Review Article

The Role of $CD4^+CD28^{null}$ T-Lymphocytes and Statins in Rheumatoid Arthritis and Unstable Atherosclerotic Plaque

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During the past few years, evidence has accumulated that implies the presence and activation of mechanisms common to both atherosclerosis and rheumatoid arthritis. Points that support this correlation are the existence of systemic and chronic inflammatory reactions in both diseases, and the fact that the therapeutic approaches to both conditions have common denominators, such as the use of aspirin in recent years and the present use of statins.

The similarities between atherosclerosis and rheumatoid arthritis mainly stem from the activation of the cellular networks of the immune system. In both disorders, macrophages are activated to produce tumour necrosis factor alpha (TNF-α), interleukin (IL)-6 and matrix metalloproteinases, T-cells express more soluble IL-2 receptors, the ratio of T-helper lymphocytes (Th-cells), Th1/Th2, is shifted, and B-cell activation leads to induction of autoantibodies targeting oxidised low density lipoproteins (oxLDL) and heat shock protein (HSP). Furthermore, levels of C-reactive protein (CRP) and endothelin are heightened, adhesion molecules (VCAM-1, ICAM-1, E-selectin, P-selectin) are readily expressed, and neutral and lysosomal enzymes are locally secreted that disrupt collagen and similarly destabilise atherosclerotic plaques and articular structures.

The immunomodulatory effects of statins in various autoimmune diseases apply equally well to cardiovascular disease, in which a surprisingly similar immune dysregulation is observed. Indeed, the inflammatory component of atherosclerosis, characterised by increased production of interferon-γ by T-cells, has led immunologists to suggest that atherosclerosis should be added to the list of organ-specific autoimmune diseases.

A major challenge of contemporary medicine is to break the traditional compartmentalisation that frequently separates apparently different fields. This holds true for basic biochemical mechanisms as well as for the practice of clinical medicine. Unexpected connections between different areas of medicine may turn out to be of great interest. A link between molecular immunology and cardiology practice, as will be presented in this review, is an example of such a connection.

The present review highlights the role of a subpopulation of T-lymphocytes, the $CD4^+CD28^{null}$ cells, which has recently been implicated in the development of acute coronary syndromes in patients with unstable angina, and also in the increased cardiovascular morbidity and mortality of patients with rheumatoid arthritis. The results of studies on the administration of statins in these diseases and their immuno-

modulatory effects, which seem to be mediated via effects on T-cells, are discussed.

$CD4^+CD28^{null}$ T-lymphocytes

Activation of T-lymphocytes (T-cells) requires two signals from the antigen-presenting cells (APCs). The first signal is the attachment of the T-cell receptor to the antigen molecule, which is secured to an APC through a major histocompatibility (MHC) class II molecule through which antigenicity is provided. The second signal is mediated by costimulating molecules, of which the B7 protein family appears to be the most efficient.[1] The costimulative pathway of the B7 family includes at least two molecules present on APCs, B7-1 (CD80) and B7-2 (CD86), which respectively interact with the CD28 and CTLA-4 receptors on the surface of T-cells.[2]

Interaction of the CD28 receptor with the B7-1 molecule induces T-cell activation and clonal expansion.[3-7] On activation, naïve $CD4^+$ T-cells evolve into at least two different types of Th-cells: Th1 cells, which secrete IL-2, interferon-γ (IFN-γ) and TNF-α, and Th2 cells, which secrete the interleukins IL-4, IL-5 and IL-10. The type of Th-cell produced will determine the course of the immune response.[8] The Th1-cells mediate cellular inflammatory responses and tissue destruction and participate in the pathophysiology of autoimmune diseases, such as rheumatoid arthritis and inflammatory vascular disease.[9] The Th2-cells mediate humoral immune responses and simultaneously decrease the magnitude of the Th1-cell inflammatory response. Differentiation of immature $CD4^+$ T-cells into Th1 or Th2 is affected by the type of APC that interacts with them, the presence of costimulatory molecules, the intensity of the T-cell receptor stimulation and the cytokine profile during the first steps of immature $CD4^+$ T-cell stimulation.[8]

Although the CD28 receptor is consistently expressed on the T-cell surface, it has been noted that its expression decreases with chronic infection and with aging.[10-12] At ages over 65, about 50% of the total population of $CD4^+$ T-cells is composed of $CD4^+CD28^{null}$ cells.[13] The $CD4^+CD28^{null}$ T-cells are uncommon in healthy persons and may represent 0.1-2.5% of the total T-cell population.[14] In contrast, high counts of these cells are found in patients with unstable angina and rheumatoid arthritis with extraarticular manifestations.[15-18] In such cases they acquire inflammatory capacity and participate in the pathogenesis of autoimmunity, or else they take on the features of Th1-cells. The $CD4^+CD28^{null}$ T-cells that are isolated from patients with rheumatoid arthritis (RA) are autoreactive[18] produce large amounts of IFN-γ,[19] which is typical of the immune response of a Th1 cell.[20] The $CD4^+$ $CD28^{null}$ T-cells are characterised by the prevalence of the Th1 phenotype, are oligoclonal and derive from persistent antigenic stimulation. The antigens responsible for such long-lasting stimulation are probably from microorganisms that chronically infect a host or otherwise may be products of a chronic stimulation of the immune system, such as human HSP 60 or oxLDL.[15] The $CD28^{null}$ T-cells have a "memory phenotype" ($CD45RO^+$), are long-lived, create large oligoclonal populations, resist apoptosis and present features of an aged immune system.[12,21-23]

The killer immunoglobulin-like receptors (KIRs) are a family of receptors that recognise the MHC type I molecules and are usually expressed on the surface of natural killer (NK) cells.[24] The family of KIRs includes inhibitory and stimulatory receptors. Inhibitory KIRs, after linking to the proper MHC class I molecule, activate the SHP-1 phosphatase,[25] which in turn inactivates the stimulatory receptor signal transduction, thus inhibiting the NK cell cytotoxic function. This observation argues for the "missing-self" hypothesis, in which the function of inhibitory KIRs is to recognise self-MHC class I molecules and to prevent self-directed NK cell cytotoxicity.[26] This implies that inhibitory KIRs promote self-tolerance by depressing the immune response provoked by stimulatory receptors.[27] The stimulatory KIRs possess a transmembrane molecule (DAP12) that activates members of the Syk family of protein tyrosine kinases, resulting in extracellular signal-regulated kinase (ERK) activation, calcium mobilisation, and NK cell cytotoxicity.[28]

Apart from NK cells, KIRs are expressed almost exclusively on $CD4^+$ and $CD8^+$ T-cells that have lost CD28 molecule expression.[29] The $CD28^-$ T-cells that express KIRs are resistant to apoptosis and long-lived.[30] The gene expression profile in $CD28^-$ T-cells also includes other types of genes that are normally expressed on NK cells but not on T-cells.[31] The possibility exists of region-specific gene activation in $CD4^+$ $CD28^{null}$ T-cells. If this is correct, the functional phenotypes of $CD4^+$ $CD28^{null}$ T-cells in healthy individuals in patients with RA and in patients with acute coronary syndromes (ACS) could represent different stages of a differentiation program, which ranges from the circumstantial expression of KIRs in healthy individuals, to frequent expression in RA patients, to the co-expression of DAP12 and KIR in ACS patients.[32] An observation that supports this view is that patients with severe RA

have a high risk of developing vasculitis; the KIR2DS2 gene and its HLA-C ligand have been identified as risk factors for rheumatoid vasculitis.[33] The similarities in the immunoregulatory defects between patients with RA and ACS imply common pathogenetic mechanisms in these two syndromes.[34] Possibly, the expression of KIRs in T-cell subsets is predetermined and does not represent an untoward effect of cell aging or T-cell maturation. It has been shown that the transcriptive machinery of naïve T-cells is fully equipped to transcribe KIR genes, although it is different from that of NK cells.[35] These data support the hypothesis that the expression of KIRs in T-cells is controlled by epigenetic mechanisms and does not require complex alterations of transcription factors.

The $CD4^+CD28^{null}$ T-cells in unstable angina and rupture of the atherosclerotic plaque

The ACS, which include unstable angina and acute myocardial infarction, represent acute complications of coronary artery atherosclerosis.[32] The presence of a fragile site on the surface of the atheromatous plaque that is prone to disruption, and of an appropriate stimulus, lead to plaque rupture and initiation of an ACS.[36] Histological studies prove that activated T-lymphocytes and macrophages accumulate at the plaque shoulder, which is the point of least resistance to disruption.[37,38]

In regard to the role of T-lymphocytes in coronary artery disease, Liuzzo et al have shown the presence of $CD4^+CD28^{null}$ T-cells in a group of patients with unstable angina, while in patients with stable angina these cells were almost absent.[39] Clonal expansion of the $CD4^+CD28^{null}$ T-cells in patients with unstable angina is accompanied by production of IFN-γ and inflammatory activity. The activation of monocytes in the plaque microenvironment could be attributed to the secretion of IFN-γ by the $CD4^+CD28^{null}$ cells.[20,39] In turn, monocyte activation could promote the expression of acute phase proteins[40] that contribute to the pathogenesis of ACS.[41] The $CD4^+CD28^{null}$ cells represent inflammatory cells that are capable of causing tissue destruction, since they can migrate from the vascular circulation to the tissues.[17] The migration of $CD4^+CD28^{null}$ T-cells to the atheromatous plaques may cause a series of devastating events, such as the IFN-γ-induced activation of metalloproteinase-secreting macrophages, which results in the rupture and detachment of the fibrous cap of the plaque.[37,39,42] Apart from their capability to secrete IFN-γ, they also express the cytolytic proteins perforin and granzyme B,[43] which participate in the reduction of the thickness of the atheromatous plaque. Therefore, it is possible that the $CD4^+CD28^{null}$ cells directly contribute to the destabilisation and rupture of the atheroma in many ways.

The $CD4^+CD28^{null}$ T-cells of patients with ACS commonly coexpress stimulatory KIRs (CD158j) with the DAP12 molecule. The $CD4^+$ T-cells that coexpress CD158j and DAP12 promote cellular activation, bypassing the requirement of T-cell receptor signalling after exogenous antigen stimulation, and thus respond to the endogenous signals of their own microenvironment and to self-structures.[32]

The acquisition of CD158j and its signalling protein DAP12 offers cytolytic capability to the $CD4^+CD28^{null}$ T-cell. The $CD158j^+DAP12^+CD4^+$ T-cells have been found exclusively in patients with ACS.[32] Preliminary data concerning plaque T-cells collected during angioplasty show that $DAP12^+CD4^+$ T-cells gather at the unstable lesion. Signalling through CD158j will bypass the need for T-cell receptor signalling and induce endothelial cell lysis. It has been proposed that deviant expression of LRC-encoded genes on $CD4^+$ T-cells can break self-tolerance, a mechanism possibly contributing to T-cell-mediated damage of the atherosclerotic plaque.[32] Deviant expression of stimulatory KIRs obviously jeopardises self-tolerance.

The $CD4^+CD28^{null}$ T-cells in rheumatoid arthritis

Autoimmune responses induced by T-cells play an important role in RA.[44] Patients with RA with multiarticular involvement and extraarticular manifestations bear the $CD4^+CD28^{null}$ T-cell subset.[3] The suppression of CD28 nuclear transcription in RA may be due to exposure to chronically increased levels of TNF-α, since the administration of an anti-TNF-α therapy will restore the expression of CD28 receptor.[45] The presence of a significant number of $CD4^+CD28^{null}$ T-cells may divert the immune response to the activation of Th1 lymphocytes, the production of IFN-γ, and the involvement of protease-secreting macrophages that will disrupt the extracellular matrix and erode the inflamed joints in RA patients.[3] Also, it has been reported that these cells are characterised by cytolytic activity, due to the secretion of the proteins perforin and granzyme B.[31] A higher incidence of $CD4^+CD28^{null}$ cells corresponds to a higher risk of extensive articular erosion in patients with RA.[3]

RA can be correlated with the early development of atherosclerosis and increased cardiovascular mortality.[46,47] Although some conventional risk factors for

cardiovascular disease may be involved, it is believed that the chronic, systemic inflammatory reaction plays a predominant role in the development of accelerated atherosclerosis in RA patients. Typical atherosclerotic disease, which is also considered a chronic inflammatory disorder, presents many similarities to RA,[48] one of which is the detection of $CD4^+CD28^{null}$ cells in a subgroup of patients with RA.[15,16] Since the $CD4^+$ $CD28^{null}$ present potent inflammatory activity and a capability to induce tissue destruction, it has been proposed that they promote atherosclerotic processes and the development of cardiovascular disease in RA patients.[15,17,48]

In a study of patients with RA who lacked apparent cardiovascular disease, the population of $CD4^+$ $CD28^{null}$ cells was correlated with indices of preclinical atherosclerotic disease.[49] The group of patients with RA and clonal expansion of the $CD4^+CD28^{null}$ cells had disorders of flow-mediated vasodilation and increased carotid artery intima-media thickness, compared to the group of RA patients without circulating $CD4^+$ $CD28^{null}$ cells. This implies that the particular T-cell subset is partially involved in the development of early atherosclerotic lesions.[49]

The *de novo* expression of KIRs on $CD4^+$ T-cells was initially shown in patients with RA.[50] The $CD4^+$ $CD28^{null}$ T-cells of patients with RA express KIRs, with a preferential expression of the stimulatory receptor CD158j. Individuals carrying the *KIR2DS2* gene, which encodes the CD158j molecule, are at greater risk of developing RA-associated vasculitis.[33] The expression of stimulatory KIRs on $CD4^+CD28^{null}$ T-cells is related to autoimmunity, providing costimulatory signalling in the absence of professional APCs.[51] This form of costimulation seems to play a significant role in the pathogenesis of RA, in which the $CD4^+$ T-cells express various regulatory molecules that induce INF-γ production and T-cell proliferation.[52]

In T-cell clones of RA patients, it has been shown that CD158j selectively activates the c-Jun NH_2-terminal kinase (JNK) signalling pathway independently of the adaptor molecule KARAP/DAP12, after stimulation of the T-cell receptor.[28] Activation of the JNK pathway by CD158j led to activation of c-Jun, a component of the transcription factor AP-1, which is known to bind to the IL-2 promoter as well as to the IFN-γ promoter.[53,54] It has been proposed that, through this JNK pathway, the CD158j receptor induces inflammation in RA and ACS, by regulating the effector functions of $CD4^+CD28^{null}$ T-cells.[28] CD158j on cytotoxic $CD4^+$ T-cells does not induce or augment a cytotoxic T-cell response, raising the possibility that the signalling pathways of stimulatory KIRs in T-cells and NK cells are different.[28] This would explain why CD158j does not induce any calcium flux and cannot induce cytotoxic activity in $CD4^+CD28^{null}$ T-cells in RA.[55]

The KIR^+CD4^+ T-cells in RA and ACS have similar phenotypes and functional features, but are not the same. In RA, the $CD158j^+CD4^+$ T-cells are not cytotoxic after CD158j stimulation, and fail to express the DAP12 gene.[32,55] In contrast, in ACS the $CD4^+CD28^{null}$ T-cells possess both DAP12 and CD158j, thus being cytotoxic. In ACS, the cytotoxicity mediated by the KIR-expressing T-cells is in part responsible for smooth muscle cell apoptosis and plaque instability (Figure 1).[32]

The immunoregulatory role of statins

The family of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) inhibitors, known as statins, are used to control increased levels of cholesterol, with a proven clinical benefit in primary and secondary prevention of cardiovascular disease.[56] However, statins may also prove beneficial in the treatment of autoimmune disorders.[57] It has been reported that the administration of pravastatin to heart transplant recipients was associated with a significant decrease in organ rejection cases and increased survival, independently of its action on blood cholesterol levels.[58] Since then, data have accumulated concerning the anti-inflammatory and immunosuppressive function of statins.

Statins bind to the HMG-CoA reductase, leading to competitive displacement of the natural substrate,

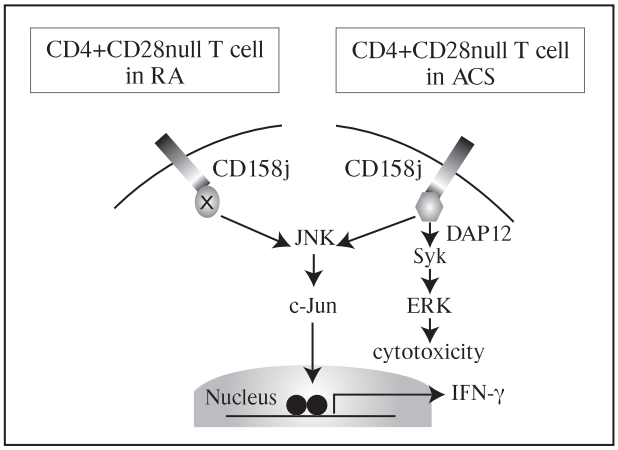

Figure 1. The functional and structural differences in $CD4^+CD28^{null}$ T-cells between rheumatoid arthritis (RA) and acute coronary syndromes (ACS). Further details provided in text.

HMG-CoA, thereby inhibiting its catalytic conversion to L-mevalonate.[59] Mevalonate is a precursor not only to cholesterol synthesis, but also to intermediary lipid donors that are important for the isoprenylation of GTP-binding proteins, such as RhoA and RhoB.[60] These small GTPases require lipid modifications for membrane tethering and subsequent interaction with downstream effector cascades. Only then are they capable of regulating cellular functions, which include cell survival, proliferation, differentiation and cytoskeletal organisation.[61] As a result of their isoprenylation requirements, GTPases are targets of statin therapy.

The mechanisms through which statins exert their immunosuppressive activities are set out in the following paragraphs.

Anergy of T-cells

Anergy imposes immune tolerance by rendering autoreactive cells functionally inactive. It was found that an induction of T-cell anergy following the *in vitro* application of atorvastatin was accompanied by an induction of IL-10 gene expression in peripheral immune cells, which could be responsible for the accumulation of the anergy factor $p27^{Kip1}$.[62,63] The negative cell cycle regulator $p27^{Kip1}$ plays a central role in the blockade of clonal expansion and represents a critical downstream target of anergy induced by blockade of costimulatory pathways.[64]

Immunohistochemistry has suggested an inverse correlation between $p27^{Kip1}$ expression and the proliferation of macrophages and vascular smooth muscle cells (VSMCs) within human atherosclerotic tissue.[65] Also, $p27^{Kip1}$ is abundantly expressed in intimal and medial VSMCs of non-atherosclerotic human coronary arteries.[66] Thus, the growth suppressor $p27^{Kip1}$ functions as an important regulator of the phenotypic response of VSMCs to mitogenic and hypertrophic stimuli, both *in vitro* and *in vivo*. In addition, p27 is reported to be a negative regulator of migration.[67] Indeed, $p27^{Kip1}$ mediates the antiproliferative and antimigratory activity of sirolimus-coated stents.[68] One downstream effect of Rho is to reduce the expression of cell cycle inhibitors such as $p27^{Kip1}$, leading to acceleration of cell cycle progression and migration.[69] Conversely, cellular levels of $p27^{Kip1}$ are increased when Rho function is inhibited by treatment with statins.[70] Collectively, agents that increase $p27^{Kip1}$ levels in vivo, such as statins, may have both an antiproliferative and an anti-migratory effect.

Inhibition of apoptosis

Activated T-cells can induce apoptosis in resident and infiltrating cells of atherosclerotic plaques by the action of the Fas-Fas ligand (FasL) system. Fas is a type I membrane protein, belonging to the tumour necrosis factor receptor family, that induces a death signal when bound to FasL.[71] FasL expression has been reported in activated T-cells.[72] Fas is expressed ubiquitously, and expression of Fas and FasL has been detected in the diseased vessel wall.[73] It has been proposed that Fas-mediated apoptosis of resident and infiltrating cells of these vascular lesions is a feature of atherogenesis and of atherosclerotic plaque instability.[74,75]

Treatment with atorvastatin downregulated FasL expression in activated T-cells.[76] The physiological importance of FasL downregulation by atorvastatin is attributed to the decreased cytotoxic activity of T-lymphocytes upon target cells. The reduction of the cytolytic effect of T-lymphocytes on target cells may contribute to the maintaining of cellularity in the lesions, favouring the stability of the plaque.[76] Also, it has been observed that treatment with statins decreases FasL expression and apoptotic cells in human carotid plaques.[77]

Suppression of Th1 immune responses

Major histocompatibility complex (MHC) class I and class II molecules play an important role in immune surveillance.[78] MHC-II molecules, expressed on the surface of specialised cells, are directly involved in the control of the immune response. Whereas a limited number of specialised cell types express MHC-II constitutively, numerous other cells become MHC-II positive on induction by the inflammatory mediator IFN-γ. This complex regulation is under the control of the transactivator CIITA, the expression of which is tightly regulated under distinct physiological conditions.[79] The MHC-II molecules play a central role in the initiation of the cellular and humoral immune responses and have also been implicated as contributing factors for a variety of autoimmune disorders.

Among the first reports of the immunologic effects of statins was the finding that they inhibit the increase in cell-surface MHC-II proteins induced by INF-γ.[80] Statins effectively repress the induction of MHC-II expression by IFN-γ in a dose-dependent manner.[80,81] This effect of statins is specific for MHC-II and does not affect MHC class I expression.

Statins inhibit IFN-γ-inducible MHC-II expression

on macrophages and block lymphocyte function-associated antigen-1 (LFA-1)-dependent stimulation of T-cells, both of which might suppress the activation of proinflammatory Th1 cells.[80,82] Treatment with atorvastatin prevents and reverses experimental autoimmune encephalomyelitis via suppression of Th1 and augmentation of Th2 immune responses.[63] Simvastatin has also been reported to inhibit the Th1 response in a murine model of RA.[9] In these studies, the beneficial effects of statins on autoimmune diseases were attributable to the suppression of Th1 response. On the other hand, statins have been reported to downregulate the expression of MHC-II, CD40, CD80 and CD86 on APCs induced by IFN-γ.[80] Statin treatment reduced the expression of CD40 on atheroma-associated cells *in vitro*, as well as on atherosclerotic lesions *in situ* in patients treated with statins.[83] CD40 and its ligand CD154 have been implicated in several crucial immunological pathways. These results provide a rationale for suggesting their use as immunosuppressors in various autoimmune diseases, including rheumatoid arthritis, and chronic inflammatory diseases, such as atherosclerosis.[81]

The association between cholesterol levels and immunologic regulation may be closer than previously realised. Cholesterol is a key component of the structure and function of cell membranes. The response of lymphocytes to exogenous signals such as antigen is orchestrated by a number of molecules that cluster in cholesterol-rich areas of the cell membrane known as lipid rafts. Lipid rafts bring together molecules essential for the activation of immune cells, but also separate them when the conditions for activation are not appropriate. Several strands of evidence suggest that the inhibition of cholesterol synthesis by statins disrupts these lipid rafts and thereby influences the function of lymphocytes.[84]

Statins in rheumatoid arthritis

Cytokines that mediate the inflammatory mechanisms in RA could partially account for the increased risk of vascular disease in these patients, through the induction of endothelial dysfunction and/or insulin resistance.[85] The existing data support the hypothesis that statins could prove beneficial for the therapy of RA patients, by suppressing these inflammatory mechanisms.[80] The benefit of statins seems to be greater when high values of CRP are detected.[87] Statins may decrease blood levels of CRP, which represent an independent risk factor for cardiovascular disease,[88] and suppress the production of IL-6.[9] A chronic increase in IL-6 could promote atherogenesis directly, through effects on the vascular wall, and indirectly, through development of insulin resistance.[89]

Arterial stiffness is an indicator of vascular dysfunction and an independent risk factor for cardiovascular disease.[90] After administration of atorvastatin to patients with RA, a significant decrease of arterial stiffness was observed. The greatest decrease in arterial stiffness was observed in the patients with the most intense activity of RA disease.[9] The immunomodulatory properties of statins may prove important in the cardiovascular complications of patients with RA – even if they have low levels of cholesterol – in particular in those patients with extraarticular manifestations of RA and high levels of CRP.[91]

Simvastatin provides a highly effective therapy in treating murine collagen-induced arthritis, a surrogate model for human RA. This drug can suppress the progression of acute to chronic inflammation *in vivo*, and was effective even after the onset of arthritis. *Ex vivo* analysis demonstrated a significant suppression of collagen-specific Th1 humoral and cellular immune responses and a reduction of IFN-γ release from mononuclear cells derived from peripheral blood and synovial fluid.[9]

Some studies have shown the important role of the matrix metalloproteinases (MMPs), particularly MMP-3, that are produced by chondrocytes, during the process of cartilage catabolism and degradation in joint diseases.[92] Recent studies have demonstrated that some statins inhibit MMP production both *in vitro* and *in vivo*.[77,93,94] Bellosta et al have demonstrated that statins reduce MMP-9 production by cultured macrophages.[94] Simvastatin inhibits MMP-3 production from cultured human chondrocytes stimulated with IL-1β, and from unstimulated cells.[95] These effects of statins could provide an additive cartilage-protective mechanism in joint diseases. It is reasonable to assume that statins may play a modulatory role in chronic inflammatory conditions, such as rheumatic diseases.[96,97]

The effects of statins on the atheromatous plaque

Vulnerable positions of the atheromatous plaque contain large amounts of lipid, a high proportion of activated inflammatory cells, increased expression of MMPs, low VSMC contents and increased VSMC apoptosis.[98] Plaque stability depends upon the VSMC content, as only these cells are capable of synthesising the structurally important collagens I and III. Increasing evidence indicates that apoptosis of VSMCs promotes pla-

que instability. Higher levels of VSMC apoptosis are seen in plaques compared with normal vessels,[99,100] In contrast, inflammatory cells (particularly macrophages) release MMPs that degrade collagen and extracellular matrix, potentially weakening the plaque. The quality of the plaque, rather than its absolute size, determines the likelihood of rupture. Fatal events in atherosclerosis are due to rupture of the thinnest part of the fibrous cap region, which is synthesised almost exclusively by VSMCs, and rupture sites are characterised by a relatively low density of VSMCs.[98]

Statins increase collagen content and decrease lipid content, inflammation, MMPs, and cell death in human carotid artery plaques.[101,102] The preoperational administration of pravastatin changed the structure of the unstable atheromatous plaque, by significantly lowering the local concentrations of oxLDL, MMP-2, T-cells and macrophages, while increasing collagen content and the inhibitory factors to MMPs.[98] Angiographic trials have indicated that statins produce little if any regression of established lesions.[103,104] Thus, the evidence suggests that statins may change the characteristics of the plaque to stabilise fragile, moderate sized plaques, with little reduction in plaque volume.

As has been mentioned previously, statins inhibit the inflammatory actions of Th1 cells. This effect, in addition to the interruption of the CD40/CD40-activator system, reveals a specific mechanism for manipulating T-cell-induced inflammation, which in turn is a cornerstone event in activating the atherosclerotic plaque and in the transition from stable to unstable prethrombotic condition.[105]

It is evident that activation of plaque rather than lumen stenosis precipitates ischaemia and infarction.[106] The activation of plaque seems to be associated with its increased temperature. Indeed, in patients with ACS an inflammatory activation is observed with increased local atherosclerotic plaque temperature.[107] This is associated with plaque containing more macrophages, fewer VSMCs, and greater MMP-9 activity.[108] Moreover, the administration of statins was shown to be associated with a reduction of local plaque thermal heterogeneity. The possible mechanism is the reduction of the macrophage content of atherosclerotic plaque due to their pleiotropic effects. Statins have a beneficial effect on plaque temperature post-myocardial infarction, as well as in patients with ACS and type 2 diabetes.[109-111] This favourable effect was also found in patients with diabetes, independently of the levels of LDL.[111] A very recent study has shown that statins reduce the numbers of $CD4^+$ $CD28^{null}$ cells, which provides a link to explain the beneficial effects of statins in these disorders.[112]

Cytokines and the induction of atherosclerosis

Cytokines (including TNF-α, IFN-γ, the various interleukins and TGF-β) mainly promote vascular disease through their pleiotropic immunoregulatory and metabolic effects. The sustained increase of cytokine levels, independently of its magnitude and aetiology, facilitates atherogenesis by aggravating risk factors such as lipoprotein metabolism disorders, insulin resistance and endothelial dysfunction. Indeed, a low-grade chronic inflammatory response in population studies correlates with many classic and novel risk factor pathways for CHD.[85]

Cytokines regulate the expression of a number of inflammatory molecules, leading to destabilisation and finally rupture of vulnerable atheromatous plaques. They also participate in the pathophysiology of ACS by direct effects on myocardial contractility and apoptosis. At a clinical level, circulating cytokines also have a prognostic role, since they are useful markers predicting future coronary events in patients with advanced atherosclerosis and in patients after ACS.[113]

Conclusions

The present review has attempted to present evidence that would correlate $CD4^+CD28^{null}$ cells with rheumatoid arthritis and unstable atherosclerotic plaque, as well as providing data concerning the effects of statins on these diseases. The beneficial effects of statins in these disorders could be linked to the reduction in the numbers of $CD4^+CD28^{null}$ cells, providing a further link between these conditions. Such findings represent leads in the research to define the molecular mechanisms of autoimmune disease pathogenesis and also argue for the use of statins to depress inflammatory processes in rheumatoid arthritis and coronary artery disease, regardless of the lipidaemic profile.

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