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

Nilofer Sorathia,[1,2,3] Hussein Al-Rubaye[3] and Benham Zal[1,2]

1. Medipathways College London, London, UK; 2. University of Buckingham, Buckingham, UK; 3. St George's, University of London, London, UK

Abstract

Acute coronary syndrome (ACS) is characterised by increased effector cells and decreased regulatory T-cells (Tregs). Statins have been shown to be clinically beneficial in ACS patients. This effect could be mediated via the induction of Tregs in ACS patients. The aim of this systemic review and meta-analysis was to evaluate whether statin therapy enhances the frequency of Tregs determined by CD4+CD25+FOXP3+ in this subset of patients. A comprehensive search of PubMed and Embase was performed. Studies were restricted to randomised controlled trials that quantified CD4+CD25+FOXP3+ cell frequency by flow cytometric analysis before and after statin treatment in adults diagnosed with ACS. A minimum of at least two of the conventional markers to identify Tregs was compulsory. Four randomised controlled trials studies (439 participants) were included, all with low-to-moderate risk of bias. Pooled data showed a significant increase in Treg frequency after statin therapy in ACS patients. A further meta-regression and subgroup analysis also showed a negative dose-related effect, and a statin type-related effect (rosuvastatin versus atorvastatin), respectively. The results confirmed that statins positively alter the frequency of Tregs, which may indicate a potential mechanism of their therapeutic effect. However, there was a risk of information bias due to the markers used to identify Tregs, which was not fully explored, therefore, further randomised controlled trials should utilise markers of Tregs, such as the FOXP3 locus (Treg-specific demethylated region), for identification.

Statins are most frequently prescribed for the primary prevention of cardiovascular disease due to their lipid-lowering properties. They are known to exert their effect by competitive inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. The reduction in the synthesis of cholesterol induces upregulation of LDL receptors in the liver, resulting in increased clearance and decreased deposits of LDL in blood vessels.[1] These LDL deposits are central to the induction of atherogenesis once oxidised by reactive oxygen species.[2] Reducing circulating LDL leads to less oxidised LDL, which causes less damage.

However, it was discovered that the therapeutic effect of statins is not solely due to the reduction of LDL. This is because statins are able to reduce mortality in healthy individuals with a normal LDL cholesterol profile.[3] Similarly, other cholesterol-lowering methods, such as ileal bypass surgery or bile acid sequestrants, do not have an immediate clinically beneficial result, despite the drop in LDL.[4]

Statins also show a significant reduction in morbidity, mortality, recurrent unstable angina incidents, non-fatal MI after 4 months and reduced rehospitalisation in just 30 days for acute coronary syndrome (ACS) patients.[5–9] Studies also demonstrated that the use of a higher statin dose strategy did not increase the clinical benefit, despite greater LDL reduction.[10]

Apart from their lipid-lowering ability, statins have various immunomodulatory properties that have recently been identified.[11] Inhibition of HMG-CoA reductase not only suppresses cholesterol synthesis, but also various inflammatory pathways due to the intermediates in the mevalonate pathway. Two isoprenoids, farnesyl-pyrophosphate and geranylgeranyl-pyrophosphate, are involved in post-translational modifications, in particular, the prenylation of GTPases, such as Ras, Rac, Rho and Cdc42.[12] These binary switches are involved in various inflammatory pathways activated by their effect on cell signalling via phophatidylinositol-3-kinase, mitogen-activated protein kinase and nuclear factor kappa-light chain-enhancer of activated B-cells.

Of note, Rho activates nuclear factor kappa-light-chain-enhancer of activated B-cells, which reduces endothelial nitric oxide synthase.[13] Inhibition by statins would increase nitric oxide bioavailability and improve vascular function, which could explain how statins are able to improve vascular endothelial function within 3 hours of treatment
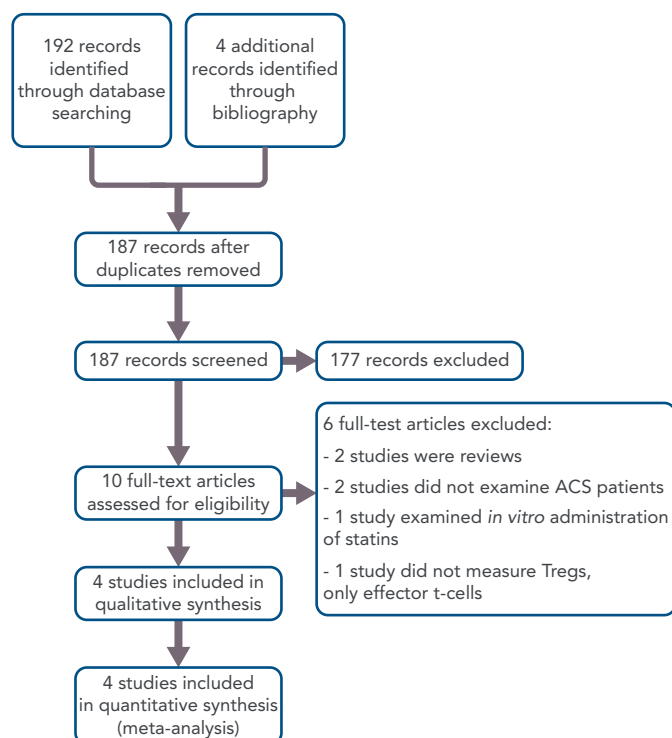
ACS = acute coronary syndrome; Tregs = regulatory T-cell.

in healthy patients.[14] Inhibition of these pathways also causes a downregulation of vascular cell adhesion protein 1 and tumour necrosis factor-alpha-induced expression of intercellular adhesion molecule 1, reducing the ability of macrophages to bind to endothelial cells.[15] Macrophage activity is also disrupted because of the reduction in chemoattractants, such as monocyte chemoattractant protein 1.

Statins have also been shown to favour expression of anti-inflammatory cytokines, such as interleukin (IL)-4, IL-10 and transforming growth factor-beta (TGF-beta), while downregulating interferon-gamma (IFN-gamma), IL-6, IL-1 and IL-8 due to a shift from T-helper cell type 1 to T-helper cell type 2 response, as shown in a mouse model of autoimmune encephalomyelitis treated with statins.[16] A similar milieu of anti-inflammatory mediators was seen to improve graft versus host disease in animal models with the treatment of atorvastatin due to its ability to inhibit protein geranylgeranylation.[17]

Independent of HMG-CoA reductase inhibition, statins have been shown to bind to an allosteric site on lymphocyte function-associated antigen 1, thereby blocking the recruitment of lymphocytes and their cytotoxicity.[18] All the effects of statins highlighted demonstrate that statins tilt the balance towards an anti-inflammatory state, so it is not surprising when statins are shown to significantly reduce regulatory T-cells (Tregs) in healthy individuals.[19]

Tregs are a subpopulation of CD4+ T-cells that are induced by an inflammatory stimulus in the peripheries from naive CD4+ cells, but they can also be derived in the thymus as CD4+CD25- cells.[20] It has been found that 5–10% of circulating CD4+ T-cells are Tregs.[21] They are characterised by the constitutive expression of CD25 and CTLA4 receptors, and FOXP3; a transcription factor that promotes T-cell differentiation into Tregs.[22,23] Although these markers may also be found on classic CD4+ T-cells,[24,25] research for a specific marker of Tregs has identified a demethylation site in a 5'untranslated region of FOXP3 called Treg-specific demethylated region, which allows for discrimination of Tregs.[26]

Tregs are regarded as the master switch of immune system regulation by exerting their effect through the modulation of both adaptive and innate immune responses through multiple mechanisms. The main four mechanisms by which Tregs exert their effects are through the release of inhibitory cytokines, antigen-dependent inhibition of immune responses, and direct contact and inhibition of APC maturation, as well as the interaction with CD80/86 complex on APC via CTLA4.

Tregs release immunosuppressive cytokines IL-10, IL-35 and TGF-beta. Secretion of IL-10 by Tregs inhibits synthesis of pro-inflammatory cytokines (such as IL-6 and IL-2)and expression of major histocompatibility complex class II by APC, preventing antigen presentation.[27] IL-35 secretion mediates induction of CD4+ cells into Tregs while also inhibiting proliferation of T-cells.[28] TGF-beta released by Tregs decreases CD28 expression (a co-stimulatory molecule required for the activation of T-cells) and induces CD4+ differentiation into Tregs.[29]

Another functional mechanism of Tregs is antigen-dependent suppression. Tregs can be activated by an antigen via its T-cell receptor, where after it may suppress a T-cell with any antigen specificity.[30] Direct contact of Tregs with APC exerts multiple actions. One of them is downregulation of co-stimulatory ligands CD80/CD86, which are required for T-cell activation.[31] They also prevent dendritic cell activation by inhibiting effector T-helper cell type 1 cells (reducing IFN-gamma and tumour necrosis factor-alpha production), which is required for antigen presentation.[32]

Tregs also modulate macrophage activity, in particular, reducing their production of matrix metalloproteinases.[33] However, the predominant mechanism by which Tregs exert their inhibitory effect on immune responses is through expression of CTLA4 molecule on its surface. CTLA4 binds the CD80/86 complex on APC, hence preventing CD28-mediated activation of CD4+T-helper cells, leading to downregulation of both B-cell and cytotoxic T-lymphocyte (CTL)-mediated inflammatory responses.[34] Binding also generates inhibitory signals to decrease the expression of CD28 while enhancing FOXP3 expression.[35]

Finally, CTLA4 and CD80/CD86 binding can also induce expression of indoleamine 2,3-dioxygenase, which catabolises tryptophan into pro-apoptotic mediators.[36] Other mechanisms include Treg-mediated cytolysis of B-cells, natural killer cells and CTL via the release of granzyme A and perforin.[37] Tregs can also cause metabolic disruption of CTL, as high levels of CD25 on Tregs cause depletion of IL-2 in the local environment, starving the effector T-cells and inducing apoptosis.[38] Overall, Tregs play a significant role in the prevention of autoimmunity and induction of peripheral tolerance, but overactivation may also prevent immunity to certain pathogens or tumours.

Vascular endothelial injury and dysfunction are the initial stages of coronary atheroma formation. The influx of LDL and its oxidisation within the coronary vessels are followed by activation and recruitment of monocyte-derived macrophages, leading to formation of lipid-laden foam cells in the vessel lumen and early plaque lesions.[2] The damaged endothelium and macrophages release cytokines and growth factors including IFN-gamma, platelet-derived growth factor and TGF-beta,

Table 1: Characteristics of Included Studies

Author	Statin		Control		Statin and dose (mg/day)	Length of administration	Treg markers used
	M	F	M	F			
Xie at al. 2014[54]	59	20	56	24	Rosuvastatin 40	24 hours	CD4+FOXP3+
Hu et al. 2007[53]	24		24		Atorvastatin 10	2 weeks	CD4+CD25-
Wang et al. 2015[52]	44	16	42	18	Atorvastatin 20	4 weeks	CD4+CD25-FOXP3+
Zhang et al. 2011[51]	42	10	52	8	Atorvastatin 80	3 months	CD4+CD25-FOXP3+
Total	**215**		**224**				

F = female, M = male, Treg = regulatory T-cell.

which signal for the recruitment of pro-inflammatory T-cells and proliferation of smooth muscle cells.[39]

The chronic vicious cycle of inflammation and proliferation of collagen-secreting smooth muscle cells gradually leads to the thickening of the lesion and formation of a fibrolipid cap covering a highly thrombogenic necrotic core.[40] Such advanced coronary plaques can, however, become destabilised and rupture through multiple inflammatory processes predominantly mediated by T-cells.[41]

In this context, an aggressive and unusual subpopulation of CD4+ T-cells, CD4+CD28- cells, has been reported to significantly contribute to coronary plaque destabilisation.[42] These T-cells are believed to be activated by an auto-antigen, namely, human heat shock protein 60, expressed within the plaque environment. Human heat shock protein 60 is a chaperone stress protein that is constitutively expressed in all cells, and its expression is significantly increased during inflammation.[42] Upon antigen exposure and activation, these T-cells are able to directly target and lyse the smooth muscle cell constituent of the plaque through the release of cytotoxic granules, resulting in plaque rupture and exposure of its thrombogenic content to the coronary blood flow. Once activated, CD4+CD28- cells also produce IFN-gamma, which activates local macrophages, leading to increased secretion of matrix metalloproteinase enzymes.[43,44] These enzymes can gradually degrade the fibrous plaque cap, and contribute to plaque destabilisation and rupture resulting in thrombosis and ACS.

ACS is characterised by heightened inflammatory status, and T-cells within the coronary environment, in particular, have been implicated in both disease initiation and progression.[43,45] It has been shown that the frequency of Tregs is reduced in patients with non-ST-segment elevation MI, ST-segment elevation MI, acute MI and unstable angina.[46,47] In addition, Wigren et al. showed that individuals with low levels of Tregs were at increased risk of a first coronary event.[48] Furthermore, Tregs are reported to have compromised functional efficacy, increased tendency for apoptosis and reduced responsiveness to T-cell receptor-mediated induction.[46,49,50] These findings have led to suggestions that Tregs are unable to modulate coronary disease progression through tilting the internal milieu in support of a pro-inflammatory state.

ACS patients have been shown to benefit from the anti-inflammatory effects of statin treatment; although, the exact mechanisms by which these effects are exerted are still debatable. Since ACS is characterised by diminished frequency and function of Tregs, their induction could potentially shift the immunomodulatory balance to an anti-inflammatory state. To date, there is no systematic review and

meta-analysis compiling results from different randomised controlled trials (RCTs) in ACS patients.

The aim of this systematic review and meta-analysis is to evaluate whether statin therapy enhances the frequency of CD4+CD25+FOXP3+ Tregs in patients with ACS.

Methods

Eligibility Criteria

Studies were restricted to RCTs, with no minimum or maximum length of follow-up. Adults diagnosed with ACS were included in this study. Studies that quantified Tregs cell frequency by flow cytometric analysis before and after statin treatment were included. A minimum of at least two of the conventional markers from CD4+CD25+FOXP3+ was compulsory to identify Tregs.

The intervention must have been any of the oral tablet forms of statins: simvastatin, atorvastatin, rosuvastatin, pravastatin and fluvastatin, of which at least a minimum of 10 mg and a maximum of 80 mg dosage were prescribed

Search Strategy

Studies from all years and languages were allowed for inclusion. MeSH terms were used to add synonyms and to increase the scope of the search (*Supplementary Material Appendix 1*). Following identification of eligibility criteria and the relevant search terms, the PubMed (1996 to search date) and EMBASE (1947 to search date) databases were searched on 13 June 2017. The detailed search strategy can also be found in *Supplementary Material Appendix 2*.

Data Collection and Analysis

The title and abstract of all studies retrieved were evaluated for relevance to the objective of this review using the population, intervention, comparator, outcomes and study type (*Supplementary Material Appendix 1*). The piloted spread sheet contained the population, intervention, comparator, outcomes and study type, p-value, power, mean, Treg percentage, SD, 95% CI and length of the study.

Quality Assessment of Included Studies

To assess the risk bias of eligible RCTs, authors (NS and HA) used the Cochrane risk of bias tool. The randomisation, allocation concealment, blinding of patients, researchers, outcome, completeness of data reported and selection of biomarkers used to identify Treg cells were evaluated. Studies should state the patient characteristics, exclusion criteria, lost to follow-up and number of patients in each group. If studies did not use all three CD25, CD4 and FOXP3 biomarkers, they were classified as high risk of information bias, as this may increase the probability of an impure yield of Tregs. Studies needed to report that
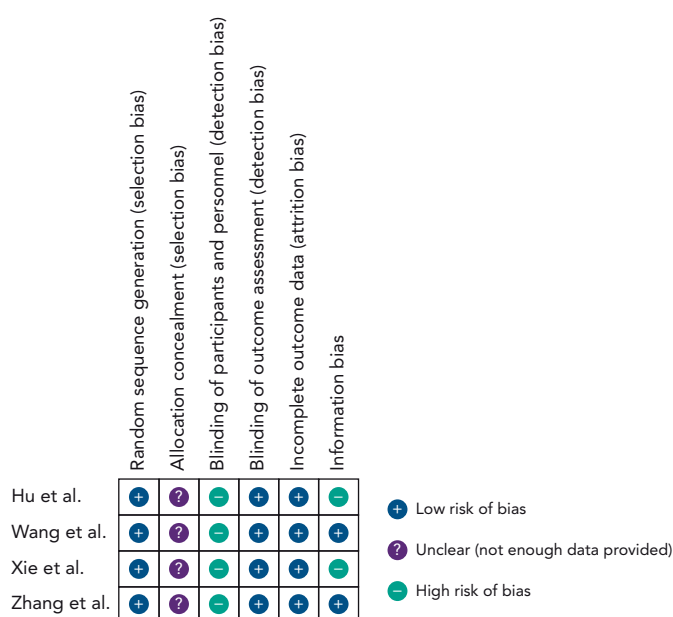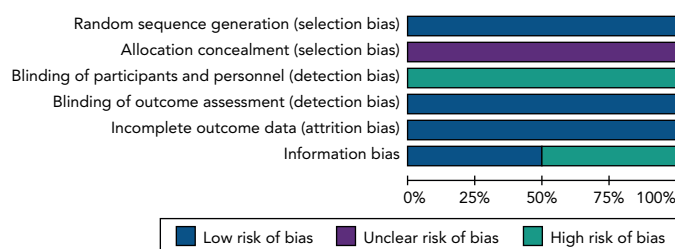
Figure 2: Risk of Bias Summary

Figure 3: Percentage Risk of Bias in Each Domain of the Risk of Bias Assessment of the Studies Included in the Review

division of participants into control or statin therapy groups had been conducted randomly. However, a detailed account of randomisation was not required, as long as the patient characteristics of both groups were included and did not differ significantly (p>0.05).

Synthesis of Results

Standardised mean difference (SMD) was used as a summary measure due to the nature of the outcomes. As the data type is continuous, the statistical method to pool the results will be inverse variance.

Assessment of Homogeneity

Chi-squared and I-squared were calculated using Review Manager 5.3. Heterogeneity was interpreted according to guidelines by the Cochrane Handbook 5.1.0. A random effects model was used, as the I-squared was significant (>50%). Due to the significant heterogeneity, a subgroup analysis was performed by the type of statin. A meta-regression was also performed to see the dose-related effect.

Results

Study Selection

Search Results

The search results retrieved 192 articles. Four additional records were identified through bibliographies. After duplicates were removed, 187 articles remained. Ten full-text articles were reviewed for inclusion,

of which four met the full eligibility criteria. *Figure 1* shows the flow diagram for study selection.

Characteristics of Included Studies

There was a total of 439 participants from all the included studies, of which 215 received statin therapy and 224 were part of the control groups. The characteristics of the included studies are summarised in *Table 1*.

All studies were RCTs reported in English. Three studies administered atorvastatin oral tablets, one at a dose of 80 mg, and the rest at 20 mg and 10 mg, respectively.[51–53] A study by Xie et al. used rosuvastatin oral tablets at a dose of 40 mg.[54] Only studies by Zhang et al. and Wang et al. used placebos in the control group, compared with Hu et al. and Xie et al., who did not administer any medication to the control group, besides conventional ACS medication.[51–54]

Risk of Bias

Random Sequence Generation

The four studies had a low risk of bias due to randomisation (*Figures 2* and *3*).[51–54]

Allocation Concealment

None of the studies provided adequate information to assign bias as low or high, consequentially the risk of bias remains unclear.[51–54]

Blinding

All studies had a high risk of bias by blinding due to the nature of the study, as blinding was not possible.[51–54]

Incomplete Outcome Data

All studies included had a low risk of incomplete outcome data, as all results were reported for outcomes identified at the start of the review.[51–54]

Information Bias

Two studies used the three known markers, CD4, CD25 and FOXP3, to identify Tregs.[51,52] Therefore, they had a low risk of bias. In contrast, Hu et al. and Xie et al. only used two markers each – CD4 and CD25, and CD4 and FOXP3, respectively.[53,54] Both Hu et al. and Xie et al. used fewer surface markers to identify Tregs, which increased the chance of an impure yield. Further details of the rationale of bias assessment are found in *Supplementary Material Appendix 3*.

Synthesis of Results

There was an increase in the mean frequency of Treg percentage in patients who received statins in all four studies (*Table 2*). Each study reported a p<0.01 for Treg frequency. Meta-analysis for Treg frequency demonstrated significantly higher values in patients treated with statins (SMD 1.93; 95% CI [1.16–2.71]; p<0.00001; *Figure 4*).

Heterogeneity of Results

The heterogeneity of effect sizes resulted in a I-squared value of 91%. These results signify that the effect sizes are heterogeneous with very high inconsistency in the data.

Subgroup Analysis

A subgroup analysis of studies that only used atorvastatin is shown in *Figure 5*. This was carried out by removing the study by Xie et al., which used rosuvastatin. The meta-analysis still indicated a

Table 2: Summary Data of Each Study Included in the Review

Study	Statin therapy		Control	
	Sample size	Mean (%) ± SD [95% CI] of Treg quantity post-intervention	Sample size	Mean (%) ± SD [95% CI] of Treg quantity post-intervention
Xie at al. 2014[54]	79	9.65 ± 2.10 [9.18–10.11]	80	4.97 ± 0.87 [4.77–5.16]
Hu et al. 2007[53]	24	6.47 ± 1.75 [5.76–7.17]	24	3.26 ± 1.71 [2.57–3.94]
Wang et al. 2015[52]	60	6.47 ± 1.75 [6.02–6.91]	60	3.26 ± 1.71 [2.82–3.69]
Zhang et al. 2011[51]	52	7.64 ± 3.16 [6.78–8.49]	60	4.56 ± 2.05 [2.4–5.07]

Treg = regulatory T-cell.

Figure 4: Forest Plot Showing the Standardised Mean Difference of Regulatory T-cells Frequency in the Control and Statin Groups
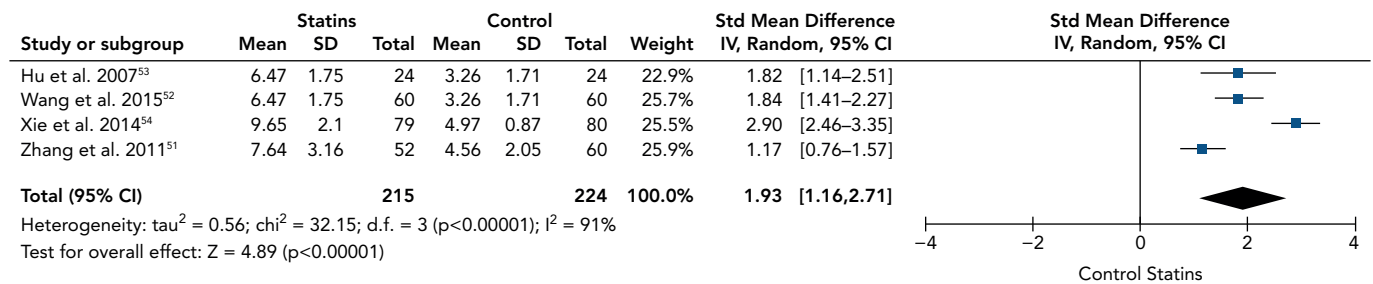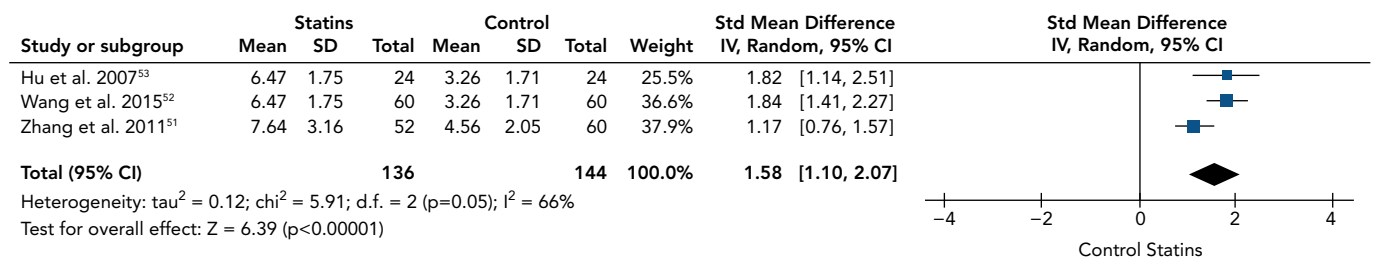

Figure 5: Forest Plot Showing the Standardised Mean Difference of Regulatory T-cells Frequency in the Control and Atorvastatin Groups, While Excluding Xie et al., Which Used Rosuvastatin

significant increase in Treg frequency compared with the control group (SMD 1.58; 95% CI [1.10–2.07]; p<0.00001). However, pooled results showed a lower SMD than that of all the studies combined. Although this subgroup analysis showed a further decrease in heterogeneity (I-squared from 91% to 66%), it still remained significant.

Meta-regression

To further explore the high heterogeneity values, dosage-related effect was explored through a meta-regression (*Figure 6*). The meta-regression including all the studies resulted in an R^2 value of 0.15 and correlation coefficient of −0.39. The graph showed a dose-related effect; however, there was an outlier – the study by Xie et al. – which used 40 mg of rosuvastatin.[54]

A meta-regression of the other three studies that used atorvastatin was conducted. It resulted in an R^2 value of 0.98 (*Figure 7*) and correlation coefficient of −0.99. These values are very close to 1, suggesting a strong negative relationship.

Discussion

The results of the present review highlighted that statins significantly increase the frequency of Tregs. However, there is a considerable

Figure 6: Meta-regression Evaluating the Dose-related Effect of Atorvastatin on Regulatory T-cells Frequency
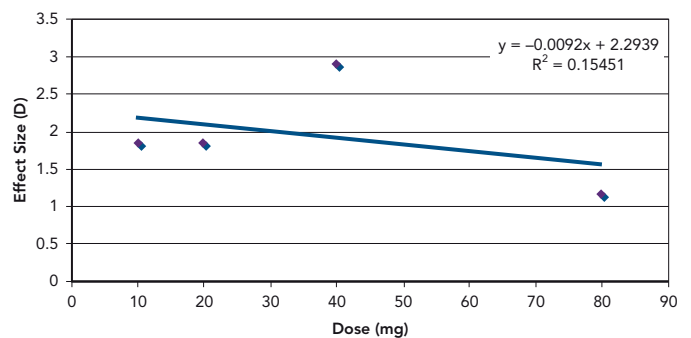
amount of heterogeneity in the pooled results, which was explored in terms of the statin type and dosage used. In terms of statin type, three of the studies used atorvastatin in the experimental group, and only Xie et al. used rosuvastatin.[54] By removing Xie et al., the heterogeneity decreased, indicating that the effect on the frequency of Tregs varies according to the statin type. The dose-related effect was evaluated by conducting a meta-regression. It was found that there is a negative

Figure 7: Meta-regression Evaluating the Dose-related Effect of Atorvastatin on Regulatory T-cells Frequency
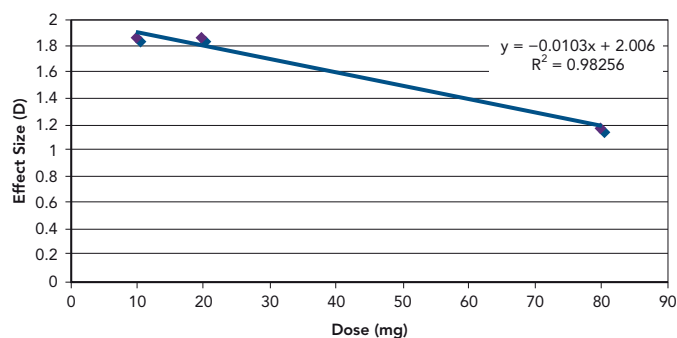
correlation with dose and the frequency of Tregs. However, there were very few studies that used the same statin type and dose, limiting the amount of data available for statistical aggregation.

The studies included in the review have a high risk of information. In terms of information bias, the studies by Hu et al. and Xie et al. have a high risk because these did not use all the Tregs markers used to identify Treg cells, which may alter the results.[53,54] However, even the studies that used all three or at least two of the classic markers (CD4, FOXP3 and CD25) may also have a risk of bias. This is because conventional CD4+ T-cells express FOXP3 transiently when activated and express CD25 upon stimulation.[55–57] ACS patients are known to have heightened CTL, which correlates with the extent of the disease. Therefore, using biomarkers that may detect CTL in such patients could further increase the impurity of the results. However, due to the lack of studies, subgroup analysis could not be performed to measure whether the effect of statins on Tregs correlated with the different patterns of Treg markers used. Future studies need to use a more accurate method in identifying Tregs to produce more reliable results. The use of the FOXP3 locus (Treg-specific demethylated region) is recommended as a specific marker of Tregs rather than the sole use of fluorescence-activated cell sorting.[26]

The present review is only applicable to the Asian population, as all studies were conducted in Asia. Moreover, it can only be applied to countries where the use of rosuvastatin and atorvastatin is approved.

Despite the limitations highlighted by the review, the potential of statins in inducing the frequency of Tregs has been shown by different studies with a plausible mechanistic effect. A similar benefit induced by statins was shown in rheumatoid arthritis, which is also characterised by

impaired Tregs functioning.[58] A RCT showed that atorvastatin treatment increases the amount of Tregs in the PBMC of rheumatoid arthritis patients and reduces disease activity.[59]

This trend was also seen in people with asthma, for whom statin therapy improved symptom control, and in vitro studies with the incubation of statins and CD4+ T-cells revealing expansion of Tregs.[60,61] The effect of statins in upregulating of Tregs and reducing disease burden was also seen in animal models of autoimmune neuritis, experimental autoimmune myasthenia gravis (Li et al.), ischaemia-reperfusion injury and apolipoprotein E (ApoE)-/- model of atherosclerosis.[62–65]

However, Hasib et al. demonstrated that patients with ACS had a low baseline frequency of Tregs, and despite 12 months of extensive statin use after an acute event, Treg frequency remained significantly unchanged, which contradicts our findings.[66] Although that was an observational study, it did not provide any strong support to the theory that statins exert a pronounced effect on Treg levels in a clinical scenario.

Finally, in terms of mechanism, Maussner-Fainberg et al. highlighted the ability of statins to induce FOXP3+ expression from peripheral CD4+CD25-FOXP3-.[67] This expression may be induced by inhibition of HMG-CoA reductase, which suppresses intermediates in the mevalonate pathway leading to prenylation of GTPases, such as Ras, Rac, Rho and Cdc42.[11–13] These small G proteins are postulated to be the reason for the induction of foxp3+ Tregs.[67]

Conclusion

ACS patients have been shown to clinically benefit from the anti-inflammatory effects of statin therapy through different pharmacological mechanisms. The current literature indicates a significant difference in Treg frequency following statin therapy in ACS patients. It is hypothesised that this is due to the stabilisation of the plaque by decreasing pro-inflammatory mediators involved in plaque destabilisation. It is proposed that future RCTs use accurate methods to identify Tregs and study the dose-related effects.

Overall, these results have considerable implications for patients who would benefit from restoring the Treg-induced immunomodulatory balance, with a view to exploring new therapeutic approaches to the management of disease progression in ACS.

Despite the limited data available and heterogeneity, the present review highlights areas for improvement, such as using more specific universal markers for Tregs, such as the Treg-specific demethylated region, and determining the statin type and dose-related effect. ■

1. Kostner GM. [Pharmacology of HMG CoA reductase inhibitors (statins)]. Wien Med Wochenschr 1999;149:120–4 [in German]. PMID: 10408002.
2. Morel DW, DiCorleto PE, Chisolm GM. Endothelial and smooth muscle cells alter LDL in vitro by free radical oxidation. Arteriosclerosis 1984;4:357–64. https://doi.org/10.1161/01.ATV.4.4.357; PMID: 6466193.
3. Shalev V, Chodick G, Silber H, et al. Continuation of statin treatment and all-cause mortality: a population-based cohort study. Arch Intern Med 2009;169:260–8. https://doi.org/10.1001/archinternmed.2008.552; PMID: 19204217.
4. Ray KK, Cannon CP. Time to benefit: an emerging concept for assessing the efficacy of statin therapy in cardiovascular disease. Crit Pathw Cardiol 2005;4:43–5. https://doi.org/10.1097/01.hpc.0000154979.98731.5d; PMID: 18340184.
5. Nordmann A, Schwartz G, Vale N, et al. Cochrane corner: early statin therapy in acute coronary syndromes--what is the clinical benefit? Heart 2016;102:653–4. https://doi.org/10.1136/heartjnl-2015-307781; PMID: 26908101.
6. Vale N, Nordmann AJ, Schwartz GG, et al. Statins for acute coronary syndrome. Cochrane Database Syst Rev 2014:CD006870.

https://doi.org/10.1002/14651858.CD006870.pub3; PMID: 21678362.
7. Hulten E, Jackson JL, Douglas K, et al. The effect of early, intensive statin therapy on acute coronary syndrome: a meta-analysis of randomized controlled trials. Arch Intern Med 2006;166:1814–21. https://doi.org/10.1001/archinte.166.17.1814; PMID: 17000936.
8. Schwartz GG, Olsson AG, Ezekowitz MD, et al. Effects of atorvastatin on early recurrent ischemic events in acute coronary syndromes: the MIRACL study: a randomized controlled trial. JAMA 2001;285:1711–8. https://doi.org/10.1001/jama.285.13.1711; PMID: 11277825.
9. Murphy SA, Cannon CP, Wiviott SD, et al. Reduction in recurrent cardiovascular events with intensive lipid-lowering statin therapy compared with moderate lipid-lowering statin therapy after acute coronary syndromes from the PROVE IT-TIMI 22 (Pravastatin or Atorvastatin Evaluation and Infection Therapy-Thrombolysis In Myocardial Infarction 22) trial. J Am Coll Cardiol 2009;54:2358–62. https://doi.org/10.1016/j.jacc.2009.10.005; PMID: 20082923.
10. de Lemos JA, Blazing MA, Wiviott SD, et al. Early intensive vs

a delayed conservative simvastatin strategy in patients with acute coronary syndromes: phase Z of the A to Z trial. JAMA 2004;292:1307–16. https://doi.org/10.1001/jama.292.11.1307; PMID: 15337732.
11. Tousoulis D, Oikonomou E, Economou EK, et al. Inflammatory cytokines in atherosclerosis: current therapeutic approaches. Eur Heart J 2016;37:1723–32. https://doi.org/10.1093/eurheartj/ehv759; PMID: 26843277.
12. Takai Y, Sasaki T, Matozaki T. Small GTP-binding proteins. Physiol Rev 2001;81:153–208. https://doi.org/10.1152/physrev.2001.81.1.153; PMID: 11152757.
13. Laufs U, Liao JK. Post-transcriptional regulation of endothelial nitric oxide synthase mRNA stability by Rho GTPase. J Biol Chem 1998;273:24,266–71. https://doi.org/10.1074/jbc.273.37.24266; PMID: 9727051.
14. Omori H, Nagashima H, Tsurumi Y, et al. Direct in vivo evidence of a vascular statin: a single dose of cerivastatin rapidly increases vascular endothelial responsiveness in healthy normocholesterolaemic subjects. Br J Clin Pharmacol 2002;54:395–9. https://doi.org/10.1046/j.1365-2125.2002.01677.x; PMID: 12392587.

15. Bergh N, Larsson P, Ulfhammer E, Jern S. Effect of shear stress, statins and TNF-alpha on hemostatic genes in human endothelial cells. *Biochem Biophys Res Commun* 2012;420:166–71. https://doi.org/10.1016/j.bbrc.2012.02.136; PMID: 22405819.

16. Youssef S, Stuve O, Patarroyo JC, et al. The HMG-CoA reductase inhibitor, atorvastatin, promotes a Th2 bias and reverses paralysis in central nervous system autoimmune disease. *Nature* 2002;420:78–84. https://doi.org/10.1038/nature01158; PMID: 12422218.

17. Zeiser R, Youssef S, Baker J, et al. Preemptive HMG-CoA reductase inhibition provides graft-versus-host disease protection by Th-2 polarization while sparing graft-versus-leukemia activity. *Blood* 2007;110:4588–98. https://doi.org/10.1182/blood-2007-08-106005; PMID: 17827390.

18. Weitz-Schmidt G, Welzenbach K, Brinkmann V, et al. Statins selectively inhibit leukocyte function antigen-1 by binding to a novel regulatory integrin site. *Nat Med* 2001;7:687–92. https://doi.org/10.1038/89058; PMID: 11385505.

19. Rodriguez-Perea AL, Montoya CJ, Olek S, et al. Statins increase the frequency of circulating CD4+ FOXP3+ regulatory T cells in healthy individuals. *J Immunol Res* 2015;2015:762506. https://doi.org/10.1155/2015/762506; PMID: 25759848.

20. Thorstenson KM, Khoruts A. Generation of anergic and potentially immunoregulatory CD25+CD4 T cells in vivo after induction of peripheral tolerance with intravenous or oral antigen. *J Immunol* 2001;167:188–95. https://doi.org/10.4049/jimmunol.167.1.188; PMID: 11418648.

21. Baecher-Allan C, Brown JA, Freeman GJ, Hafler DA. CD4+CD25 high regulatory cells in human peripheral blood. *J Immunol* 2001;167:1245–53. https://doi.org/10.4049/jimmunol.167.3.1245; PMID: 11466340.

22. Sakaguchi S, Sakaguchi N, Asano M, et al. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J Immunol* 1995;155:1151–64. PMID: 7636184.

23. Brunkow ME, Jeffery EW, Hjerrild KA, et al. Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse. *Nat Genet* 2001;27:68–73. https://doi.org/10.1038/83784; PMID: 11138001.

24. Kmieciak M, Gowda M, Graham L, et al. Human T cells express CD25 and Foxp3 upon activation and exhibit effector/memory phenotypes without any regulatory/suppressor function. *J Transl Med* 2009;7:89. https://doi.org/10.1186/1479-5876-7-89; PMID: 19849846.

25. Wang J, Ioan-Facsinay A, van der Voort EI, et al. Transient expression of FOXP3 in human activated nonregulatory CD4+ T cells. *Eur J Immunol* 2007;37:129–38. https://doi.org/10.1002/eji.200636435; PMID: 17154262.

26. Baron U, Floess S, Wieczorek G, et al. DNA demethylation in the human FOXP3 locus discriminates regulatory T cells from activated FOXP3(+) conventional T cells. *Eur J Immunol* 2007;37:2378–89. https://doi.org/10.1002/eji.200737594; PMID: 17694575.

27. Kearley J, Barker JE, Robinson DS, Lloyd CM. Resolution of airway inflammation and hyperreactivity after in vivo transfer of CD4+CD25+ regulatory T cells is interleukin 10 dependent. *J Exp Med* 2005;202:1539–47. https://doi.org/10.1084/jem.20051166; PMID: 16314435.

28. Collison LW, Workman CJ, Kuo TT, et al. The inhibitory cytokine IL-35 contributes to regulatory T-cell function. *Nature* 2007;450:566–9. https://doi.org/10.1038/nature06306; PMID: 18033300.

29. Nakamura K, Kitani A, Strober W. Cell contact-dependent immunosuppression by CD4(+)CD25(+) regulatory T cells is mediated by cell surface-bound transforming growth factor beta. *J Exp Med* 2001;194:629–44. https://doi.org/10.1084/jem.194.5.629; PMID: 11535631.

30. von Herrath MG, Harrison LC. Antigen-induced regulatory T cells in autoimmunity. *Nat Rev Immunol* 2003;3:223–32. https://doi.org/10.1038/nri1029; PMID: 12658270.

31. Cederbom L, Hall H, Ivars F. CD4+CD25+ regulatory T cells down-regulate co-stimulatory molecules on antigen-presenting cells. *Eur J Immunol* 2000;30:1538–43. https://doi.org/10.1002/1521-4141(200006)30:6<1538::AID-IMMU1538>3.0.CO;2-X; PMID: 10898488.

32. Liang B, Workman C, Lee J, et al. Regulatory T cells inhibit dendritic cells by lymphocyte activation gene-3 engagement of MHC class II. *J Immunol* 2008;180:5916–26. https://doi.org/10.4049/jimmunol.180.9.5916; PMID: 18424711.

33. Taams LS, van Amelsfort JM, Tiemessen MM, et al. Modulation of monocyte/macrophage function by human CD4+CD25+ regulatory T cells. *Hum Immunol* 2005;66:222–30. https://doi.org/10.1016/j.humimm.2004.12.006; PMID: 15784460.

34. Read S, Malmstrom V, Powrie F. Cytotoxic T lymphocyte-associated antigen 4 plays an essential role in the function of CD25(+)CD4(+) regulatory cells that control intestinal inflammation. *J Exp Med* 2000;192:295–302. https://doi.org/10.1084/jem.192.2.295; PMID: 10899916.

35. Oderup C, Cederbom L, Makowska A, et al. Cytotoxic T lymphocyte-antigen-4-dependent down-modulation of costimulatory molecules on dendritic cells in CD4+CD25+ regulatory T-cell-mediated suppression. *Immunology* 2006;118:240–9. https://doi.org/10.1111/j.1365-2567.2006.02362.x; PMID: 16771859.

36. Fallarino F, Grohmann U, Hwang KW, et al. Modulation of tryptophan catabolism by regulatory T cells. *Nat Immunol* 2003;4:1206–12. https://doi.org/10.1038/ni1003; PMID: 14578884.

37. Grossman WJ, Verbsky JW, Tollefsen BL, et al. Differential expression of granzymes A and B in human cytotoxic lymphocyte subsets and T regulatory cells. *Blood* 2004;104:2840–8. https://doi.org/10.1182/blood-2004-03-0859; PMID: 15238416.

38. Pandiyan P, Zheng L, Ishihara S, et al. CD4+CD25+Foxp3+ regulatory T cells induce cytokine deprivation-mediated apoptosis of effector CD4+ T cells. *Nat Immunol* 2007;8:1353–62. https://doi.org/10.1038/ni1536; PMID: 17982458.

39. Jonasson L, Holm J, Skalli O, et al. Regional accumulations of T cells, macrophages, and smooth muscle cells in the human atherosclerotic plaque. *Arteriosclerosis* 1986;6:131–8. https://doi.org/10.1161/01.ATV.6.2.131; PMID: 2937395.

40. Amento EP, Ehsani N, Palmer H, Libby P. Cytokines and growth factors positively and negatively regulate interstitial collagen gene expression in human vascular smooth muscle cells. *Arterioscler Thromb* 1991;11:1223–30. https://doi.org/10.1161/01.ATV.11.5.1223; PMID: 1911708.

41. van der Wal AC, Becker AE, van der Loos CM, Das PK. Site of intimal rupture or erosion of thrombosed coronary atherosclerotic plaques is characterized by an inflammatory process irrespective of the dominant plaque morphology. *Circulation* 1994;89:36–44. https://doi.org/10.1161/01.CIR.89.1.36; PMID: 8281670.

42. Zal B, Kaski JC, Akiyu JP, et al. Differential pathways govern CD4+ CD28- T cell proinflammatory and effector responses in patients with coronary artery disease. *J Immunol* 2008;181:5233–41. https://doi.org/10.4049/jimmunol.181.8.5233; PMID: 18832677.

43. Liuzzo G, Kopecky SL, Frye RL, et al. Perturbation of the T-cell repertoire in patients with unstable angina. *Circulation* 1999;100:2135–9. https://doi.org/10.1161/01.CIR.100.21.2135; PMID: 10571971.

44. Huang WC, Sala-Newby GB, Susana A, et al. Classical macrophage activation up-regulates several matrix metalloproteinases through mitogen activated protein kinases and nuclear factor-kappaB. *PLoS One* 2012;7:e42507. https://doi.org/10.1371/journal.pone.0042507; PMID: 22880008.

45. Liuzzo G, Goronzy JJ, Yang H, et al. Monoclonal T-cell proliferation and plaque instability in acute coronary syndromes. *Circulation* 2000;101:2883–8. https://doi.org/10.1161/01.CIR.101.25.2883; PMID: 10869258.

46. Mor A, Luboshits G, Planer D, et al. Altered status of CD4(+)CD25(+) regulatory T cells in patients with acute coronary syndromes. *Eur Heart J* 2006;27:2530–7. https://doi.org/10.1093/eurheartj/ehl222; PMID: 16954132.

47. Han SF, Liu P, Zhang W, et al. The opposite-direction modulation of CD4+CD25+ Tregs and T helper 1 cells in acute coronary syndromes. *Clin Immunol* 2007;124:90–7. https://doi.org/10.1016/j.clim.2007.03.546; PMID: 17512253.

48. Wigren M, Bjorkbacka H, Andersson L, et al. Low levels of circulating CD4+FoxP3+ T cells are associated with an increased risk for development of myocardial infarction but not for stroke. *Arterioscler Thromb Vasc Biol* 2012;32:2000–4. https://doi.org/10.1161/ATVBAHA.112.251579; PMID: 22628434.

49. Zhang WC, Wang J, Shu YW, et al. Impaired thymic export and increased apoptosis account for regulatory T cell defects in patients with non-st segment elevation acute coronary syndrome. *J Biol Chem* 2012;287:34157–66. https://doi.org/10.1074/jbc.M112.382978; PMID: 22872639.

50. Flego D, Severino A, Trotta F, et al. Increased PTPN22 expression and defective CREB activation impair regulatory T-cell differentiation in non-ST-segment elevation acute coronary syndromes. *J Am Coll Cardiol* 2015;65:1175–86. https://doi.org/10.1016/j.jacc.2015.01.027; PMID: 25814225.

51. Zhang D, Wang S, Guan Y, et al. 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* 2011;57:536–41. https://doi.org/10.1097/FJC.0b013e318211d016; PMID: 21297491.

52. Wang ZX, Wang CQ, Li XY, et al. 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* 2015;56:163–9. https://doi.org/10.1536/ihj.14-245; PMID: 25740578.

53. Hu Z, Li D, Hu Y, Yang K. 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* 2007;27:524–7. https://doi.org/10.1007/s11596-007-0512-4; PMID: 18060626.

54. Xie W, Li P, Wang Z, et al. 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* 2014;32:276–82. https://doi.org/10.1111/1755-5922.12098; PMID: 25319951.

55. Gavin MA, Torgerson TR, Houston E, et al. Single-cell analysis of normal and FOXP3-mutant human T cells: FOXP3 expression without regulatory T cell development. *Proc Natl Acad Sci U S A* 2006;103:6659–64. https://doi.org/10.1073/pnas.0509484103; PMID: 16617117.

56. Ziegler SF. FOXP3: not just for regulatory T cells anymore. *Eur J Immunol* 2007;37:21–3. https://doi.org/10.1002/eji.200636929; PMID: 17183612.

57. Walker MR, Kasprowicz DJ, Gersuk VH, et al. Induction of FoxP3 and acquisition of T regulatory activity by stimulated human CD4+CD25- T cells. *J Clin Invest* 2003;112:1437–43. https://doi.org/10.1172/JCI19441; PMID: 14597769.

58. Ehrenstein MR, Evans JG, Singh A, et al. Compromised function of regulatory T cells in rheumatoid arthritis and reversal by anti-TNFalpha therapy. *J Exp Med* 2004;200:277–85. https://doi.org/10.1084/jem.20040165; PMID: 15280421.

59. Tang TT, Song Y, Ding YJ, et al. Atorvastatin upregulates regulatory T cells and reduces clinical disease activity in patients with rheumatoid arthritis. *J Lipid Res* 2011;52:1023–32. https://doi.org/10.1194/jlr.M010876; PMID: 21368320.

60. Zeki AA, Oldham J, Wilson M, et al. Statin use and asthma control in patients with severe asthma. *BMJ Open* 2013;3:e003314. https://doi.org/10.1136/bmjopen-2013-003314; PMID: 23943778.

61. Maneechotesuwan K, Kasetsinsombat K, Wamanuttajinda V, et al. Statins enhance the effects of corticosteroids on the balance between regulatory T cells and Th17 cells. *Clin Exp Allergy* 2013;43:212–22. https://doi.org/10.1111/cea.12067; PMID: 23331562.

62. Li XL, Dou YC, Liu Y, et al. Atorvastatin ameliorates experimental autoimmune neuritis by decreased Th1/Th17 cytokines and up-regulated T regulatory cells. *Cell Immunol* 2011;271:455–61. https://doi.org/10.1016/j.cellimm.2011.08.015; PMID: 21889126.

63. Li XL, Liu Y, Cao LL, et al. 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* 2013;56:85–95. https://doi.org/10.1016/j.mcn.2013.03.005; PMID: 23541702.

64. Ke D, Fang J, Fan L, Chen Z, Chen L. Regulatory T cells contribute to rosuvastatin-induced cardioprotection against ischemia-reperfusion injury. *Coron Artery Dis* 2013;24:334–41. https://doi.org/10.1097/MCA.0b013e3283608c12; PMID: 23531479.

65. Meng X, Zhang K, Li J, et al. Statins induce the accumulation of regulatory T cells in atherosclerotic plaque. *Mol Med* 2012;18:598–605. https://doi.org/10.2119/molmed.2011.00471; PMID: 22331026.

66. Hasib L, Lundberg AK, Zachrisson H, et al. Functional and homeostatic defects of regulatory T cells in patients with coronary artery disease. *J Intern Med* 2016;279:63–77. https://doi.org/10.1111/joim.12398; PMID: 26260103.

67. Mausner-Fainberg K, Luboshits G, Mor A, et al. The effect of HMG-CoA reductase inhibitors on naturally occurring CD4+CD25+ T cells. *Atherosclerosis* 2008;197:829–39. https://doi.org/10.1016/j.atherosclerosis.2007.07.031; PMID: 17826781.