Journal Pre-proof

Regulatory T cells: possible mediators for the anti-inflammatory action of statins

Sanaz Keshavarz Shahbaz, Mahvash Sadeghi, Khadije koushki, Peter E. Penson, Amirhossein Sahebkar

PII: S1043-6618(19)31177-6

DOI: https://doi.org/10.1016/j.phrs.2019.104469

Reference: YPHRS 104469

To appear in: *Pharmacological Research*

Received Date: 24 June 2019

Revised Date: 26 September 2019

Accepted Date: 27 September 2019

Please cite this article as: Shahbaz SK, Sadeghi M, koushki K, Penson PE, Sahebkar A, Regulatory T cells: possible mediators for the anti-inflammatory action of statins, *Pharmacological Research* (2019), doi: https://doi.org/10.1016/j.phrs.2019.104469

Regulatory T cells: possible mediators for the anti-inflammatory action of statins

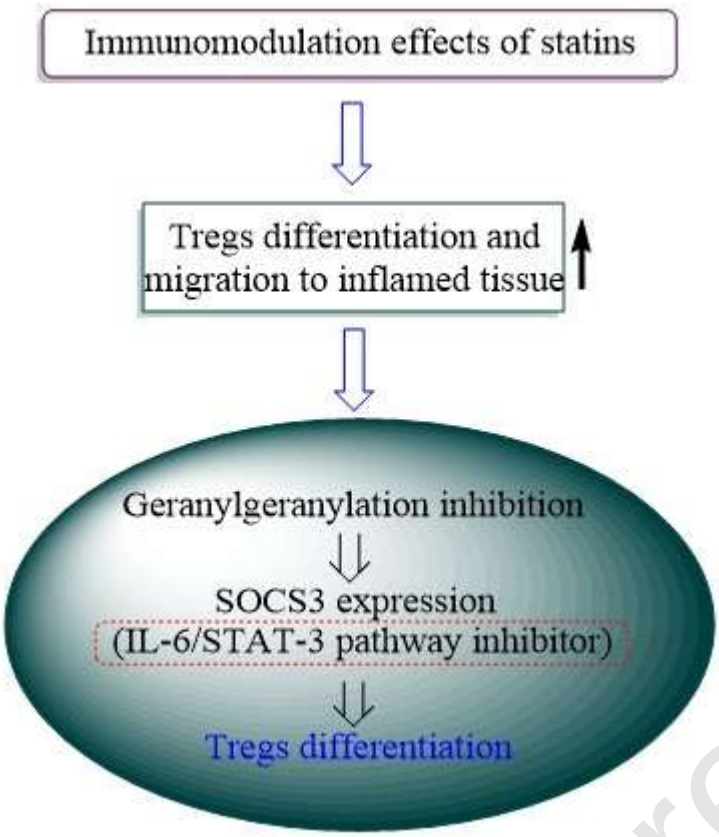
Running title: Statins and Tregs

Sanaz Keshavarz Shahbaz[1], Mahvash Sadeghi[1], Khadije koushki[1], Peter E. Penson[2], Amirhossein Sahebkar[3,4,5*]

[1]*Department of Immunology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
[2]*School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Liverpool, UK*
[3]*Halal Research Center of IRI, FDA, Tehran, Iran*
[4]*Biotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran*
[5]*Neurogenic Inflammation Research Center, Mashhad University of Medical Sciences, Mashhad, Iran*

***Corresponding author**:
Amirhossein Sahebkar, PharmD, PhD, Department of Medical Biotechnology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran, P.O. Box: 91779-48564, Iran. Tel: 985118002288; Fax: 985118002287; E-mail: sahebkara@mums.ac.ir; amir_saheb2000@yahoo.com

Graphical abstract



Abstract

Statins beside their main effect on reducing the progression of cardiovascular disease through pharmacological inhibition of the endogenous cholesterol synthesis, have additional pleiotropic effects including antiinflammatory effects mediated through the induction of suppressor regulatory T cells (Tregs). Statin-induced expansion of Tregs reduces chronic inflammation and may have beneficial effects in autoimmune diseases. However, statins could represent a double-edged sword in immunomodulation. Drugs that act by increasing the concentration of Tregs could enhance the risk of cancers, particularly in the elderly and may have adverse effects in neurodegenerative disorders and infectious diseases. In the present paper, we review the experimental studies that evaluate the effects of statins on Treg cells in autoimmune and inflammatory diseases and we discuss potential therapeutic applications of statins in this setting.

Key words: Statins; Treg; Immune system; Autoimmunity

Abbreviations

ACS	Acute coronary syndromes
APC	Antigen presenting cells
ApoE	Apolipoprotein (a)
ATP	Adenosine triphosphate
ATRA	All-Trans Retinoic Acid
BALF	Bronchoalveolar lavage fluid
BCG	Bacille Calmette–Guérin
BMDC	Bone marrow-derived DCs
CNS	Central nervous system
CRP	C-reactive protein
DTH	Delayed-type hypersensitivity
EAE	Experimental autoimmune encephalomyelitis
EAMG	Experimental autoimmune myasthenia gravis
ERK	Extracellular signal-regulated kinases
FP	Fluticasone propionate
FPP	Farnesyl pyrophosphate
GARP	Glycoprotein A Repetitions Predominant
GBM	Glomerular basement membrane
GGPP	Geranylgeranyl pyrophosphate
HFFD	High Fat Fructose Diet
HIV	Human immunodeficiency virus
HMG-CoAR	Hydroxy methyl glutaryl coenzyme A reductase
IDO	Indoleamine 2,3-dioxygenase
IPEX	Immune dysregulation, poly endocrinopathy and enteropathy, X-linked syndrome
IRI	Ischemia-reperfusion injury
LDL	Low-density lipoprotein
MBP	Myelin basic protein
MHC	Major histocompatibility complex
MOG	Myelin oligodendrocyte glycoprotein
MS	Multiple sclerosis
NK	Natural Killer (cells)
PBMC	Peripheral blood mononuclear cells
PCI	Percutaneous coronary intervention
PMI	Periprocedural myocardial infarction
RA	Rheumatoid arthritis
ROCK	Rho Associated kinase
ROR	Related Orphan Receptors
RRMS	Relapsing-remitting multiple sclerosis
SCID	Severe combined immunodeficiency

STEMI	ST-Segment Elevated Myocardial Infarction
TNF	Tumour necrosis factor
TolDC	Tolerogenic dendritic cell

Introduction

Statins are amongst the most commonly used medicines in preventative cardiology. In addition to their well-documented lipid-lowering effects meditated through inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A, considerable evidence suggests that additional 'pleiotropic' effects may contribute to the anti-atherosclerotic effects of these drugs [1-6]. In particular, anti-inflammatory effects mediated through the induction of suppressor regulatory T cells (Tregs). Statin-induced expansion of Tregs reduces chronic inflammation and may have beneficial effects in autoimmune diseases. The aim of this review is to describe the experimental studies which have evaluated the effects of statins on Treg cells in autoimmune and inflammatory diseases and to discuss potential therapeutic applications of statins in this setting.

The effects of statins on cholesterol and vascular health.

Cholesterol is a fundamental component of cell membranes and a chemical precursor of steroid hormones and bile acids [7]. Nevertheless an elevated circulating concentrations of cholesterol (particularly that contained within apolipoprotein-B containing lipoproteins such as low-density lipoprotein (LDL)) is an important risk factor for atherosclerotic cardiovascular disease, an observation which has been repeatedly documented in epidemiological studies such as the Framingham Heart Study [8, 9] and the Multiple Risk Factor Intervention Trial [10, 11]. Atherosclerosis is characterized by the deposition and oxidation of cholesterol-rich lipoproteins in the vascular wall, disrupting endothelial function and ultimately leding to the generation of plaques which obstruct blood flow and are vulnerable to rupture, precipitating intravascular thrombosis leading to myocardial infarction and ischemis stroke. The liver enzyme HMG-CoA reductase is the rate-limiting step in the biosynthesis of cholesterol by the mevalonate pathway [7]. This enzyme catalyzes the conversion of HMG-CoA to mevalonic acid, a precursor of cholesterol [12].

Statins reversibly inhibit HMG-CoA reductase through side chains that bind to the active site of the enzyme and prevent the substrate-product transition state of the enzyme [13].

The vascular endothelium is an essential autocrine and paracrine mediator, which modulates the contractile tone and cellular composition of the vascular wall. Endothelial function is impaired in hypercholesterolemia, and endothelial dysfunction is one of the earliest indications of atherosclerosis, occurring before angiographic evidence of disease becomes evident [14, 15]. Endothelial function is influenced by the impaired production, release, and activity of endothelial-derived nitric oxide (NO). Endothelial NO has a variety of beneficial effects in the context of athrosclerosis, such as promoting vascular smooth-muscle relaxation [16] and inhibiting platelet aggregation [17], vascular smooth muscle proliferation [18], and the interactions of endothelial cells and leukocytes [19, 20]

In addition to their major role in reducing serum cholesterol concentration, statins exert a variety of potentially beneficial including as immunoregulatory, anti-inflammatory, and neuroprotective agents [21] which are commonly described as pleiotropic effects [22-27] (**Figure 1**). Statins stimulate and upregulate endothelial NO synthase (eNOS) [28, 29] and have been shown to restore eNOS activity during conditions which have adverse effects upon endothelial function, including hypoxia [30] and the presence of oxidized LDL [28]. Elevation of tissue-type plasminogen activator expression [31] and the suppression of endothelin-1, a potent vasoconstrictor and mitogen, have been noted following statin administration in vitro [32]. Statins enhance the number of circulating endothelial progenitor cells and stimulate their proliferation, migration, and survival [33] and hence induce angiogenesis [34].

Statins confer beneficial effects by inhibiting vascular smooth muscle proliferation; this is accomplished by the downregulation of DNA synthesis, mediated by the inhibition of platelet-derived growth factor and RhoA [35, 36]. The result is an improvement in the stability of atherosclerotic plaques stability, rendering them less likely to rupture. This occurs as a result of alterations to the physiochemical features of the lipid core and reduction of the size of the plaque, together with reduced circulating lipid concentrations [37, 38].

Anti-inflammatory and immunoregulatory effects of statins

Statins have been shown to reduce vascular inflammation, as demonstrated by a reduction in the number and activity of inflammatory cells [39] and decreased circulating concentrations of c-reactive protein (CRP) level [40, 41]. This is achieved in part by statin interference with endothelial adhesion and leukocyte migration to sites of inflammation [42]. Suppression of HMG-CoA reductase has been shown to reduce all mevalonate pathway products. Nonsteroidal isoprenoid compounds, such as geranylgeranyl pyrophosphate (GGPP) and farnesyl pyrophosphate (FPP), are important lipid mediators for intracellular signaling molecules, such as Rho, Rac, and Cdc42. In addition to being a precursor for cholesterol, mevalonate is necessary for the synthesis of these nonsteroidal isoprenoid compounds (**Figure 2**). Protein farnesylation and geranylgeranylation are catalyzed by farnesyltransferase (FTase) and geranylgeranyl transferase (GGTase), respectively [43-45]. Statin administration leads to *in vivo* consumption of GGPP and FPP which both have a central role in posttranslational prenylation of several important cell signaling proteins associated with inflammation [46]

Statins have been demonstrated to modulate the immune system (**Figure 3**) is by interfering with the maturation and function of dendritic cells (DCs) [47-49]. Statins inhibit the expression of MHC-II, induced by cytokine and co-stimulatory molecules of antigen presenting cells (APC), and prevent antigen presentation to CD4 T cells. Furthermore, statins inhibit Th1 differentiation by the inhibition of STAT-4 and T-bet and enhance secretion of the anti-inflammatory Th2 cytokine through STAT-6 and GATA-3 activation. Hence, statins suppress the secretion of pro-inflammatory cytokines. Therefore, statins can alter the T-cell profile by changing the Th1/Th2 balance [50].

Concerning the immune modulatory effects of statins, initial studies show that statins suppress autoimmune diseases by preventing Th1 cell differentiation. The severity and duration of both active and passive experimental autoimmune encephalomyelitis (EAE) have been shown to reduce after atorvastatin administration in mice [51, 52]. The results of a small human open-label trial showed that oral administration of simvastatin reduced lesion severity in patients with multiple sclerosis (MS) [53]. It has also been reported that atorvastatin reduced rheumatoid arthritis (RA) disease severity scores [54]. More recently, it has been shown that the development of Th17 cells is blocked by statins [55] and that statins may also suppress the differentiation of Th17 cells. Zhang et al. have recently reported that simvastatin reduces the expression of IL-17A and its transcription factor, Retinoic Acid-Related Orphan Receptors (RORs) that regulates IL-17A production in human CD4+ T cells [55].

An *in-vitro and in-vivo* study performed by Mira et al. demonstrated that that lovastatin exert effects on the regulatory T cells CD4+ and CD25+, potentially increasing the number of cells, migration towards inflamed tissues and enhancing their suppressive function through induction of the transcription factor, FOXP3 [56]. It has also been demonstrated that *in vitro* treatment of

atorvastatin in a dose-dependent manner (2 and 10 M) decreased the number and function of these CD4+CD25+ cells. However, the findings were not replicated in an *in vivo* mouse model [57]. Geranylgeranylation inhibition is assumed to be the mechanism that leads to the expression of SOCS3 (suppressor of cytokine signaling 3), an IL-6/STAT-3 pathway inhibitor, which promotes the differentiation of T cells towards Tregs [58]. Consequently, it is reasonable to conclude that Treg modulation is one aspect of the pleiotropic effects of statins.

Impact of statins on the microenvironment required for Treg cell differentiation

Regulatory T cells (Tregs) are anti-inflammatory lymphocytes which regulate immune responses. FoxP3 is the main transcription factor of the CD4+ Treg subset. These cells work through various mechanisms to inhibit the proliferation and activation of proinflammatory leukocytes [59, 60]. According to recent studies, during IL-6 signaling, STAT-3 activation leads to the suppression of Foxp3 transcription; therefore, statins could indirectly increase Foxp3 expression inhibiting IL-6 signaling and consequently increase the number of Tregs [61]. IL-6 has various roles in modulating responses of the immune system. The initial effects of IL-6 increased expression of chemoattractant, for example, MCP-1, and IL-6/sIL-6R engagement mediated via monocyte chemotaxis. Several studies have demonstrated that statins suppress MCP-1 expression in cultured human aortic endothelial cells and thereby inhibited monocyte chemotaxis. Statins inhibit the progression of inflammation by suppression of prenylation and the phosphorylation of numerous important cell signaling proteins such as JAK1, JAK2, TYK2, STAT1, and STAT3, and by inhibiting the translocation of STAT3 IL-6/sIL-6R to the nucleus. Consequently, statins efficiently block the effects of IL-6 by attenuating prenylation of STAT3, an important downstream signaling molecule in the IL-6 receptor pathway [62]. Statins may play a major role in modulating antigen

presenting cells (APC) and thereby promote Treg development. Chen et al. showed that atorvastatin-modified dendritic cells pulsed with myelin oligodendrocyte glycoprotein (MOG) autoantigen had a beneficial effect on EAE, consequent upon their steady semimature phenotype which included a low level of costimulatory molecules and proinflammatory cytokines. These DCs have a tolerogenic phenotype (TolDCs). Intraperitoneal injection of TolDCs pulsed with MOG (TolDCs-MOG) into EAE mice significantly reduced the disease severity and modulated the balance of Th17/Tregs, with a significant reduction in Th17 cells and an increase in the number of Tregs [63]. Li et al showed that modification of DCs with atorvastatin *in vitro* could shift DCs towards the tolerogenic DC (TolDC) phenoype. Their results demonstrated that the levels of CD86 and MHC-II on the endogenous DCs are decreased after atorvastatin modification, which could turn Th1/Th17 into the Th2 response and increase Treg cell production during the progress of experimental autoimmune myasthenia gravis (EAMG) [64]. In experimental autoimmune neuritis, Xu et al reported that central and peripheral immune tolerance could be induced by atorvastatin-modified DCs to suppress disease development. During central tolerance, T cells differentiate into Treg cells after administration of atorvastatin-modified DCs; during peripheral tolerance, atorvastatin-modified DCs suppressed the proliferation of CD4+ T cells to regulate the immune response via reduced co-stimulatory molecules and MHC class II, decreased secretion of Th1 and Th17 cytokines, diminished proinflammatory cytokines including IFN-γ, TNF-α, and IL-17A, and increased Treg numbers *in vivo* [65]. Statin-Dex (statin-DCs-derived exosomes) *in vivo*, showed higher levels of IDO (indoleamine 2,3-dioxygenase) than control-Dex. Statin-Dex with high levels of IDO may expand Treg cell number and/or increase function while also being involved in modulating the immune system. In line with the hypothesis, administration of statin-Dex to EAMG

rats significantly increased the frequency of Foxp3+ cells in the thymus and the population of CD4+Foxp3+ T cells in the lymph nodes [66].

Statins increase the development of Treg cells

A large body of evidence supports the function of statins as not only having anti-inflammatory effects but also promoting Treg development. The cardiovascular protective effects of statins may occur by reducing the progression of atherosclerosis by modulation of lipoprotein metabolism, in which Tregs play a central role [67]. Klingenberg et al. reported that Treg depletion results in reduced clearance of large lipoprotein particles. This leads to increased levels of plasma cholesterol and, consequently, accelerated atherosclerosis [68]. The *in vitro* culture of mice and human peripheral blood mononuclear cells (PBMCs) with rosuvastatin and atorvastatin [69, 70] demonstrated the direct induction of Tregs by statins.

Short-term atorvastatin treatment in patients with acute coronary syndromes (ACS) *in vivo* resulted in an increased number of Treg cells and a reduction in T cell proliferation. Concurrently, pro-inflammatory cytokines including IFN-γ and CRP were reduced and, by contrast, anti-inflammatory cytokines (IL-10 and TGF-β1) were considerably elevated [71]. It is not known how atorvastatin influences Treg development and function. Since immature DCs can induce differentiation of Tregs, atorvastatin could affect Tregs by suppression of DC maturation [72].

Xiao et al. confirmed that simvastatin treatment resulted in increased expression of Foxp3 and accumulation of Tregs in atherosclerotic lesions of ApoE knockout mice. Moreover, simvastatin treatment increased IL-4 mRNA and protein expression, and reduced IL-1β, IFN-γ and IL-17 expression in carotid plaques and also elevated Treg number and inhibitory function in patients

with acute coronary syndrome. Accordingly, Treg activation by statins may attenuate atherosclerotic plaque development [73].

The Treg number in heart transplant recipient rats was reported to be significantly increased by treatment with a combination of simvastatin and aspirin. This combination therapy also decreased vascular damage, delayed the expansion of pathological changes in the myocardium and prolonged the maintenance of the cardiac allograft. The mechanism of this benefit was thought to be related to the induction of immune tolerance via Treg cells and improved vascular endothelial cell protection [74].

Rodriguez-Perea A et al. evaluated *in vitro* the function of Tregs (expression of Treg functional markers, activation levels, cytokine secretion and calcium flux) after treatment with atorvastatin at 1 or 10 μM concentration. They reported that the higher dose of atorvastatin hindered the suppressive function of Tregs and also diminished the FoxP3, PD-1, and CTLA-4 expression.[75]

It has also been demonstrated that the prophylaxis of an allergic asthma mice models by intraperitoneal injection oftorvastatin (40 mg/kg) for 7 or 15 days increased the frequency of Tregs in mediastinal lymph nodes (MLN) and the interleukin (IL)-10 in lungs. Also, the specific IgE in the serum and Th2 cytokines in the lungs were decreased, resulting in a reduction of peribronchial inflammation during allergic asthma.[76]

It has been reported that atorvastatin *in vivo* and also *in vitro* significantly increased T reg numbers and suppressive functions in a dose-dependent manner, which resulted in reducing the clinical disease activity in patients with rheumatoid arthritis.[77]

Administration of rosuvastatin before an episode of ischemia-reperfusion injury (IRI), enhanced the accumulation of IR-induced Tregs in the heart and spleen *in vivo*. Myocardial necrosis was alleviated, related to a reduced rise in circulating concentrations of cardiac troponin I (cTnI). This

effect was reversed by co-treatment with mevalonate. Moreover, there was a strong negative correlation between inflammatory cell accumulation (the levels of FoxP3 protein) and the number of Tregs in the myocardium. This study was the first to demonstrate the cardioprotective effect of Tregs against IRI [78].

In hypertensive patients with carotid atherosclerosis, a telmisartan/rosuvastatin combination treatment *in vivo* reduced both the Th17 cell number and the Th17/Treg cell ratio and increased the Treg cell frequency. The telmisartan/rosuvastatin combination also significantly reduced the levels of IL-17, IL-23, IL-6 and TNF, and increased TGF- β and IL-10 levels, while RORɣt mRNA was reduced and Foxp3 mRNA was increased [79].

Atorvastatin therapy in patients with acute coronary syndromes increased circulating natural Tregs (nTreg) frequency and activity, elevated the expression of Foxp3, mRNA and protein, and enhanced Treg suppressive function *in vivo.* Thus, nTregs may have a protective role in atherosclerosis [80]. High dose rosuvastatin therapy significantly enhanced the number of CD4+FoxP3+ Tregs *in vivo*. Inflammatory responses were inhibited by rosuvastatin through the miR-155/SHIP-1 signaling pathway [81].

Zhao et al in an *in vivo and in vitro* study showed that atorvastatin increased the aggregation of Glycoprotein A Repetitions Predominant (GARP), which is an orphan toll-like receptor and has been considered as a potential surface marker of activated Tregs, as well as the number of Foxp3+ Treg cells and secretion of TGF-??1 in atherosclerotic plaques. Moreover, they confirmed the ability of atorvastatin to improve atherosclerotic plaque stability and to delay the atherosclerotic process. Indeed, upregulation of GARP expression on Tregs by atorvastatin improves, albeit slightly, the inflammatory response in atherosclerosis [82].

Atorvastatin, administered to patients with ST-Segment Elevated Myocardial Infarction (STEMI) prior to receiving a primary percutaneous coronary intervention (PCI), increased the numbers of CD4+CD25+ Tregs *in vivo* and the expression of Foxp3 in PBMCs, and this was associated with increased production of TGF-?? and decreased INF-gamma. Therefore, early oral administration of atorvastatin before PCI could significantly repress inflammation in patients with STEMI and could improve their prognosis [83].

Statins can exert immunomodulatory effects by altering various properties of Treg cells, such as differentiation and survival, through regulation of transcription factors which are necessary to Treg development. Kim et al. demonstrated that simvastatin synergizes with low levels of TGF-?? *in vitro* could induce Foxp3+ Treg cells. The inhibition of protein geranylgeranylation at late time-points after T-cell activation and demethylation of the Foxp3 promoter are proposed simvastatin mechanisms. One of the main effects of simvastatin was suppression of Smad6 and Smad7 induction, Smads that inhibit TGF-β signaling. This study indicates that one probable mechanism for the immunosuppressive effects of statins is the ability to promote Foxp3+ Treg cell development.[84]

Simvastatin also enhanced the number and function of Tregs in HFFD (High Fat Fructose Diet) mice compared to HFFD alone *in vivo* [85]. Simvastatin increases IL-10 and TGF-?? levels, IDO production and Foxp3 expression in cancer cells *in vitro* and expands the development of Tregs *in vivo* [86]. Statins significantly enhanced the number of Tregs and FOXP3 mRNA levels in male subjects with normal levels of cholesterol who have followed lovastatin or atorvastatin regimens daily for 45 days *in vivo*. Also, there was a positive correlation between Treg cell number and plasma levels of high-density lipoprotein cholesterol (HDL-c), implying that HDL-c plays a role in Treg homeostasis. Therefore, the influence of statins on immune modulation is independent of

inflammation and the immunomodulatory effect of statins via increasing Treg cell numbers occurs even in healthy individuals [58].

In low-density lipoprotein receptor-knockout (LDLR-/-) transgenic mice following simvastatin treatment, the number of CD83+ DCs in plaque was significantly reduced, whereas the frequency of Tregs was increased compared to control plaques [87]. Another study, however, showed conflicting results about the effects of satins on Tregs subsets. Guasti et al reported that the frequency of CD4 + CD25 high FoxP3+ T cells was not altered following 3-month atorvastatin therapy (10-20 mg/day) in dyslipidemic patients, which might be explained by the low dose of atorvastatin and the relatively short duration of treatment as well as the small size and non-randomized design of the mentioned study [88].

Simvastatin has been shown to improve *in vivo* T-cell function in cecal ligation and puncture-induced abdominal sepsis mouse models. Simvastatin increased the proliferation of T cells and cytokine production in vitro and diminished apoptosis of T cells, Treg cell development, and systemic inflammation in septic animals [89].

The roles of Treg cells in health and disease

Regulatory T (Treg) cells, are the primary mediators of peripheral immune tolerance and play a principal role in maintaining immune homeostasis, preventing autoimmunity and regulating inflammation induced by pathogens and environmental insults. Immune regulation by Tregs suppresses the development of autoimmune diseases, such as type 1 diabetes [90, 91], restrains beneficial responses by limiting the immune response evoked by pathogens [92, 93], regulates antitumor immunity [94], and reduces the development of chronic inflammatory diseases such as asthma and inflammatory bowel disease [95, 96]. The development, maintenance and function of

Tregs are dependent upon the transcription factor, forkhead box P3 (FOXP3) [97, 98]. FOXP3 is the main regulator of the development and function of Tregs and acts by controlling the expression of multiple genes that modulate their regulatory activity. However, other transcriptional events also exert influence upstream of, and concurrently with FOXP3 to mediate the development of Tregs. Immune dysregulation, poly endocrinopathy and enteropathy, X-linked syndrome (IPEX) are severe autoimmune disorders caused by Foxp3 dysfunction [99], and serve to highlight the importance of this regulatory pathway. Treg cells exert their functions via four basic modes of action:

i) Secretion of inhibitory cytokines such as interleukin-10 (IL-10), IL‑35 and transforming growth factor‑β (TGFβ).

ii) Cytolysis, mediated via granzyme‑A and granzyme‑B and perforin-dependent killing mechanisms.

iii) Metabolic disruption including apoptosis mediated via high-affinity CD25 (also known as IL-2 receptor α)-dependent cytokine deprivation, inhibition by cyclic adenosine monophosphate (cAMP), and immunosuppression mediated via CD39- and CD73 and adenosine receptor 2A.

iv) Modulation of dendritic cell (DC) maturation and function, such as via lymphocyte-activation gene 3 (LAG3) –MHC-class-II mediated suppression of DC maturation, and cytotoxic T-lymphocyte antigen‑4 (CTLA4)–CD80/CD86-mediated induction of indoleamine 2,3-dioxygenase (IDO), which is an immunosuppressive molecule produced by DCs [100].

Tregs can be divided into subsets based upon the cytokines that they produce, and the expression of various cell surface markers, The most common types of Tregs are naturally occurring (nTreg) and induced (iTreg).[101] The suppression mechanism of nTreg is mainly cytokine-independent,

and generation occurs in the thymus. FoxP3, glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR) and intracellular CTLA-4 are the main markers of nTregs. [102]. Conversely, the suppression mechanisms of the antigen-induced or adaptive iTregs, CD4+ Tr1 and Th3 cells mediated through cytokine (TGF-β and IL-10) and induced in the periphery in a process involving cognate antigens and cytokines.[103-106]

Statins enhance the migration and homing of Treg cells to inflamed tissue

Leukocyte migration is influenced by statins. During the early stages of inflammation, migration of leukocytes requires the engagement of LFA-1 (Lymphocyte Function Associated Antigen-1) which is expressed on hematopoietic cells by ICAM-1(Intracellular Adhesion Molecule-1 which is expressed on the surface of endothelial cells. Weitz-Schmidt et al. reported that simvastatin, mevastatin and lovastatin prevent the leukocyte/endothelium interaction and consequently neutrophil migration in a peritonitis mouse model by binding them to an allosteric site of LFA-1 and blocking its attachment to ICAM-1 [107].

Moreover, the migration of Tregs to inflamed tissues is influenced by statins. Lovastatin treatment in a model of *Candida albicans*-induced delayed-type hypersensitivity *in vivo and in vitro* and resulted in the enhancement of expression of the CCL1 chemokine, which plays a role in the chemotaxis of Tregs. This resulted in the number of migrated Tregs being increased in the skin lesions. In fact, lovastatin therapy markedly up-regulated only CCL1 ligand amongst various Treg cell associated-inflammatory chemokine receptors (CCR2, CCR4, CCR5, and CCR8) and enhanced Treg migration to the region of inflammation.

Conversely, FoxP3+ cells have been observed in the footpad of CCL1 knockout transgenic mice. This indicates that other chemokine-receptor pairs also participate in Treg cell migration, independent of the effects of statins. These results suggest that statins respond to CCL1 and induce a selective Treg infiltration into the inflamed region [108].

Simvastatin treatment in an apolipoprotein E knockout (ApoE−/−) atherosclerotic mouse model increased the Foxp3, TGF-?? and IL-10 expression in both the atherosclerotic plaque and in circulating cells. Moreover, atherosclerotic plaque analyses showed high levels of IL-4 mRNA and protein expression and low levels of IL-17, IL1-?? and IFN-?? [73].

Atorvastatin administration reduced glial cell activation and neurological damage, along with a decline in the circulating and cervical lymph node Treg population, while concurrently increasing the CD4+CD25+ T cell population in the brain. Therefore, atorvastatin decreased the peripheral tissue Treg cell frequency and promoted their accumulation in the brain, thereby exerting neuroprotective effects, possibly through the attenuation of glial cell activation [109]. Acute treatment with low dose atorvastatin decreased T cell, neutrophil and NK cell invasion into the brain, and was accompanied by a reduced *in vivo* production of pro-inflammatory cytokines and chemokines. Additionally, the peripheral spleen and brain Treg cells were increases as was the expression of the cytokines (IL-10 and TGF-β1). Therefore, neuronal destruction was reduced, and the behavioral deficiency ameliorated [110].

Suppression of the Th1 immune response by statins.

The suppression of the Th1 immune response, a primary function of Tregs, could be influenced by statins. Statins modulate the expression of CD40, and alter CD40 to CD40L engagement, and

thereby influence IL-12 expression which improves Th1 cell differentiation and clonal expansion [111]. Atorvastatin decreases expression of CD40 and TNF-?? in monocytes and human endothelial cells [112]. Statins have been shown to act as farnesyltransferase inhibitors and to improve the bacterial clearance and survival of septic mice. Sepsis enhances farnesyltransferase activity and thereby produces high levels of farnesylated proteins that suppress Tregs and IFN-?? secretion, and attenuate T-cell proliferation [113]. Statins therapy has been shown to have beneficial effects on prognosis in patients with sepsis [114, 115]. Simvastatin has been shown to reduce CD4 T-cell destruction induced by sepsis. The expansion of Tregs, enhances CD4 T-cell proliferation and cytokine expression and has been associated with a reduction in bacterial load in models of sepsis. These results suggest that simvastatin mediated-Treg reduction contributes to the effectiveness of statins in improving severe infections [89].

Clarification has been provided by recent research in the field of sepsis. A well-conducted systematic and meta-analysis evaluated 2628 patients in fourteen trials demonstrated that statins did not decrease 30-day all-cause mortality in the subgroup of patients with severe sepsis (RR 0.97, 95% CI 0.84e1.12), so therefore statins are not recommended for the treatment of sepsis [116].

The geranylgeranyltransferase inhibitor, GGTI-289, induces SOCS3 expression in CD4 T cells which inhibits STAT3 phosphorylation following signaling of IL-6. Consequently, protein geranylgeranylation through induction of SOCS3 could participate in differentiating the naive T cells to Th17 and Foxp3+ Tregs [117].

It has also been demonstrated that statins have a potent immunomodulatory effect in ameliorating rheumatoid arthritis [118], unstable angina, unstable coronary plaques. These disorders result in the development of CD28null T cells via increased circulating levels of cytokines such as TNF.

These CD4+ CD28 null T cell subsets have a cytolytic effect on endothelial cells [119] which results from the increase of the levels of the proinflammatory cytokines such as IFN-γ which stimulate the macrophages producing tissue destructive metalloproteinases, perforin, and granzyme B. [120] Brugaletta and et al. observed that statin therapy (atorvastatin, simvastatin or pravastatin) reduced the population of this particular of T lymphocytes in patients referred to the coronary care unit with unstable angina.[121]

The effect of statins on suppression of Th17 induction

The balance of Treg/Th17 cells may be involved in the pathogenesis of many chronic inflammatory diseases. Statins may suppress Th17 cell development and increase Treg function. Simvastatin shifted CD4+T cell differentiation toward Treg cells in mice spleen cells cultured under various polarizing conditions. The differentiation of Treg/Th17 cells was regulated by protein geranylgeranylation, since GGTI-298 (like simvastatin) inhibited the immune response through induction of Treg cell development in a Severe combined immunodeficiency (SCID) mouse induced colitis model [117].

Simvastatin has been investigated as a potential treatment for relapsing-remitting multiple sclerosis (RRMS). In this setting, it was demonstrated that co-culture of CD14+ cells (collected from the PBMCs of patients) and simvastatin *in vitro* could induce the expression of SOCS3 and SOCS7. This resulted in a reduction of STAT1 and STAT3 phosphorylation (both are involved in the regulation of IL-23 and IL-6). Simvastatin suppressed the expression of the transcription factor RORc and secretion of IL-17, but induced INF-??, IL-4, and IL-27 production [55]. Co-culture of

naive CD4+ T cells from RRMS patients with simvastatin suppressed the production of cytokines IL-17, IL-21, and IL-22, and therefore suppressed Th17 differentiation.

IRF4 (IFN regulatory factor 4) is a central molecule in the development of human Th17 cell differentiation which, following phosphorylation by the Rho Associated kinase (ROCK) 2, binds to the IL-17A and IL-21 promoters and thus induces their activation. IRF4 gene knockout suppressed Th17 cell differentiation. Simvastatin has been shown to suppress Rho Associated kinase (ROCK) and, consequently, it causes inhibition of IRF4 phosphorylation, which is the main component in the regulation of Th17 differentiation [122]. The treatment of CD4+ T cells and monocyte-derived dendritic cells (mDC) from asthmatic patients with fluticasone propionate (FP) and simvastatin *in vitro* showed a sharp increase in Treg differentiation and a reduced Th17 cell population. These effects of FP combined with simvastatin occurred in a dose-dependent manner. This combination therapy caused a synergistic effect, significantly reducing IL-23 production and increasing IL-10 and IDO production [123].

It has been demonstrated that simvastatin treatment in a dose-dependent manner *in vitro,* decreased the number of cancer cells and also increased the production of the immune regulatory markers IL-10, TGF-b and expression of IDO and forkhead box P3 (FoxP3) transcription factor in cancer cells [124].

In a Guillain-Barre model, atorvastatin treatment considerably improved the manifestations of the disease by limiting inflammatory cell infiltration, for example IL-17 and INF-?? producing lymphocytes, into the peripheral nervous system, enhancing the Treg number in lymph nodes and decreasing the expression of costimulatory T-cell activation markers like CD80.[125]

The role of statins in the treatment of chronic inflammatory and autoimmune diseases

The anti-inflammatory properties of statins can be utilized for therapy in chronic inflammatory and autoimmune diseases. Simvastatin enhanced the frequency of regulatory T cells, and anti-inflammatory cytokine IL-10 production in the mesenteric lymph nodes, ileum and spleen of treated animals. Following simvastatin treatment, mucosal T cells and neutrophilic granulocyte number along with pro-inflammatory cytokines (IL-23, IFN- γ, TNF-a, IL-6, MCP-1) was reduced in the ileum of treated animals [126]. In an MS animal model, combination therapy with atorvastatin and ATRA (All-Trans Retinoic Acid) was synergistic, ameliorating clinical and neuropathological outcomes of disease. Atorvastatin decreased IL-17 cytokine production and, conversely, elevated the production of the anti-inflammatory cytokine IL-10. This study also showed that atorvastatin and ATRA in combination significantly increased the FoxP3+Treg cells number [127]. Brahmachari et al. reported that NO reduced the CD4+CD25+ and CD25+FoxP3+ phenotypes of Tregs during myelin basic protein (MBP) priming. They demonstrated that blocking of NO by inhibiting inducible NO synthases using drugs such as pravastatin enhanced the expression of Foxp3 in MBP-primed T cells. Consequently, specific targeting of NO by statins, which inhibit iNOS, may be helpful in inducing Foxp3+ Tregs and eliminating autoreactive T cells in MS and other autoimmune disorders [128].

Atorvastatin and rapamycin combination therapy improved clinical and neuropathologic outcomes of EAE. The Treg number, secretion of Th2 and Treg cytokines were increased and the migration of inflammatory Th17 cells, proinflammatory Th1/Th17 cytokines production were decreased in EAE mice. The addition of atorvastatin to rapamycin attenuated rapamycin-reactivated extracellular signal-regulated kinases (ERK). These results show that immunomodulating drugs can be combined to potentiate the effects of one another, enabling improved treatment of

autoimmune conditions in the CNS [129]. The anti-inflammatory effects of atorvastatin are mediated via a reduction of the activation markers of CD4+T cells, such as HLA-DR and CD38, reducing T cell proliferation, limiting expression of CCR-5 (HIV-1 co-receptor), enhancing Treg development and improving the suppressive function of Tregs *in vitro* in a dose-dependent manner . Moreover, atorvastatin, through upregulation of p21 as a cyclin-dependent kinase inhibitor in the CD4+ T cells, could decrease their susceptibility to HIV-1 infection and replication. These data may explain how statins affect the CD4+ T cell resistance to HIV-1 infection through p21 overexpression, and could represent a novel option for therapy in HIV-infected patients [130]. Oral treatment with atorvastatin caused a reduction in Th1/Th17 responses in the local draining lymph nodes of the kidneys and decreased the migration of inflammatory CD4+ T/Th17 cells, macrophages and neutrophils into the kidneys. Treg frequency was not changed but the suppressive activity, Foxp3 expression and IL-10 secretion from Tregs was significantly increased following atorvastatin administration. Atorvastatin therapy improved the survival of mice by protecting them against the progression of anti-glomerular basement membrane (GBM) nephritis [131]. Atorvastatin also induced immature bone marrow-derived DCs (BMDCs) *in vitro*, and atorvastatin- bone marrow-derived DCs and ameliorated ongoing EAMG. These DCs have tolerogenic properties, through their inhibition of the antibody response, reduction in Tfh cell numbers, production of the cytokine IL-21, and increase in the numbers of Breg cells. Hence, Atorvastatin-BMDC might have therapeutic potential for myasthenia gravis and other autoimmune diseases [25].

Atorvastatin treatment in IL-4/GFP (green fluorescent protein)-enhanced transcript (4-GET) mice (a reporter model designed to monitor immune responses) did not induce Th2 cells *in vivo,* but *in vitro* co-culture of T cells from 4-GET mice with atorvastatin preferentially differentiated T cells

into Th2 cells, whereas antigen-specific T-cell proliferation and proinflammatory cytokine (IFN-ɣ, IL-17, TNF, and IL-12) secretion were reduced. Oral atorvastatin administration inhibited Th2 differentiation, resulting in the prevention of EAE development, and did not induce Foxp3+ Tregs in transgenic STAT6 knockout mice. As a result, the suppression of encephalitogenicity of proinflammatory T cells by atorvastatin could improve the clinical outcomes of neurogenic autoimmune disease [132]. Pravastatin also increased the Treg population, elevated IL-4 production, decreased IFN-γ levels and improved lung pathology in asthma models [133].

Atorvastatin therapy in hypercholesterolemic patients significantly decreased lymphocyte proliferation by 15 %, increasing adenosine triphosphate (ATP) levels by 16 %, and showed a trend toward increased number of Treg cells. However, in renal transplant recipients, atorvastatin therapy did not alter any of the biomarkers of immunosuppression that were studied, suggesting that statin treatment cannot be considered to be a means to reduce the dose of immunosuppressive agents [134].

Al-Husein et al showed that the use of statins in patients with colorectal cancer (CRC) was associated with more extensive Treg infiltration in an advanced stage of the disease, less angiogenesis, especially in early disease stage, but no difference in TGF-β1 concentration in tumor tissue. Hence, statins might be appropriate for prophylaxis and treatment of CRC via anti-angiogenic effects, and reduction of tumor aggressiveness .[135]

A Systematic Review and Meta-analysis of Randomised Controlled Trials was conducted which included four randomised controlled trials (439 participants). Their results showed a significant increase in Treg frequency after statin therapy in Acute Coronary Syndrome (ACS) patients. The authors noted dose-related effects and heterogeneity of responses between different statins

(rosuvastatin and atorvastatin). The results verified that statins positively modify the frequency of Tregs, which may present a possible mechanism of their therapeutic effect. [136]

The side effects of statin immunomodulation

In the follow-up of a large randomized trial of statin-treated patients, LDL cholesterol levels were found to be negatively correlated with cancer risk [137]. Statins enhance the development of Tregs by inducing Foxp3 [70]. Statins could exert an effect on the immune system through various mechanisms, such as by limiting effector T cell response in atheroma to improve atherosclerotic plaque stability [138]. However, despite their many beneficial effects on the immune system, statins may also have some side effects. Statins suppressed tumor-specific T-cell responses, which attenuated the host anti-tumor immune responses and could theoretically increase the risk of developing cancer [139, 140]. Statin administration has been associated with certain tumors, such as breast [141], ovarian [142] and hepatocellular carcinoma [143]. Pravastatin treatment of women with average cholesterol levels increased the Treg number could potentially have contributed to breast cancer recurrence [144].

Evidence suggests that statins increase the risk of new-onset diabetes, and many mechanisms for this effect have been suggested [145]. As described above, one of the main immunomodulatory effects of statins is increasing the number and functionality of CD4+CD25+ regulatory T cells (Tregs) in vivo by inducing the transcription factor Foxp3[57]. Recently, it has been reported that during the aging process, fat-resident Tregs accumulate in adipose tissue and their selective exhaustion enhances adipose tissue insulin sensitivity. These dysfunctional Tregs do not maintain an optimal immune state in the aged adipose tissue. This results in metabolic dysfunctions like insulin resistance and age-associated diabetes [146].

Because of the important role of Treg in diabetogenesis, patients on statin therapy especially those who are elderly, should receive long-term follow-up to manage patientsfor whom the deleterious diabetogenic immunomodulatory effects of statins overweigh their cardiovascular benefits [147].

Pravastatin therapy in men with hypercholesterolemia for an average of 5 years appeared to increase the risk of prostate cancer while concurrently decreasing the risk of coronary events [148]. Elderly persons are predisposed to develop cancer, due to their advanced age and associated immunosenescence [149]. The use of pravastatin for an average of 3.2 years in elderly subjects (average age 75 years) [150] and, in a separate study, the use of pravastatin therapy of elderly subjects (65 to 75 years) for 6 years, both showed an increase in the prevalence of cancer [151]. Furthermore, there may be a dose-response effect, as higher daily dose of atorvastatin were associated with greater cancer mortality. In conclusion, statin therapy in elderly individuals resulted in an increase in Tregs, which could potentially play a role in increasing cancer risk, and this risk may be dose-dependent [152].

Statin therapy during Bacille Calmette–Guérin (BCG) immunotherapy of patients with bladder cancer appeared to exacerbate tumor progression [153]. Statin therapy is associated with the development of amyotrophic lateral sclerosis by increasing the Treg population and function [154]. The glutamate level in the central nervous system (CNS) plays a major role in modulating neurotoxicity through T cell-mediated immune system induction [155]. Auto-reactive Th1 cells, by increasing IFN-γ production, might induce a beneficial phenotype in microglial cells by promoting high expression of GLT-1(Glutamate transporter) (which mediates the clearance of glutamate) and, thus, may decrease glutamate-induced neurotoxicity [156].

T cell-mediated neuroprotective autoimmunity for resistance to CNS injury could be abrogated by the suppressive function of regulatory T cells [157].

Statins might worsen many infectious diseases by eliciting regulatory T cell expansion [158], Human immunodeficiency virus (HIV) infection being one example [159]. The Tregs isolated from PBMCs have been shown to promote CD4+ T cell responses against HIV, and this could allow for disease progression [160]. Interestingly, subjects randomized to statins demonstrated an increase in respiratory infectious diseases [161]. Both immunocompetent and immunocompromised adults showed an increased incidence of herpes zoster (as a latent viral infection) and hepatitis B and C virus infections (as chronic infection), and this could be associated with Treg development and compromised immune responses resulting from statin therapy [158, 162, 163]. Moreover, it is probable that statins may impair the response to vaccination by increasing the number of Tregs [149]. Consequently, care must be taken in prescribing statins to immunocompetent patients who have latent and/or chronic infectious diseases, those with degenerative neurological disorder, neoplastic diseases and the elderly because of their potential side effects.

Conclusion

Recent studies have confirmed that statins are able to potentiate the suppressor function of Tregs, which could result in a reduction of the inflammatory response. Statins have a major role in modulating Th17/Treg differentiation, through inhibition of Th17 differentiation/activation and, conversely, by increasing the Treg differentiation/suppressive action in autoimmune disease. These pleiotropic properties of statins in modulating immune responses make them attractive candidates for the treatment of autoimmune and inflammatory diseases. Nevertheless, despite their many beneficial effects, statins may also have some negative effects, particularly in immunocompromised individuals. Consequently, statin combination therapy, as an

accompaniment to other immunomodulatory drugs, could augment the statin-induced Tregs. Understanding the side effects of such combination therapy is vital for the better management of autoimmune and inflammatory diseases.

Funding

No funding was received for preparing this review.

Conflict of interests

Dr. Penson owns four shares in Astra Zeneca PLC and has received travel/speaker's fees from Amgen Inc. Other authors have no competing interests to declare.

References

[1] P. Chruściel, A. Sahebkar, M. Rembek-Wieliczko, M.C. Serban, S. Ursoniu, D.P. Mikhailidis, S.R. Jones, S. Mosteoru, M.J. Blaha, S.S. Martin, J. Rysz, P.P. Toth, G.Y.H. Lip, M.J. Pencina, K.K. Ray, M. Banach, Impact of statin therapy on plasma adiponectin concentrations: A systematic review and meta-analysis of 43 randomized controlled trial arms, Atherosclerosis 253 (2016) 194-208.

[2] S.M.R. Parizadeh, M.R. Azarpazhooh, M. Moohebati, M. Nematy, M. Ghayour-Mobarhan, S. Tavallaie, A.A. Rahsepar, M. Amini, A. Sahebkar, M. Mohammadi, G.A.A. Ferns, Simvastatin therapy reduces prooxidant-antioxidant balance: Results of a placebo-controlled cross-over trial, Lipids 46(4) (2011) 333-340.

[3] A. Sahebkar, K. Kotani, C. Serban, S. Ursoniu, D.P. Mikhailidis, S.R. Jones, K.K. Ray, M.J. Blaha, J. Rysz, P.P. Toth, P. Muntner, G.Y.H. Lip, M. Banach, Statin therapy reduces plasma endothelin-1 concentrations: A meta-analysis of 15 randomized controlled trials, Atherosclerosis 241(2) (2015) 433-442.

[4] A. Sahebkar, C. Serban, D.P. Mikhailidis, A. Undas, G.Y.H. Lip, P. Muntner, V. Bittner, K.K. Ray, G.F. Watts, G.K. Hovingh, J. Rysz, J.J.P. Kastelein, M. Banach, Lipid, G. Blood Pressure Meta-analysis Collaboration, Association between statin use and plasma d-dimer levels: A systematic review and meta-analysis of randomised controlled trials, Thrombosis and Haemostasis 114(3) (2015) 546-557.

[5] A. Sahebkar, C. Serban, S. Ursoniu, D.P. Mikhailidis, A. Undas, G.Y.H. Lip, V. Bittner, K.K. Ray, G.F. Watts, G. Kees Hovingh, J. Rysz, J.J.P. Kastelein, M. Banach, Lipid, G. Blood Pressure Meta-analysis Collaboration, The impact of statin therapy on plasma levels of von Willebrand

factor antigen: Systematic review and meta-analysis of Randomised placebo-controlled trials, Thrombosis and Haemostasis 115(3) (2016) 520-532.

[6] C. Serban, A. Sahebkar, S. Ursoniu, D.P. Mikhailidis, M. Rizzo, G.Y.H. Lip, G. Kees Hovingh, J.J.P. Kastelein, L. Kalinowski, J. Rysz, M. Banach, A systematic review and meta-analysis of the effect of statins on plasma asymmetric dimethylarginine concentrations, Scientific Reports 5 (2015).

[7] J.L. Goldstein, M.S. Brown, Regulation of the mevalonate pathway, Nature 343(6257) (1990) 425-30.

[8] T. Gordon, W.B. Kannel, Premature mortality from coronary heart disease. The Framingham study, JAMA 215(10) (1971) 1617-25.

[9] W.B. Kannel, W.P. Castelli, T. Gordon, P.M. McNamara, Serum cholesterol, lipoproteins, and the risk of coronary heart disease. The Framingham study, Ann Intern Med 74(1) (1971) 1-12.

[10] Multiple Risk Factor Intervention Trial Research Group, Multiple Risk Factor Intervention Trial: risk factor changes and mortality results, Jama 248 (1982) 1465-1477.

[11] H. Iso, D.R. Jacobs, Jr., D. Wentworth, J.D. Neaton, J.D. Cohen, Serum cholesterol levels and six-year mortality from stroke in 350,977 men screened for the multiple risk factor intervention trial, N Engl J Med 320(14) (1989) 904-10.

[12] V.W. Rodwell, J.L. Nordstrom, J.J. Mitschelen, Regulation of HMG-CoA reductase, Adv Lipid Res 14 (1976) 1-74.

[13] E.S. Istvan, J. Deisenhofer, Structural mechanism for statin inhibition of HMG-CoA reductase, Science 292(5519) (2001) 1160-4.

[14] P. Libby, Molecular bases of the acute coronary syndromes, Circulation 91(11) (1995) 2844-50.

[15] J.K. Liao, M.A. Bettmann, T. Sandor, J.I. Tucker, S.M. Coleman, M.A. Creager, Differential impairment of vasodilator responsiveness of peripheral resistance and conduit vessels in humans with atherosclerosis, Circ Res 68(4) (1991) 1027-34.

[16] L.J. Ignarro, G.M. Buga, K.S. Wood, R.E. Byrns, G. Chaudhuri, Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide, Proceedings of the National Academy of Sciences 84(24) (1987) 9265-9269.

[17] M.W. Radomski, D.D. Rees, A. Dutra, S. Moncada, S-nitroso-glutathione inhibits platelet activation in vitro and in vivo, Br J Pharmacol 107(3) (1992) 745-9.

[18] U.C. Garg, A. Hassid, Nitric oxide-generating vasodilators and 8-bromo-cyclic guanosine monophosphate inhibit mitogenesis and proliferation of cultured rat vascular smooth muscle cells, J Clin Invest 83(5) (1989) 1774-7.

[19] T.W. Gauthier, R. Scalia, T. Murohara, J.P. Guo, A.M. Lefer, Nitric oxide protects against leukocyte-endothelium interactions in the early stages of hypercholesterolemia, Arterioscler Thromb Vasc Biol 15(10) (1995) 1652-9.

[20] P. Kubes, M. Suzuki, D.N. Granger, Nitric oxide: an endogenous modulator of leukocyte adhesion, Proc Natl Acad Sci U S A 88(11) (1991) 4651-5.

[21] P.J. van der Most, A.M. Dolga, I.M. Nijholt, P.G. Luiten, U.L. Eisel, Statins: mechanisms of neuroprotection, Prog Neurobiol 88(1) (2009) 64-75.

[22] P. Chrusciel, A. Sahebkar, M. Rembek-Wieliczko, M.C. Serban, S. Ursoniu, D.P. Mikhailidis, S.R. Jones, S. Mosteoru, M.J. Blaha, S.S. Martin, J. Rysz, P.P. Toth, G.Y. Lip, M.J. Pencina, K.K. Ray, M. Banach, Lipid, G. Blood Pressure Meta-analysis Collaboration, Impact of statin therapy on plasma adiponectin concentrations: A systematic review and meta-analysis of 43 randomized controlled trial arms, Atherosclerosis 253 (2016) 194-208.

[23] G. Ferretti, T. Bacchetti, A. Sahebkar, Effect of statin therapy on paraoxonase-1 status: A systematic review and meta-analysis of 25 clinical trials, Prog Lipid Res 60 (2015) 50-73.

[24] S.M. Parizadeh, M.R. Azarpazhooh, M. Moohebati, M. Nematy, M. Ghayour-Mobarhan, S. Tavallaie, A.A. Rahsepar, M. Amini, A. Sahebkar, M. Mohammadi, G.A. Ferns, Simvastatin therapy reduces prooxidant-antioxidant balance: results of a placebo-controlled cross-over trial, Lipids 46(4) (2011) 333-40.

[25] C. Serban, A. Sahebkar, S. Ursoniu, D.P. Mikhailidis, M. Rizzo, G.Y. Lip, G. Kees Hovingh, J.J. Kastelein, L. Kalinowski, J. Rysz, M. Banach, A systematic review and meta-analysis of the effect of statins on plasma asymmetric dimethylarginine concentrations, Sci Rep 5 (2015) 9902.

[26] A. Sahebkar, C. Serban, D.P. Mikhailidis, A. Undas, G.Y. Lip, P. Muntner, V. Bittner, K.K. Ray, G.F. Watts, G.K. Hovingh, J. Rysz, J.J. Kastelein, M. Banach, Lipid, G. Blood Pressure Meta-analysis Collaboration, Association between statin use and plasma D-dimer levels. A systematic review and meta-analysis of randomised controlled trials, Thromb Haemost 114(3) (2015) 546-57.

[27] A. Sahebkar, C. Serban, S. Ursoniu, D.P. Mikhailidis, A. Undas, G.Y. Lip, V. Bittner, K. Ray, G.F. Watts, G.K. Hovingh, J. Rysz, J.J. Kastelein, M. Banach, Lipid, G. Blood Pressure Meta-analysis Collaboration, The impact of statin therapy on plasma levels of von Willebrand factor antigen. Systematic review and meta-analysis of randomised placebo-controlled trials, Thromb Haemost 115(3) (2016) 520-32.

[28] U. Laufs, V. La Fata, J. Plutzky, J.K. Liao, Upregulation of endothelial nitric oxide synthase by HMG CoA reductase inhibitors, Circulation 97(12) (1998) 1129-35.

[29] Y. Kureishi, Z. Luo, I. Shiojima, A. Bialik, D. Fulton, D.J. Lefer, W.C. Sessa, K. Walsh, The HMG-CoA reductase inhibitor simvastatin activates the protein kinase Akt and promotes angiogenesis in normocholesterolemic animals, Nat Med 6(9) (2000) 1004-10.

[30] U. Laufs, V.L. Fata, J.K. Liao, Inhibition of 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase blocks hypoxia-mediated down-regulation of endothelial nitric oxide synthase, J Biol Chem 272(50) (1997) 31725-9.

[31] M. Essig, G. Nguyen, D. Prie, B. Escoubet, J.D. Sraer, G. Friedlander, 3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors increase fibrinolytic activity in rat aortic endothelial cells. Role of geranylgeranylation and Rho proteins, Circ Res 83(7) (1998) 683-90.

[32] O. Hernandez-Perera, D. Perez-Sala, J. Navarro-Antolin, R. Sanchez-Pascuala, G. Hernandez, C. Diaz, S. Lamas, Effects of the 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors, atorvastatin and simvastatin, on the expression of endothelin-1 and endothelial nitric oxide synthase in vascular endothelial cells, J Clin Invest 101(12) (1998) 2711-9.

[33] J. Llevadot, S. Murasawa, Y. Kureishi, S. Uchida, H. Masuda, A. Kawamoto, K. Walsh, J.M. Isner, T. Asahara, HMG-CoA reductase inhibitor mobilizes bone marrow--derived endothelial progenitor cells, J Clin Invest 108(3) (2001) 399-405.

[34] S. Dimmeler, A. Aicher, M. Vasa, C. Mildner-Rihm, K. Adler, M. Tiemann, H. Rutten, S. Fichtlscherer, H. Martin, A.M. Zeiher, HMG-CoA reductase inhibitors (statins) increase endothelial progenitor cells via the PI 3-kinase/Akt pathway, J Clin Invest 108(3) (2001) 391-7.

[35] U. Laufs, D. Marra, K. Node, J.K. Liao, 3-Hydroxy-3-methylglutaryl-CoA reductase inhibitors attenuate vascular smooth muscle proliferation by preventing rho GTPase-induced down-regulation of p27(Kip1), J Biol Chem 274(31) (1999) 21926-31.

[36] Z. Yang, T. Kozai, B. van der Loo, H. Viswambharan, M. Lachat, M.I. Turina, T. Malinski, T.F. Luscher, HMG-CoA reductase inhibition improves endothelial cell function and inhibits smooth muscle cell proliferation in human saphenous veins, J Am Coll Cardiol 36(5) (2000) 1691-7.

[37] Y. Fukumoto, P. Libby, E. Rabkin, C.C. Hill, M. Enomoto, Y. Hirouchi, M. Shiomi, M. Aikawa, Statins alter smooth muscle cell accumulation and collagen content in established atheroma of watanabe heritable hyperlipidemic rabbits, Circulation 103(7) (2001) 993-9.

[38] K.K. Koh, Effects of statins on vascular wall: vasomotor function, inflammation, and plaque stability, Cardiovasc Res 47(4) (2000) 648-57.

[39] C.J. Vaughan, A.M. Gotto, Jr., C.T. Basson, The evolving role of statins in the management of atherosclerosis, J Am Coll Cardiol 35(1) (2000) 1-10.

[40] P.M. Ridker, N. Rifai, M. Clearfield, J.R. Downs, S.E. Weis, J.S. Miles, A.M. Gotto, Jr., I. Air Force/Texas Coronary Atherosclerosis Prevention Study, Measurement of C-reactive protein for the targeting of statin therapy in the primary prevention of acute coronary events, N Engl J Med 344(26) (2001) 1959-65.

[41] P.M. Ridker, N. Rifai, S.P. Lowenthal, Rapid reduction in C-reactive protein with cerivastatin among 785 patients with primary hypercholesterolemia, Circulation 103(9) (2001) 1191-1193.

[42] L. Diomede, D. Albani, M. Sottocorno, M.B. Donati, M. Bianchi, P. Fruscella, M. Salmona, In vivo anti-inflammatory effect of statins is mediated by nonsterol mevalonate products, Arteriosclerosis, thrombosis, and vascular biology 21(8) (2001) 1327-1332.

[43] F.L. Zhang, P.J. Casey, Protein prenylation: molecular mechanisms and functional consequences, Annu Rev Biochem 65(1) (1996) 241-69.

[44] S. Etienne-Manneville, A. Hall, Rho GTPases in cell biology, Nature 420(6916) (2002) 629-35.

[45] A. Corsini, N. Ferri, M. Cortellaro, Are pleiotropic effects of statins real?, Vasc Health Risk Manag 3(5) (2007) 611-3.

[46] J.K. Liao, Isoprenoids as mediators of the biological effects of statins, The Journal of clinical investigation 110(3) (2002) 285-288.

[47] E. Mira, S. Manes, Immunomodulatory and anti-inflammatory activities of statins, Endocr Metab Immune Disord Drug Targets 9(3) (2009) 237-47.

[48] A. Tsakiri, D. Tsiantoulas, J. Frederiksen, I.M. Svane, Increased immunopotency of monocyte derived dendritic cells from patients with optic neuritis is inhibited in vitro by simvastatin, Experimental neurology 221(2) (2010) 320-328.

[49] H. Li, C.C. Wang, M. Zhang, X.L. Li, P. Zhang, L.T. Yue, S. Miao, S. Wang, Y. Liu, Y.B. Li, R.S. Duan, Statin-modified dendritic cells regulate humoral immunity in experimental autoimmune myasthenia gravis, Mol Cell Neurosci 68 (2015) 284-92.

[50] J. Greenwood, L. Steinman, S.S. Zamvil, Statin therapy and autoimmune disease: from protein prenylation to immunomodulation, Nat Rev Immunol 6(5) (2006) 358-70.

[51] S. Youssef, O. Stuve, J.C. Patarroyo, P.J. Ruiz, J.L. Radosevich, E.M. Hur, M. Bravo, D.J. Mitchell, R.A. Sobel, L. Steinman, S.S. Zamvil, The HMG-CoA reductase inhibitor, atorvastatin, promotes a Th2 bias and reverses paralysis in central nervous system autoimmune disease, Nature 420(6911) (2002) 78-84.

[52] O. Aktas, S. Waiczies, A. Smorodchenko, J. Dorr, B. Seeger, T. Prozorovski, S. Sallach, M. Endres, S. Brocke, R. Nitsch, F. Zipp, Treatment of relapsing paralysis in experimental encephalomyelitis by targeting Th1 cells through atorvastatin, J Exp Med 197(6) (2003) 725-33.

[53] T. Vollmer, L. Key, V. Durkalski, W. Tyor, J. Corboy, S. Markovic-Plese, J. Preiningerova, M. Rizzo, I. Singh, Oral simvastatin treatment in relapsing-remitting multiple sclerosis, Lancet 363(9421) (2004) 1607-8.

[54] D.W. McCarey, I.B. McInnes, R. Madhok, R. Hampson, O. Scherbakov, I. Ford, H.A. Capell, N. Sattar, Trial of Atorvastatin in Rheumatoid Arthritis (TARA): double-blind, randomised placebo-controlled trial, Lancet 363(9426) (2004) 2015-21.

[55] X. Zhang, J. Jin, X. Peng, V.S. Ramgolam, S. Markovic-Plese, Simvastatin inhibits IL-17 secretion by targeting multiple IL-17-regulatory cytokines and by inhibiting the expression of IL-17 transcription factor RORC in CD4+ lymphocytes, J Immunol 180(10) (2008) 6988-96.

[56] E. Mira, B. León, D.F. Barber, S. Jiménez-Baranda, I. Goya, L. Almonacid, G. Márquez, A. Zaballos, C. Martínez-A, J.V. Stein, Statins induce regulatory T cell recruitment via a CCL1 dependent pathway, The Journal of Immunology 181(5) (2008) 3524-3534.

[57] K. Mausner-Fainberg, G. Luboshits, A. Mor, S. Maysel-Auslender, A. Rubinstein, G. Keren, J. George, The effect of HMG-CoA reductase inhibitors on naturally occurring CD4+ CD25+ T cells, Atherosclerosis 197(2) (2008) 829-839.

[58] A.L. Rodriguez-Perea, C.J. Montoya, S. Olek, C.A. Chougnet, P.A. Velilla, Statins increase the frequency of circulating CD4+ FOXP3+ regulatory T cells in healthy individuals, J Immunol Res 2015 (2015) 762506.

[59] J.H. Buckner, S.F. Ziegler, Regulating the immune system: the induction of regulatory T cells in the periphery, Arthritis Res Ther 6(5) (2004) 215-22.

[60] Y.P. Rubtsov, J.P. Rasmussen, E.Y. Chi, J. Fontenot, L. Castelli, X. Ye, P. Treuting, L. Siewe, A. Roers, W.R. Henderson Jr, Regulatory T cell-derived interleukin-10 limits inflammation at environmental interfaces, Immunity 28(4) (2008) 546-558.

[61] S.Z. Josefowicz, L.F. Lu, A.Y. Rudensky, Regulatory T cells: mechanisms of differentiation and function, Annu Rev Immunol 30 (2012) 531-64.

[62] M. Jougasaki, T. Ichiki, Y. Takenoshita, M. Setoguchi, Statins suppress interleukin-6-induced monocyte chemo-attractant protein-1 by inhibiting Janus kinase/signal transducers and activators of transcription pathways in human vascular endothelial cells, Br J Pharmacol 159(6) (2010) 1294-303.

[63] Z. Chen, D. Yang, X. Peng, J. Lin, Z. Su, J. Li, X. Zhang, Y. Weng, Beneficial effect of atorvastatin-modified dendritic cells pulsed with myelin oligodendrocyte glycoprotein autoantigen on experimental autoimmune encephalomyelitis, Neuroreport 29(4) (2018) 317-327.

[64] X.L. Li, Y. Liu, L.L. Cao, H. Li, L.T. Yue, S. Wang, M. Zhang, X.H. Li, Y.C. Dou, R.S. Duan, Atorvastatin-modified dendritic cells in vitro ameliorate experimental autoimmune myasthenia gravis by up-regulated Treg cells and shifted Th1/Th17 to Th2 cytokines, Mol Cell Neurosci 56 (2013) 85-95.

[65] H. Xu, X.L. Li, L.T. Yue, H. Li, M. Zhang, S. Wang, C.C. Wang, R.S. Duan, Therapeutic potential of atorvastatin-modified dendritic cells in experimental autoimmune neuritis by decreased Th1/Th17 cytokines and up-regulated T regulatory cells and NKR-P1(+) cells, J Neuroimmunol 269(1-2) (2014) 28-37.

[66] X.L. Li, H. Li, M. Zhang, H. Xu, L.T. Yue, X.X. Zhang, S. Wang, C.C. Wang, Y.B. Li, Y.C. Dou, R.S. Duan, Exosomes derived from atorvastatin-modified bone marrow dendritic cells ameliorate experimental autoimmune myasthenia gravis by up-regulated levels of IDO/Treg and partly dependent on FasL/Fas pathway, J Neuroinflammation 13(1) (2016) 8.

[67] P. Meier, R. Meier, E. Blanc, Influence of CD4+/CD25+ regulatory T cells on atherogenesis in patients with end-stage kidney disease, Expert Rev Cardiovasc Ther 6(7) (2008) 987-97.

[68] R. Klingenberg, N. Gerdes, R.M. Badeau, A. Gistera, D. Strodthoff, D.F. Ketelhuth, A.M. Lundberg, M. Rudling, S.K. Nilsson, G. Olivecrona, S. Zoller, C. Lohmann, T.F. Luscher, M.

Jauhiainen, T. Sparwasser, G.K. Hansson, Depletion of FOXP3+ regulatory T cells promotes hypercholesterolemia and atherosclerosis, J Clin Invest 123(3) (2013) 1323-34.

[69] D. Ke, J. Fang, L. Fan, Z. Chen, L. Chen, Regulatory T cells contribute to rosuvastatin-induced cardioprotection against ischemia-reperfusion injury, Coron Artery Dis 24(4) (2013) 334-41.

[70] K. Mausner-Fainberg, G. Luboshits, A. Mor, S. Maysel-Auslender, A. Rubinstein, G. Keren, J. George, The effect of HMG-CoA reductase inhibitors on naturally occurring CD4+CD25+ T cells, Atherosclerosis 197(2) (2008) 829-39.

[71] Z. Hu, D. Li, Y. Hu, K. Yang, Changes of CD4+CD25+ regulatory T cells in patients with acute coronary syndrome and the effects of atorvastatin, J Huazhong Univ Sci Technolog Med Sci 27(5) (2007) 524-7.

[72] P. Verginis, H.S. Li, G. Carayanniotis, Tolerogenic semimature dendritic cells suppress experimental autoimmune thyroiditis by activation of thyroglobulin-specific CD4+CD25+ T cells, J Immunol 174(11) (2005) 7433-9.

[73] X. Meng, K. Zhang, J. Li, M. Dong, J. Yang, G. An, W. Qin, F. Gao, C. Zhang, Y. Zhang, Statins induce the accumulation of regulatory T cells in atherosclerotic plaque, Mol Med 18(4) (2012) 598-605.

[74] J. Zhu, B. Gao, Simvastatin combined with aspirin increases the survival time of heart allograft by activating CD4(+)CD25(+) Treg cells and enhancing vascular endothelial cell protection, Cardiovasc Pathol 24(3) (2015) 173-8.

[75] A. Rodríguez-Perea, M. Rojas, P. Velilla-Hernández, High concentrations of atorvastatin reduce in-vitro function of conventional T and regulatory T cells, Clinical & Experimental Immunology 196(2) (2019) 237-248.

[76] Y. Blanquiceth, A.L. Rodríguez-Perea, J.H. Tabares Guevara, L.A. Correa, M.D. Sánchez, J.R. Ramírez-Pineda, P.A. Velilla, Increase of frequency and modulation of phenotype of regulatory T cells by atorvastatin is associated with decreased lung inflammatory cell infiltration in a murine model of acute allergic asthma, Frontiers in immunology 7 (2016) 620.

[77] T.-T. Tang, Y. Song, Y.-J. Ding, Y.-H. Liao, X. Yu, R. Du, H. Xiao, J. Yuan, Z.-H. Zhou, M.-Y. Liao, Atorvastatin upregulates regulatory T cells and reduces clinical disease activity in patients with rheumatoid arthritis, Journal of lipid research 52(5) (2011) 1023-1032.

[78] D. Ke, J. Fang, L. Fan, Z. Chen, L. Chen, Regulatory T cells contribute to rosuvastatin-induced cardioprotection against ischemia-reperfusion injury, Coronary Artery Disease 24(4) (2013) 334-341.

[79] Z. Liu, Y. Zhao, F. Wei, L. Ye, F. Lu, H. Zhang, Y. Diao, H. Song, Z. Qi, Treatment with telmisartan/rosuvastatin combination has a beneficial synergistic effect on ameliorating Th17/Treg functional imbalance in hypertensive patients with carotid atherosclerosis, Atherosclerosis 233(1) (2014) 291-9.

[80] Z.X. Wang, C.Q. Wang, X.Y. Li, Y. Ding, G.K. Feng, X.J. Jiang, Changes of naturally occurring CD4(+)CD25(+) FOXP3(+) regulatory T cells in patients with acute coronary syndrome and the beneficial effects of atorvastatin treatment, Int Heart J 56(2) (2015) 163-9.

[81] W. Xie, P. Li, Z. Wang, J. Chen, Z. Lin, X. Liang, Y. Mo, Rosuvastatin may reduce the incidence of cardiovascular events in patients with acute coronary syndromes receiving percutaneous coronary intervention by suppressing miR-155/SHIP-1 signaling pathway, Cardiovasc Ther 32(6) (2014) 276-82.

[82] X. Zhao, Y. Liu, Y. Zhong, B. Liu, K. Yu, H. Shi, R. Zhu, K. Meng, W. Zhang, B. Wu, Q. Zeng, Atorvastatin Improves Inflammatory Response in Atherosclerosis by Upregulating the Expression of GARP, Mediators Inflamm 2015 (2015) 841472.

[83] D. Zhang, S. Wang, Y. Guan, L. Wang, W. Xie, N. Li, P. Zhao, G. Su, Effect of oral atorvastatin on CD4+CD25+ regulatory T cells, FoxP3 expression, and prognosis in patients with ST-segment elevated myocardial infarction before primary percutaneous coronary intervention, J Cardiovasc Pharmacol 57(5) (2011) 536-41.

[84] Y.C. Kim, K.K. Kim, E.M. Shevach, Simvastatin induces Foxp3+ T regulatory cells by modulation of transforming growth factor-beta signal transduction, Immunology 130(4) (2010) 484-93.

[85] M.F. Atho'Illah, S. Widyarti, M. Rifa'I, Elicited soybean (Glycine max L.) extract improves regulatory T cell activity in high fat-fructose diet mice, AIP Conference Proceedings, 2017.

[86] K.J. Lee, J.Y. Moon, H.K. Choi, H.O. Kim, G.Y. Hur, K.H. Jung, S.Y. Lee, J.H. Kim, C. Shin, J.J. Shim, K.H. In, S.H. Yoo, K.H. Kang, S.Y. Lee, Immune regulatory effects of simvastatin on regulatory T cell-mediated tumour immune tolerance, Clin Exp Immunol 161(2) (2010) 298-305.

[87] G. Song, C. Zong, Z. Zhang, Y. Yu, S. Yao, P. Jiao, H. Tian, L. Zhai, H. Zhao, S. Tian, X. Zhang, Y. Wu, X. Sun, S. Qin, Molecular hydrogen stabilizes atherosclerotic plaque in low-density lipoprotein receptor-knockout mice, Free Radic Biol Med 87 (2015) 58-68.

[88] L. Guasti, A.M. Maresca, L. Schembri, E. Rasini, F. Dentali, A. Squizzato, C. Klersy, L. Robustelli Test, C. Mongiardi, L. Campiotti, W. Ageno, A.M. Grandi, M. Cosentino, F. Marino, Relationship between regulatory T cells subsets and lipid profile in dyslipidemic patients: a longitudinal study during atorvastatin treatment, BMC Cardiovasc Disord 16(1) (2016) 26.

[89] S. Zhang, L. Luo, Y. Wang, M. Rahman, M. Lepsenyi, I. Syk, B. Jeppsson, H. Thorlacius, Simvastatin protects against T cell immune dysfunction in abdominal sepsis, Shock 38(5) (2012) 524-531.

[90] S. Sakaguchi, N. Sakaguchi, J. Shimizu, S. Yamazaki, T. Sakihama, M. Itoh, Y. Kuniyasu, T. Nomura, M. Toda, T. Takahashi, Immunologic tolerance maintained by CD25+ CD4+ regulatory T cells: their common role in controlling autoimmunity, tumor immunity, and transplantation tolerance, Immunological reviews 182(1) (2001) 18-32.

[91] E.M. Shevach, R.A. DiPaolo, J. Andersson, D.M. Zhao, G.L. Stephens, A.M. Thornton, The lifestyle of naturally occurring CD4+ CD25+ Foxp3+ regulatory T cells, Immunol Rev 212(1) (2006) 60-73.

[92] Y. Belkaid, Regulatory T cells and infection: a dangerous necessity, Nat Rev Immunol 7(11) (2007) 875-88.

[93] B.T. Rouse, P.P. Sarangi, S. Suvas, Regulatory T cells in virus infections, Immunol Rev 212(1) (2006) 272-86.

[94] K. Kretschmer, I. Apostolou, E. Jaeckel, K. Khazaie, H. von Boehmer, Making regulatory T cells with defined antigen specificity: role in autoimmunity and cancer, Immunol Rev 212(1) (2006) 163-9.

[95] E. Xystrakis, S.E. Boswell, C.M. Hawrylowicz, T regulatory cells and the control of allergic disease, Expert Opin Biol Ther 6(2) (2006) 121-33.

[96] J.L. Coombes, N.J. Robinson, K.J. Maloy, H.H. Uhlig, F. Powrie, Regulatory T cells and intestinal homeostasis, Immunol Rev 204(1) (2005) 184-94.

[97] S. Hori, T. Nomura, S. Sakaguchi, Control of regulatory T cell development by the transcription factor Foxp3, Science 299(5609) (2003) 1057-61.

[98] J.D. Fontenot, M.A. Gavin, A.Y. Rudensky, Foxp3 programs the development and function of CD4+CD25+ regulatory T cells, Nat Immunol 4(4) (2003) 330-6.

[99] S.E. Allan, S.Q. Crome, N.K. Crellin, L. Passerini, T.S. Steiner, R. Bacchetta, M.G. Roncarolo, M.K. Levings, Activation-induced FOXP3 in human T effector cells does not suppress proliferation or cytokine production, International Immunology 19(4) (2007) 345-354.

[100] D.A. Vignali, L.W. Collison, C.J. Workman, How regulatory T cells work, Nature Reviews Immunology 8(7) (2008) 523.

[101] S. Sakaguchi, T. Yamaguchi, T. Nomura, M. Ono, Regulatory T cells and immune tolerance, Cell 133(5) (2008) 775-787.

[102] S. Sakaguchi, Naturally arising CD4+ regulatory T cells for immunologic self-tolerance and negative control of immune responses, Annu. Rev. Immunol. 22 (2004) 531-562.

[103] C.A. Piccirillo, E.M. Shevach, Naturally-occurring CD4+ CD25+ immunoregulatory T cells: central players in the arena of peripheral tolerance, Seminars in immunology, Elsevier, 2004, pp. 81-88.

[104] N. Cools, P. Ponsaerts, V.F. Van Tendeloo, Z.N. Berneman, Regulatory T cells and human disease, Clinical and Developmental Immunology 2007 (2008).

[105] C. Ustun, J.S. Miller, D.H. Munn, D.J. Weisdorf, B.R. Blazar, Regulatory T cells in acute myelogenous leukemia: is it time for immunomodulation?, Blood 118(19) (2011) 5084-5095.

[106] K. Wing, Z. Fehérvári, S. Sakaguchi, Emerging possibilities in the development and function of regulatory T cells, International immunology 18(7) (2006) 991-1000.

[107] G. Weitz-Schmidt, K. Welzenbach, V. Brinkmann, T. Kamata, J. Kallen, C. Bruns, S. Cottens, Y. Takada, U. Hommel, Statins selectively inhibit leukocyte function antigen-1 by binding to a novel regulatory integrin site, Nat Med 7(6) (2001) 687-92.

[108] E. Mira, B. Leon, D.F. Barber, S. Jimenez-Baranda, I. Goya, L. Almonacid, G. Marquez, A. Zaballos, A.C. Martinez, J.V. Stein, C. Ardavin, S. Manes, Statins induce regulatory T cell recruitment via a CCL1 dependent pathway, J Immunol 181(5) (2008) 3524-34.

[109] A.L. Rodriguez-Perea, J. Gutierrez-Vargas, G.P. Cardona-Gomez, C.J. Guarin, M. Rojas, P.A. Hernandez, Atorvastatin Modulates Regulatory T Cells and Attenuates Cerebral Damage in a Model of Transient Middle Cerebral Artery Occlusion in Rats, J Neuroimmune Pharmacol 12(1) (2017) 152-162.

[110] X. Xu, W. Gao, S. Cheng, D. Yin, F. Li, Y. Wu, D. Sun, S. Zhou, D. Wang, Y. Zhang, R. Jiang, J. Zhang, Anti-inflammatory and immunomodulatory mechanisms of atorvastatin in a murine model of traumatic brain injury, J Neuroinflammation 14(1) (2017) 167.

[111] G. Trinchieri, Proinflammatory and immunoregulatory functions of interleukin-12, Int Rev Immunol 16(3-4) (1998) 365-96.

[112] A.H. Wagner, M. Gebauer, B. Guldenzoph, M. Hecker, 3-hydroxy-3-methylglutaryl coenzyme A reductase-independent inhibition of CD40 expression by atorvastatin in human endothelial cells, Arterioscler Thromb Vasc Biol 22(11) (2002) 1784-9.

[113] W. Yang, M. Yamada, Y. Tamura, K. Chang, J. Mao, L. Zou, Y. Feng, K. Kida, M. Scherrer-Crosbie, W. Chao, F. Ichinose, Y.M. Yu, A.J. Fischman, R.G. Tompkins, S. Yao, M. Kaneki, Farnesyltransferase inhibitor FTI-277 reduces mortality of septic mice along with improved bacterial clearance, J Pharmacol Exp Ther 339(3) (2011) 832-41.

[114] P. Kopterides, M.E. Falagas, Statins for sepsis: a critical and updated review, Clin Microbiol Infect 15(4) (2009) 325-34.

[115] F. Qiang, C. Naiqiang, Y. Wenli, D. Chao, Percentages of CD4+ T regulatory cells and HLA-DR expressing monocytes in severe intra-abdominal infections, Scandinavian journal of infectious diseases 42(6-7) (2010) 475-478.

[116] B. Pertzov, N. Eliakim-Raz, H. Atamna, A.Z. Trestioreanu, D. Yahav, L. Leibovici, Hydroxymethylglutaryl-CoA reductase inhibitors (statins) for the treatment of sepsis in adults–a systematic review and meta-analysis, Clinical Microbiology and Infection (2018).

[117] S.-i. Kagami, T. Owada, H. Kanari, Y. Saito, A. Suto, K. Ikeda, K. Hirose, N. Watanabe, I. Iwamoto, H. Nakajima, Protein geranylgeranylation regulates the balance between Th17 cells and Foxp3+ regulatory T cells, International immunology 21(6) (2009) 679-689.

[118] D. Schmidt, J.J. Goronzy, C.M. Weyand, CD4+ CD7-CD28-T cells are expanded in rheumatoid arthritis and are characterized by autoreactivity, The Journal of clinical investigation 97(9) (1996) 2027-2037.

[119] T. Nakajima, S. Schulte, K.J. Warrington, S.L. Kopecky, R.L. Frye, J.r.J. Goronzy, C.M. Weyand, T-cell–mediated lysis of endothelial cells in acute coronary syndromes, Circulation 105(5) (2002) 570-575.

[120] G. Liuzzo, J.r.J. Goronzy, H. Yang, S.L. Kopecky, D.R. Holmes, R.L. Frye, C.M. Weyand, Monoclonal T-cell proliferation and plaque instability in acute coronary syndromes, Circulation 101(25) (2000) 2883-2888.

[121] S. Brugaletta, L.M. Biasucci, M. Pinnelli, G. Biondi-Zoccai, G. Di Giannuario, G. Trotta, G. Liuzzo, F. Crea, Novel anti-inflammatory effect of statins: reduction of CD4⁺CD28^{null} T lymphocyte frequency in patients with unstable angina, Heart 92(2) (2006) 249-250.

[122] X. Zhang, Y. Tao, L. Troiani, S. Markovic-Plese, Simvastatin inhibits IFN regulatory factor 4 expression and Th17 cell differentiation in CD4+ T cells derived from patients with multiple sclerosis, J Immunol 187(6) (2011) 3431-7.

[123] K. Maneechotesuwan, K. Kasetsinsombat, V. Wamanuttajinda, A. Wongkajornsilp, P. Barnes, Statins enhance the effects of corticosteroids on the balance between regulatory T cells and Th17 cells, Clinical & Experimental Allergy 43(2) (2013) 212-222.

[124] K. Lee, J. Moon, H. Choi, H. Kim, G. Hur, K. Jung, S. Lee, J. Kim, C. Shin, J. Shim, Immune regulatory effects of simvastatin on regulatory T cell-mediated tumour immune tolerance, Clinical & Experimental Immunology 161(2) (2010) 298-305.

[125] X.L. Li, Y.C. Dou, Y. Liu, C.W. Shi, L.L. Cao, X.Q. Zhang, J. Zhu, R.S. Duan, Atorvastatin ameliorates experimental autoimmune neuritis by decreased Th1/Th17 cytokines and up-regulated T regulatory cells, Cell Immunol 271(2) (2011) 455-61.

[126] S. Bereswill, M. Munoz, A. Fischer, R. Plickert, L.M. Haag, B. Otto, A.A. Kuhl, C. Loddenkemper, U.B. Gobel, M.M. Heimesaat, Anti-inflammatory effects of resveratrol, curcumin and simvastatin in acute small intestinal inflammation, PLoS One 5(12) (2010) e15099.

[127] S.M. Abtahi Froushani, N. Delirezh, R. Hobbenaghi, G. Mosayebi, Synergistic effects of atorvastatin and all-trans retinoic acid in ameliorating animal model of multiple sclerosis, Immunol Invest 43(1) (2014) 54-68.

[128] S. Brahmachari, K. Pahan, Myelin basic protein priming reduces the expression of Foxp3 in T cells via nitric oxide, J Immunol 184(4) (2010) 1799-809.

[129] Z. Li, L. Chen, X. Niu, J. Liu, M. Ping, R. Li, X. Xie, L. Guo, Immunomodulatory synergy by combining atorvastatin and rapamycin in the treatment of experimental autoimmune encephalomyelitis (EAE), J Neuroimmunol 250(1-2) (2012) 9-17.

[130] S. Elahi, R.H. Weiss, S. Merani, Atorvastatin restricts HIV replication in CD4+ T cells by upregulation of p21, AIDS 30(2) (2016) 171-83.

[131] P. Eller, K. Eller, A.M. Wolf, S.J. Reinstadler, A. Tagwerker, J.R. Patsch, G. Mayer, A.R. Rosenkranz, Atorvastatin attenuates murine anti-glomerular basement membrane glomerulonephritis, Kidney Int 77(5) (2010) 428-35.

[132] M.S. Weber, T. Prod'homme, S. Youssef, S.E. Dunn, L. Steinman, S.S. Zamvil, Neither T-helper type 2 nor Foxp3+ regulatory T cells are necessary for therapeutic benefit of atorvastatin in treatment of central nervous system autoimmunity, J Neuroinflammation 11 (2014) 29.

[133] S. Wu, G. Wang, R. Yang, Y. Cui, Anti-inflammatory effects of Boletus edulis polysaccharide on asthma pathology, Am J Transl Res 8(10) (2016) 4478-4489.

[134] D. Guillen, F. Cofan, E. Ros, O. Millan, M. Cofan, M. Brunet, Biomarker assessment of the immunomodulator effect of atorvastatin in stable renal transplant recipients and hypercholesterolemic patients, Mol Diagn Ther 14(6) (2010) 357-66.

[135] B. Al-Husein, Abstract 1713: Immunomodulatory effect of statins on Tregs within human colorectal cancer is determined by the stage of disease, Cancer Research 78(13 Supplement) (2018) 1713-1713.

[136] N. Sorathia, H. Al-Rubaye, B. Zal, The effect of statins on the functionality of CD4+ CD25+ FOXP3+ regulatory T-cells in acute coronary syndrome: a systematic review and meta-analysis of randomised controlled trials in Asian populations, European Cardiology Review 14(2) (2019) 123.

[137] A.A. Alsheikh-Ali, P.V. Maddukuri, H. Han, R.H. Karas, Effect of the magnitude of lipid lowering on risk of elevated liver enzymes, rhabdomyolysis, and cancer - Insights from large randomized statin trials, Journal of the American College of Cardiology 50(5) (2007) 409-418.

[138] Z. Mallat, H. Ait-Oufella, A. Tedgui, Regulatory T cell responses: potential role in the control of atherosclerosis, Curr Opin Lipidol 16(5) (2005) 518-24.

[139] E. Yakirevich, M.B. Resnick, Regulatory T lymphocytes: pivotal components of the host antitumor response, J Clin Oncol 25(18) (2007) 2506-8.

[140] T.J. Curiel, Tregs and rethinking cancer immunotherapy, J Clin Invest 117(5) (2007) 1167-74.

[141] G.J. Bates, S.B. Fox, C. Han, R.D. Leek, J.F. Garcia, A.L. Harris, A.H. Banham, Quantification of regulatory T cells enables the identification of high-risk breast cancer patients and those at risk of late relapse, Journal of Clinical Oncology 24(34) (2006) 5373-5380.

[142] T.J. Curiel, G. Coukos, L. Zou, X. Alvarez, P. Cheng, P. Mottram, M. Evdemon-Hogan, J.R. Conejo-Garcia, L. Zhang, M. Burow, Y. Zhu, S. Wei, I. Kryczek, B. Daniel, A. Gordon, L. Myers, A. Lackner, M.L. Disis, K.L. Knutson, L. Chen, W. Zou, Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival, Nat Med 10(9) (2004) 942-9.

[143] N. Kobayashi, N. Hiraoka, W. Yamagami, H. Ojima, Y. Kanai, T. Kosuge, A. Nakajima, S. Hirohashi, FOXP3+ regulatory T cells affect the development and progression of hepatocarcinogenesis, Clin Cancer Res 13(3) (2007) 902-11.

[144] F.M. Sacks, M.A. Pfeffer, L.A. Moye, J.L. Rouleau, J.D. Rutherford, T.G. Cole, L. Brown, J.W. Warnica, J.M. Arnold, C.C. Wun, B.R. Davis, E. Braunwald, The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. Cholesterol and Recurrent Events Trial investigators, N Engl J Med 335(14) (1996) 1001-9.

[145] N. Sattar, D. Preiss, H.M. Murray, P. Welsh, B.M. Buckley, A.J. de Craen, S.R.K. Seshasai, J.J. McMurray, D.J. Freeman, J.W. Jukema, Statins and risk of incident diabetes: a collaborative meta-analysis of randomised statin trials, The Lancet 375(9716) (2010) 735-742.

[146] S.P. Bapat, J.M. Suh, S. Fang, S.H. Liu, Y. Zhang, A. Cheng, C. Zhou, Y.Q. Liang, M. LeBlanc, C. Liddle, A.R. Atkins, R.T. Yu, M. Downes, R.M. Evans, Y. Zheng, Depletion of fat-resident T-reg cells prevents age-associated insulin resistance, Nature 528(7580) (2015) 137-+.

[147] L. Mascitelli, M.R. Goldstein, Statins, regulatory T cells and diabetes, Diabetic Medicine 33(8) (2016) 1152-1153.

[148] I. Ford, H. Murray, C.J. Packard, J. Shepherd, P.W. Macfarlane, S.M. Cobbe, G. West of Scotland Coronary Prevention Study, Long-term follow-up of the West of Scotland Coronary Prevention Study, N Engl J Med 357(15) (2007) 1477-86.

[149] A. Gruver, L. Hudson, G. Sempowski, "Immunosenescence of ageing." J Pathol. 211: 144-156, (2007).

[150] J. Shepherd, G.J. Blauw, M.B. Murphy, E.L. Bollen, B.M. Buckley, S.M. Cobbe, I. Ford, A. Gaw, M. Hyland, J.W. Jukema, Pravastatin in elderly individuals at risk of vascular disease (PROSPER): a randomised controlled trial, The Lancet 360(9346) (2002) 1623-1630.

[151] D. Hunt, P. Young, J. Simes, W. Hague, S. Mann, D. Owensby, G. Lane, A. Tonkin, Benefits of pravastatin on cardiovascular events and mortality in older patients with coronary heart disease are equal to or exceed those seen in younger patients: results from the LIPID trial, Annals of internal medicine 134(10) (2001) 931-940.

[152] N.K. Wenger, S.J. Lewis, D.M. Herrington, V. Bittner, F.K. Welty, Outcomes of using high- or low-dose atorvastatin in patients 65 years of age or older with stable coronary heart disease, Annals of internal medicine 147(1) (2007) 1-9.

[153] P. Hoffmann, T. Roumeguère, C. Schulman, R. Van Velthoven, Use of statins and outcome of BCG treatment for bladder cancer, New England Journal of Medicine 355(25) (2006) 2705-2707.

[154] I.R. Edwards, K. Star, A. Kiuru, Statins, neuromuscular degenerative disease and an amyotrophic lateral sclerosis-like syndrome: an analysis of individual case safety reports from vigibase, Drug Saf 30(6) (2007) 515-25.

[155] R. Pacheco, T. Gallart, C. Lluis, R. Franco, Role of glutamate on T-cell mediated immunity, J Neuroimmunol 185(1-2) (2007) 9-19.

[156] J. Kipnis, T. Mizrahi, E. Yoles, A. Ben-Nun, M. Schwartz, Myelin specific Th1 cells are necessary for post-traumatic protective autoimmunity, J Neuroimmunol 130(1-2) (2002) 78-85.

[157] J. Kipnis, T. Mizrahi, E. Hauben, I. Shaked, E. Shevach, M. Schwartz, Neuroprotective autoimmunity: naturally occurring CD4+CD25+ regulatory T cells suppress the ability to withstand injury to the central nervous system, Proc Natl Acad Sci U S A 99(24) (2002) 15620-5.

[158] Y. Belkaid, B.T. Rouse, Natural regulatory T cells in infectious disease, Nat Immunol 6(4) (2005) 353-60.

[159] B.T. Rouse, S. Suvas, Regulatory cells and infectious agents: detentes cordiale and contraire, J Immunol 173(4) (2004) 2211-5.

[160] E.M. Aandahl, J. Michaelsson, W.J. Moretto, F.M. Hecht, D.F. Nixon, Human CD4+ CD25+ regulatory T cells control T-cell responses to human immunodeficiency virus and cytomegalovirus antigens, J Virol 78(5) (2004) 2454-9.

[161] M.R. Goldstein, L. Mascitelli, F. Pezzetta, The double-edged sword of statin immunomodulation, Int J Cardiol 135(1) (2009) 128-30.

[162] J.N. Stoop, R.G. van der Molen, C.C. Baan, L.J. van der Laan, E.J. Kuipers, J.G. Kusters, H.L. Janssen, Regulatory T cells contribute to the impaired immune response in patients with chronic hepatitis B virus infection, Hepatology 41(4) (2005) 771-8.

[163] R. Cabrera, Z. Tu, Y. Xu, R.J. Firpi, H.R. Rosen, C. Liu, D.R. Nelson, An immunomodulatory role for CD4(+)CD25(+) regulatory T lymphocytes in hepatitis C virus infection, Hepatology 40(5) (2004) 1062-71.

[164] S. Kagami, T. Owada, H. Kanari, Y. Saito, A. Suto, K. Ikeda, K. Hirose, N. Watanabe, I. Iwamoto, H. Nakajima, Protein geranylgeranylation regulates the balance between Th17 cells and Foxp3+ regulatory T cells, Int Immunol 21(6) (2009) 679-89.

[165] S. Zhang, L. Luo, Y. Wang, M. Rahman, M. Lepsenyi, I. Syk, B. Jeppsson, H. Thorlacius, Simvastatin protects against T cell immune dysfunction in abdominal sepsis, Shock 38(5) (2012) 524-31.

[166] S. Wu, R. Yang, G. Wang, Anti-asthmatic effect of pitavastatin through aerosol inhalation is associated with CD4+ CD25+ Foxp3+ T cells in an asthma mouse model, Sci Rep 7(1) (2017) 6084.

[167] K. Mausner-Fainberg, G. Luboshits, A. Mor, S. Maysel-Auslender, A. Rubinstein, G. Keren, J. George, The effect of HMG-CoA reductase inhibitors on naturally occurring CD4+CD25+T cells, Atherosclerosis 197(2) (2008) 829-839.

[168] Z.X. Wang, C.Q. Wang, X.Y. Li, Y. Ding, G.K. Feng, X.J. Jiang, Changes of naturally occurring CD4⁺CD25⁺ FOXP3⁺ regulatory T cells in patients with acute coronary syndrome and the beneficial effects of atorvastatin treatment, International Heart Journal 56(2) (2015) 163-169.

Figure legends

Figure 1. Pleiotropic effects of statins. In addition to their lipid-lowering effects, statins have many pleiotropic effects such as improved endothelial function, angiogenesis, plaque stability, platelet aggregation, anti-thrombotic, anti-oxidant, anti-inflammatory effect and immune modulation.

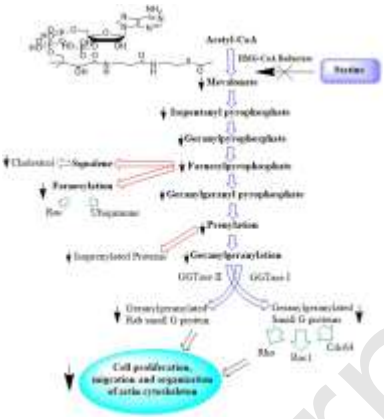


Figure 2. Mechanisms of actions of statins. Statins inhibit the HMG-CoA reductase that result in reducing all mevalonate pathway products like cholesterol and nonsteroidal isoprenoid compounds (geranylgeranyl pyrophosphate (GGPP) and farnesyl pyrophosphate (FPP), such as Rho, Rac, and Cdc42. These molecules are signaling proteins involved in cell proliferation, migration and muscle contraction mediated via actin.

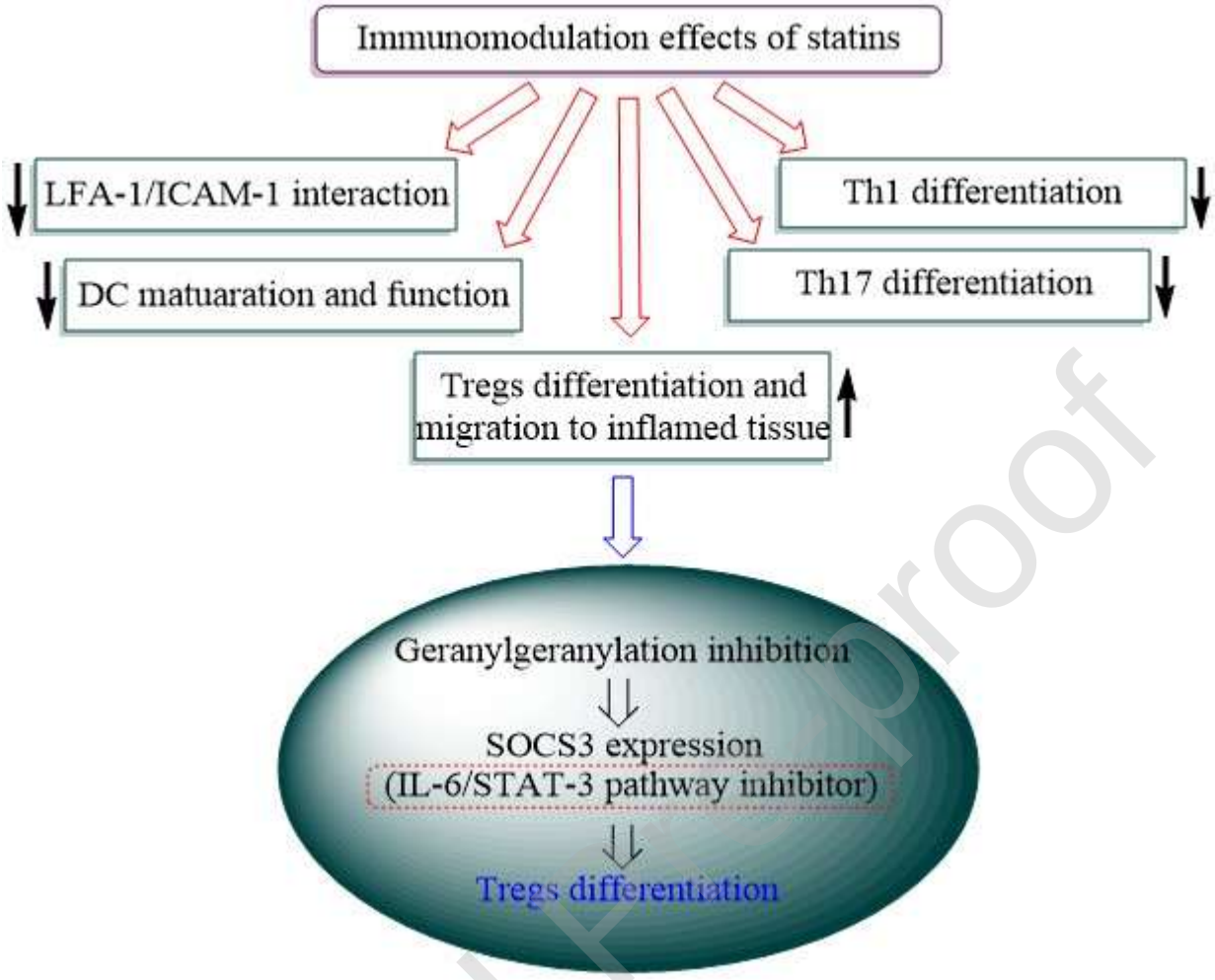


Figure 3. Immune modulation effects of statins. Immune regulatory effects of statins include inhibition of LFA-1/ICAM-1 interaction, suppression of DC maturation (decrease of MHC-II and co-stimulatory molecules expression), inhibition of Th1/Th17 and differentiation and induction of Tregs.

Table 1. Details of studies investigating the regulatory effects of statins (alone, or in combination with other therapies) in experimental models

Intervention		**1st Author**	**Year**	**Ref**	**Species / Strain / Cell-line**	**Disease model**	**Effect on of statins on Treg-cells**	**Effect on other mediators**	**Other observations and interpretation**
Statin	**Other experimental drugs**								
Atorvastatin	All-trans retinoic acid (ATRA)	Abtahi Froushani	2014	[127]	Mouse	EAE	Cell number ↑ by combination therapy:		No evidence of an independent effect of atorvastatin on Tregs
Atorvastatin	None	Chen Z.et al,	2018	[63]	Mouse	Experimental autoimmune encephalomyelitis	Treg (FOXP3 expression) ↑		Atorvastatin suppressed of EAE development
Atorvastatin	None	Eller	2010	[131]	Mouse	Autologous model of anti-GBM GN	Tregs number and FoxP3 expression not changed		Statin teharapy was associated with

									antiinflammatory effects (IL-10 ↑) and reduced renal damage.
Atorvastatin	None	Elahi	2016	[130]	CD4 T cells	Cells infected with HIV-1 ± atorvastatin	Tregs number and expression ↑ by atorvastatin		Atorvastatin induced resistance of CD4 T cells to HIV-1 infection via p21 upregulation
Atorvastatin	None	Li	2016	[66]	Rat	Experimental autoimmune myasthenia gravis (EGAM)	Foxp3↑ Tregs ↑		Suppressed the clinical symptoms of EAMG
Atorvastatin	None	Li	2013	[64]	Rat	EGAM	Foxp3↑ Tregs ↑		Immune tolerance

									induced in EAMG rats.
Atorvastatin	Rapamycin	Li	2012	[129]	Mouse	EAE	Foxp3↑ Tregs ↑		EAE development attenuated
Atorvastatin	None	Rodríguez-Perea	2016	[109]	Rat	Transient Middle Cerebral Artery Occlusion	Cervical/lymph node Tregs ↑, brain regs↓		Infarct volume, neurological deficit, glial cell activation↓
Atorvastatin	None	Weber	2014	[132]	Mouse	EAE		None	Beneficial effects on EAE
Atorvastatin	None	Zhao	2015	[82]	Mouse (ApoE-/-)	Atherosclerosis	Foxp3↑ Tregs ↑		Delayed the development of atherosclerosis and improved plaque Stability.

Atorvastatin	None	Xu	2017	[110]	Mouse (C57BL/6)	Traumatic brain injury murine model	Tregs ↑		Total microglia/ macrophage activation and neuronal apoptosis ↓
Atorvastatin	None	Xu H.et al,	2014	[65]	Rat	Autoimmune neuritis (atorvastatin-modified dendritic cells)	Tregs in lymph node ↑ Foxp3 in thymus ↑		Reduced severity of clinical autoimmune neuritis
Simvastatin	Soybean extract (Glycine max L.)	Atho'illah	2017	[85]	Mouse (Balb/C)	High Fat Fructose Diet (HFFD)	Cell number ↑ by simvastatin alone		Simvastatin demonstrated potentially anti-inflammatory effects
Simvastatin	Resveratrol, Curcumin	Bereswill	2010	[126]	Mouse	Th1-type ileitis	Cell number ↑ by simvastatin alone		Simvastatin demonstrated potentially anti-

									inflammatory effects
Simvastatin	None	Kagami S.	2009	[164]	BALB/c mice and SCID mice	Treg/Th17 differentiation	Treg differentiation↑ by simvastatin		protein geranylgeranylation ↓ differentiation of Foxp31 Tregs
Simvastatin	None	Kim	2010	[84]	Mouse	T transgenic RAG2 -/-, Foxp3 -GFP Knock-in (Foxp3gfp) models	Tregs ↑		Inhibition of protein geranylgeranylation, Methylation of Foxp3 promoter↓
Simvastatin	None	Lee	2010	[86]	Mouse	Tumor induction by 3LL line cells	Foxp3↑ Tregs ↑		No effect on tumor growth

Simvastatin	None	Meng	2012	[73]	Mouse (ApoE–/–)	Atherosclerosis	Foxp3↑ Tregs ↑		Potential contribution of Treg to antiatherosclerotic effect of statins
Simvastatin	None	Song G.	2015	[87]	Mouse (LDLR-/-)	Atherosclerosis	Tregs ↑		Improved atherosclerotic plaque stability
Simvastatin	None	Zhang	2012	[165]	Mouse (C57BL/6)	Polymicrobial sepsis	Treg development↓		Prevention of systemic inflammation, Decrease of CLP-induced bacteremia
Simvastatin	Aspirin	Zhu	2015	[74]	Rat	Cardiac transplant	Circulating Tregs ↑ by atorvastatin alone and combination therapy		Survival time of heart allografts ↑ by

									combination therapy
Lovastatin	None	Mira E.et al,	2008	[108]	Mouse	Candida albicans-induced delayed-type hypersensitivity (DTH)	Foxp3↑ Tregs ↑		Immune system modulation
Pitavastatin	None	Wu	2017	[166]	Mouse	Asthma	Tregs ↑ in (BALF)		Allergen-induced airway resistance ↓
Pravastatin	Boletus edulis polysac-charide	Wu	2016	[133]	Mouse	Asthma	Tregs ↑ in bronchoalveolar lavage fluid (BALF) by pravastatin alone		Combination therapy improved pathology, airway resistance, and ↓ anti-

									inflammatory markers
Pravastatin	L-N6-(1-iminoethyl)-lysine hydrochloride	Brahmachari S.et al,	2010	[128]	Mouse	MBP immunisation	Splenic markers of cells ↑ by pravastatin alone		Pravastatin demonstrated potentially anti-inflammatory effects

MMCS: maximum mean clinical score, **AMCS**: average mean clinical score, **Anti-GBM GN**: anti-glomerular basement membrane, **TIGIT**: T-cell immunoglobulin and Immunoreceptor tyrosine-based inhibitory motif domain, **3LL**: Lewis lung cancer, **EAMG**: Experimental autoimmune

Table2. Characteristics of studies on regulatory effects of statins in humans and human tissues

Intervention		**1st author**	**Year**	**Ref**	**Study design**	**n**	**Follow-up**	**Participants**	**Findings**
Statin	**Comparator**								
Atorvastatin	No drug	Guasti	2016	[88]	Prospective, non-randomised	30	3 months	Patients initiating statin therapy v. healthy controls	Dyslipidaeimic patients had ↑ viability and proliferation, of CD4+ , CD25+ and FoxP3+ cells which was unaffected by atorvastatin therapy
Atorvastatin	None	Guillen	2010	[134]	Prospective, non-randomised	50	3 months	Stable Renal Transplant Recipients v. Hypercholesterolemic Patients	In hypercholesterolemic, but not stable renal transpant patients there was a trend towards ↑ tregs after statin therapy. Authors conclude statin treatment cannot be considered as a means to

									reduce the dose of immunosuppressive agents.
Atorvastatin	No drug	Hu	2007	[71]	Prospective, non-randomised	66	2 weeks	Acute Coronary Syndrome (ACS) patients v. healthy controls	Treg percentage was lower in ACS than healthy control. Number and and inhibitory function of Treg in ACS patients was↑ after statin therapy
Atorvastatin, Pravastatin, Mevastatin	N/A	Mausner-Fainberg.et al,	2008	[167]	Experimental non-randomised	5	N/A	PBMCs from healthy donors	In vitro and Ex-vivo treatment of atorvastatin ↑ Tregs and ↑ Foxp3

Atorvastatin, lovastatin	N/A	Rodríguez-Perea	2015	[58]	Prospective, non-randomised	18	45 days	Subjects with normal cholesterol levels	Treg frequency and FOXP3 mRNA levels ↑ at 30 days, but not 45 days
Atorvastatin	No drug	Tang	2011	[77]	Randomised	97	12 weeks	Rheumatiod Arthritis patients and healthy controls	Atorvastatin upregulated Tregs and reduces clinical disease activity in patients with rheumatoid arthritis.
Atorvastatin + conventional therapy	Conventional therapy only	Wang	2014	[168]	Randomised	180	4 weeks	ACS patients and healthy controls	In ACS patients, the percentage and inhibitory properties of nTregs were reduced, nTreg number and function was increased in the atorvastatin group compared with the non-atorvastatin group.
Atorvastatin	Placebo	Zhang	2011	[83]	Randomised	112		Patients With STEMI receiving PCI	Tregs number and the mRNA level of FoxP3 and TGF- ?? ↑, INF-ɣ levels ↓

									Suppress of inflammation and improve the prognose of diseases
Rosuvastatin	No drug, Telmisartan, Telmisartan + rosuvastatin	Liu	2014	[79]	Randomized, 2x2 factorial	159	3 months	Hypertensive patients with carotid atherosclerosis	Both rosuvastatin and telmisartan improve Th17/Treg functional imbalance. Combination therapy is synergistic and could augment the clinical treatment of hypertensive patients with atherosclerosis.
Rosuvastatin	Placebo	Xie	2014	[81]	Randomised	159	24 hours, 30 days	Patients with ACS receiving PCI	Rosuvastatin treatment was associated with ↑ Treg cells at 24 hours and ↓ major cardiac adverse events than placebo at 30 days.
Simvastatin	Fluticasone propionate,	Maneechotesuwan. 2012	2012	[123]	Experimental non-randomised	8	N/A	Peripheral blood mononuclear cells from asthmatic patients	Simvastatin enhances the effect of FP on Treg/Th17 ratio

	Fluticasone propionate imvastatin								

myasthenia gravis, **DTH**: delayed-type hypersensitivity reaction, **LDLR**: Low-density lipoprotein receptor-knockout, **BALF**: bronchoalveolar lavage, **GARP**: Glycoprotein A Repetitions Predominant fluid **ACS**: Acute Coronary Syndrome, **RORɣT**: retinoic acid receptor related orphan receptor, **STEMI**: ST-Segment Elevated Myocardial Infarction, **PCI**: Primary Percutaneous Coronary Intervention, **cTnI**: cardiac troponin I.