, eventually leading to the development of acute coronary syndromes. This allows us to postulate the possible role of IL-10 as a therapeutic agent whose exogenous administration restrains the development of lesions and confers stability, improving the clinical course of the patient. IL-10 could also be a new risk marker that allows us to predict plaque instability and its propensity toward complications.

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