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Review

The Immunomodulatory Effects of Statins on Macrophages

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Abstract: Statins are 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors used worldwide to manage dyslipidaemia and thus limit the development of atherosclerotic disease and its complications. These atheroprotective drugs are now known to exert pleiotropic actions outside of their cholesterol-lowering activity, including altering immune cell function. Macrophages are phagocytic leukocytes that play critical functional roles in the pathogenesis of atherosclerosis and are directly targeted by statins. Early studies documented the anti-inflammatory effects of statins on macrophages, but emerging evidence suggests that these drugs can also enhance pro-inflammatory macrophage responses, creating an unresolved paradox. This review comprehensively examines the in vitro, in vivo, and clinical literature to document the statin-induced changes in macrophage polarization and immunomodulatory functions, explore the underlying mechanisms involved, and offer potential explanations for this paradox. A better understanding of the immunomodulatory actions of statins on macrophages should pave the way for the development of novel therapeutic approaches to manage atherosclerosis and other chronic diseases and conditions characterised by unresolved inflammation.

Keywords: statins; macrophages; atherosclerosis; inflammation; cholesterol; atorvastatin; simvastatin; rosuvastatin; fluvastatin; lovastatin; pitavastatin; cerivastatin; metavastatin; pravastatin

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1. Introduction

1.1. Statins Are the Most Widely Prescribed Medications for the Prevention of Cardiovascular Disease

Cardiovascular disease (CVD) is the leading cause of mortality worldwide, accounting for an estimated 17.9 million deaths in 2019 [1], which equates to 32% of all global deaths. Atherosclerosis, a chronic inflammatory disease characterised by a narrowing of the arteries, is the main underlying cause of CVD [2] and is driven by an imbalance in lipid metabolism and a maladaptive immune response [3]. Despite its causal role in deaths globally, CVD-related mortality in the UK and other industrialised countries has declined over the last 40 years [4,5], and statins, which have revolutionized the prevention of atherosclerotic CVD, have significantly contributed to this change [6]. The efficacy of statins in the preventative treatment of CVD has led to them becoming one of the most prescribed medications worldwide, with over 200 million people taking them [7].

The clinical benefit of statins in CVD prevention is thought to be primarily driven by their lipid-lowering effects [8,9], as epidemiological studies have revealed high plasma levels of low-density lipoprotein cholesterol (LDL-C) to be a significant risk factor for atherosclerosis [10]. Mechanistically, statins inhibit cellular cholesterol biosynthesis through the inhibition of the mevalonate pathway via the rate-limiting enzyme 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) reductase. In addition, statins upregulate hepatic low-density lipoprotein receptor transcription, increasing blood LDL-C removal. Together, these factors result in a 20–60% reduction in circulating LDL-C depending on the particular statin type and dose administered (Table 1).

There are several statins clinically available, with atorvastatin, simvastatin, and rosuvastatin being the most popular and most widely prescribed [11,12]. The different statins

vary in their lipophilicity, metabolism, elimination half-lives, and potency and evidence suggests that these distinct characteristics may lead to differential effects on their efficacy (Table 1). For example, studies have suggested that the variability in different statins' solubility affects their ability to enter cells, with lipophilic statins being found to passively diffuse into numerous cell types, whilst hydrophilic statins are hypothesized to be more liver-selective due to their dependence on membrane transporters [13,14]. These different properties have been suggested to potentially result in varying distributions of the drugs in different tissues, thereby resulting in differential effects on the mevalonate pathway [15].

1.2. The Central Role of Macrophages in Inflammation and CVD

Atherosclerosis is recognised as a chronic inflammatory disease characterised by a lipid imbalance and maladaptive inflammation exacerbated by the accumulation of inflammatory cells in the arterial wall. Cholesterol-laden macrophages (known as foam cells) are protagonists in the development and progression of atherosclerosis, making up the main immune cellular constituents of atherosclerotic lesions [16,17]. Foam cells contribute to the maintenance of the local endothelial inflammatory response by secreting proinflammatory cytokines and chemokines, as well as producing reactive oxygen and nitrogen species. Macrophages also engage in crosstalk with vascular smooth muscle cells, amplifying the inflammatory cycle by producing additional proinflammatory signals, promoting the growth of lipid-rich lesions [18]. Over time, these lesions can undergo further remodelling and form a fibrous cap, a layer of connective tissue that shields the lesion from the lumen (together with the lesion, this is known as an atherosclerotic plaque) [19]. Plaques can become unstable and rupture unexpectedly, exposing the lipid core to the blood and triggering thrombosis, which can result in partial or complete vessel occlusion and culminate in myocardial infarction, stroke, and other ischemic events.

Table 1. Pharmacokinetic properties of different statins.

Statin Name	Brand Name	Daily Dose (mg)	Effect on LDL Cholesterol (% Decrease)	Lipophilicity	Marketed Drug Form	Half-Life (h)	Primary Metabolizing Enzyme(s)
Atorvastatin	Lipitor	10–80 [20]	37–55 [21,22]	Lipophilic [15]	Acid [15]	14 [15,20]	CYP3A4 [15]
Cerivastatin [a]	Baycol	0.02–0.8 [23]	12–42 [24]	Lipophilic [15]	Acid [15]	2–4 [23]	CYP3A4, 2C8 [15,23]
Fluvastatin	Lescol	20–80 [25]	21–33 [21,22]	Lipophilic [15]	Acid [15]	3 [25]	CYP2C9 [15,25]
Lovastatin	Mevacor	10–80 [26]	21–45 [21]	Lipophilic [15]	Lactone [15]	3 [15]	CYP3A4 [15,26]
Metavastatin [b]				Lipophilic [27]			
Pitavastatin	Livalo	1–4 [28]	33–44 [29]	Lipophilic [15]	Acid [15]	12 [28]	CYP2C8, 2C9 [15,28]
Pravastatin	Pravachol	10–80 [30]	20–33 [21]	Hydrophilic [15]	Acid [15]	1.8 [15,30]	Non-CYP [15]
Rosuvastatin	Crestor	5–40 [31]	38–53 [21,22]	Hydrophilic [15]	Acid [15]	19 [15,31]	CYP2C9 [31]
Simvastatin	Zocor	5–80 [32]	23–42 [21,22]	Lipophilic [15]	Lactone [15]	2 [15]	CYP3A4 [15,32]

[a] Cerivastatin was voluntarily withdrawn from the clinical market [33]. [b] Metavastatin was never brought to the clinical market [34].

Macrophages are tissue-resident leukocytes present in virtually all tissues of the body and have diverse roles, acting as both pro and anti-inflammatory mediators and being associated with the resolution of infections, tissue development, homeostasis, repair, and remodelling [35]. Macrophages display remarkable plasticity, which is shaped by their specific microenvironment [36]. Following their differentiation from monocytes, macrophages are often classified into one of two distinct functional polarization states (based on surface expression markers), M1, classically activated, or M2, alternatively activated [37].

These states represent the two extremes of a spectrum of macrophage phenotypes, describing a pro-inflammatory and anti-inflammatory/pro-resolving phenotype, respectively. Additionally, M0 is used to denote resting/non-activated cells.

M1-like activated macrophages are induced by microbial products, such as lipopolysaccharides (LPS) and toll-like receptor (TLR) ligands, or by cytokines secreted from other immune cells, such as interferon (IFN)-gamma (IFN-γ) [38] (Figure 1). These inflammatory signals trigger both transmembrane receptors (e.g., TLRs and IFN-γ receptor (IFN-γR)) and cytoplasmic receptors (e.g., nucleotide-binding and oligomerization domain (NOD)-like receptors (NLRs)). Traditionally, M1-like macrophages are functionally associated with pathogen clearance and antigen presentation to T cells to initiate the adaptive immune response, which they achieve by secreting high levels of pro-inflammatory cytokines, such as tumour necrosis factor-alpha (TNFα), interleukin (IL) 1β (IL-1β), IL-6, and IL-12, and by expressing activation markers including cluster of differentiation (CD)80, CD86, class II transactivator (CIITA), and major histocompatibility complex class II receptor (MHC-II). Pro-inflammatory macrophages also express high levels of inducible nitric oxide synthase, which enables the synthesis of nitric oxide (NO) that can, in turn, form reactive oxygen species (ROS) with microbicidal properties. The expression of these inflammatory mediators is predominantly controlled by the activation and nuclear translocation of transcription factors in response to initial receptor recognition of inflammatory stimuli. NF-κB (nuclear factor kappa-light-chain enhancer of B-cell) [39], together with STAT1 (Signal transducer and activator of transcription) [38], STAT3 [40], IRF (IFN-γ regulatory factor) [41], and AP-1 (activator protein 1) [42] are all associated with the polarization of macrophages to an M1-like phenotype.

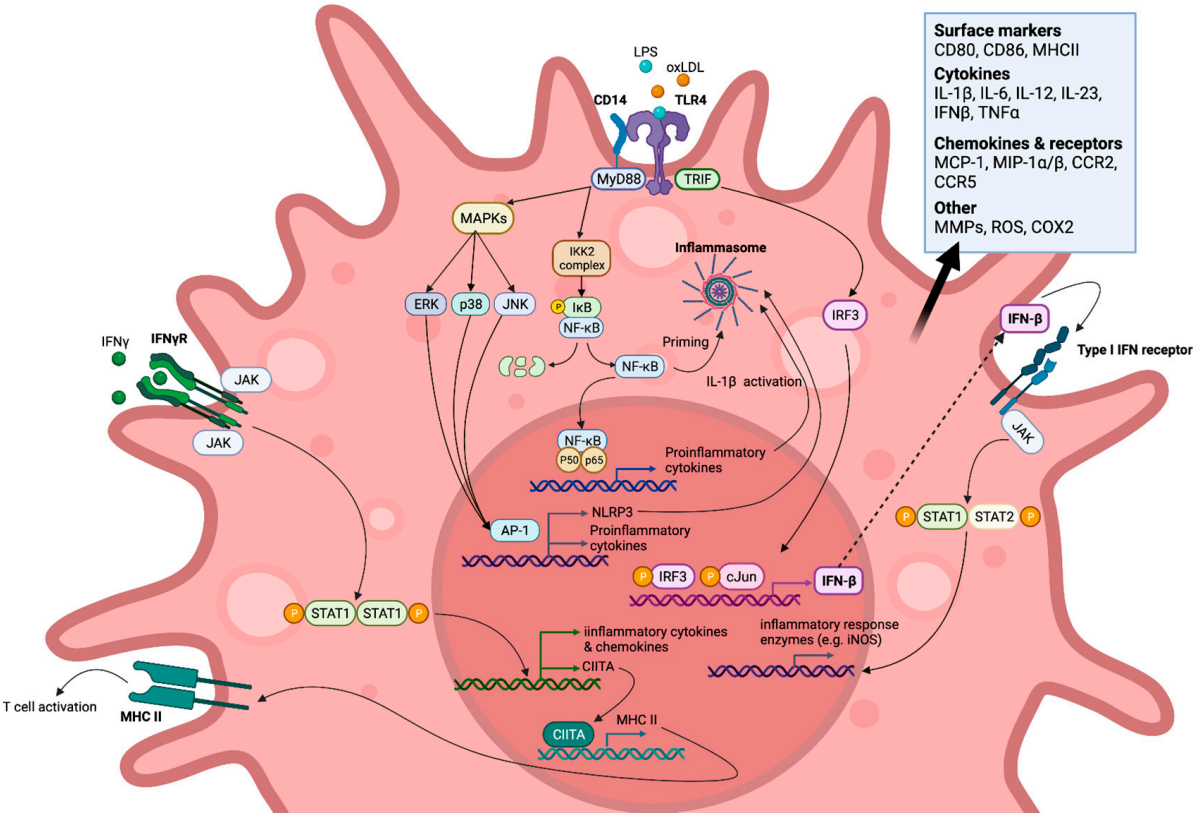

Figure 1. M1-like polarised macrophage signalling pathways (simplified) induced by toll-like receptor (TLR) and IFN-γ receptor (IFN-γR) endogenous and exogenous agonists. Created with BioRender.com, accessed on 7 March 2022.

The switch to M2-like, or alternatively activated, macrophages is mediated by factors such as IL-4 and IL-13 released from innate and adaptive immune cells [38]. M2-like macrophages are considered to be anti-inflammatory as they are noted to resolve inflammation and stimulate tissue repair. They exhibit increased expression of pro-inflammatory cytokine decoy and scavenger receptors, such as IL-1R [43], which act as molecular traps, preventing canonical signalling and thereby regulating inflammation. In addition, they secrete high levels of IL-10, transforming growth factor β, and vascular endothelial growth factor, which ameliorate the excessive activity of both innate and adaptive immune cells, stimulate fibroblast and endothelial cell proliferation, and promote blood-vessel development, allowing wound healing [38,44]. M2 polarization is also characterised by the expression of the transcription factors STAT6, SOCS1 (suppressor of cytokine signalling), and PPARγ (peroxisome proliferator-activated receptor gamma), along with the markers CD163 and CD36.

Recent evidence suggests that the M1/M2 classification system greatly oversimplifies macrophage heterogeneity. Instead, research indicates that macrophages exist on an activation spectrum with a wide array of phenotypes between these M1 and M2 extremes, dependent on their exposure to biochemical stimuli. We refer the reader to recent reviews [45–47] for detailed discussion. Despite the evolving views of macrophage polarization, to better compare the findings of the literature referenced in this review, the simplified M1/M2 nomenclature will be used as appropriate.

Atherosclerotic lesions house a heterogeneous population of macrophages, although M1-like cells are the predominant sub-type [48,49]. M1-like macrophages, expressing pro-inflammatory markers, are known to be associated with unstable and rupture-prone areas, whilst M2-like macrophages are found in stable regions [48]. M2-like macrophages have also been implicated in plaque regression in several different models suggesting that this polarization state's enrichment may aid the resolution of atherosclerosis [50–52]. Therefore, therapeutic agents that encourage this switch from an M1 to an M2-like state, suppressing inflammation, could be a promising treatment strategy to reduce cardiovascular events [53]. Macrophages also play a central role in many other disease states and have therefore emerged as important therapeutic targets in several other pathologies, such as the development and progression of cancerous tumours [54], autoimmune disorders [55] and sepsis [56].

1.3. Statins Have Immunomodulatory Effects

Beyond cholesterol-lowering, statins have a range of other pleiotropic effects [57]. These actions were first proposed when additional clinical benefits not anticipated from statin-induced changes to LDL-C levels alone became evident, including the modulation of the immune response. Clinical trials have revealed that the plasma levels of C-reactive protein (CRP—an inflammatory marker) are a powerful predictor of future cardiovascular events [58–63]. Interest in the potential of statins as anti-inflammatory agents was piqued when clinical data showed that CRP levels decrease following statin treatment [64–66], an idea which was reinforced by the finding that statin-treated patients have improved survival and reduced rejection episodes after heart transplantation [67]. Statin therapy has also been found to increase atherosclerotic plaque stability and instigate plaque regression, which some suggest may result from their immunomodulatory actions [17]. More recently, statin therapy has been evaluated in the attenuation of other immune-associated conditions, and anti-inflammatory responses have been reported for periodontal inflammation [68] and rheumatoid arthritis [69,70].

It is important to note that mevalonate pathway inhibition by statins not only impairs cholesterol production but also limits the synthesis of other downstream metabolites, such as isoprenoids (Figure 2). Isoprenoids are essential for protein prenylation (the irreversible addition of isoprenyl lipids to proteins) and the appropriate folding of certain proteins [71]. In particular, the isoprenoids farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP) are essential for the post-translational modification of

small guanosine triphosphate (GTP)-binding proteins, such as members of the Ras, Rho, and Rab families [72]. It is generally considered that Ras GTPases require FPP for their correct post-translational modification, whilst GGPP is necessary for Rho and Rab GTPases. However, there are exceptions to this, as some Rho GTPases require both FPP and GGPP isoprenylation for appropriate intracellular localization and function [73]. Disturbance of isoprenoid synthesis has been implicated as a mediator of statin-induced pleiotropic effects, with several studies demonstrating the importance of GTPases in various cell signalling pathways by their action as molecular switches, including those that regulate cell growth, proliferation, and notably inflammation [74–78].

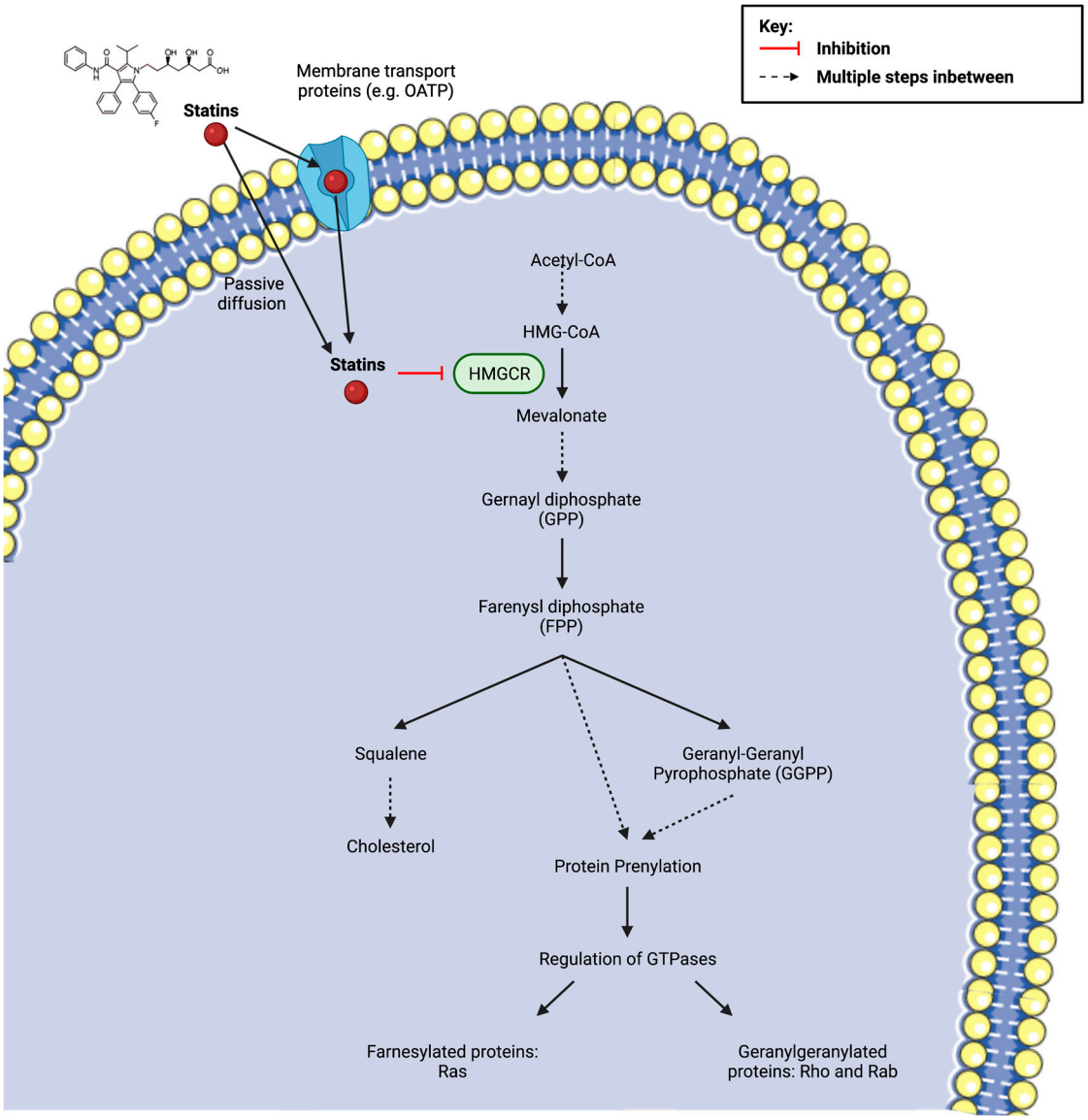

Figure 2. Statin inhibition of 3-hydroxy-3methylglutaryl coenzyme A (HMG-CoA) reductase (HMGCR) and the subsequent implications on downstream metabolites of the mevalonate pathway, including the synthesis of cholesterol and the isoprenoids farnesyl pyrophosphate (FPP) and geranyl-geranyl pyrophosphate (GGPP). Protein prenylation, via isoprenoids, is essential for the activation of small guanosine triphosphate (GTP)-binding proteins (Ras, Rho, Rac). The cellular uptake of the drug depends on its solubility. Lipophilic statins are more likely to enter the cell via passive diffusion, whereas hydrophilic statins require protein transporters, such as organic anion transporting polypeptides (OATPs) in hepatocytes. Created with BioRender.com (accessed on 7 March 2022) and Smart.Servier.com (accessed on 7 March 2022).

The finding that statins possess immunomodulatory activity, as well as the critical role of macrophages in atherosclerotic CVD development and progression, has directed research efforts towards characterizing statins' effects on macrophage functions. Here, we review evidence that has emerged from cell culture experiments, animal studies, and clinical trials, showing that statins can affect macrophage inflammatory responses. However, the findings from many of these studies are conflicting (Table 2), with pro- and anti-inflammatory roles reported, and to date, there has been no focused review of this area. This article consolidates the findings of these macrophage-centred studies, highlighting statin-mediated macrophage inflammatory responses and exploring the mechanistic basis of the paradoxical findings.

Table 2. The effects of statins on macrophages in vitro. Abbreviations: PBMC, peripheral blood mononuclear cells; BMDMs, bone-marrow derived macrophages; iNOS, inducible nitric oxide synthase; COX-2, cyclooxygenase-2; uPAR, urokinase plasminogen activator receptor; AdipoR, adiponectin receptors; mTOR, mechanistic target of rapamycin; mRNA, messenger ribonucleic acid; MWCNT, multi-walled carbon nanotubes; TF, tissue factor; GILZ, glucocorticoid-induced leucine zipper; TPA, 12-O-tetradecanoyl-phorbol-13-acetate; PMA, phorbol myristate acetate; acLDL, acetylated LDL; M-CSF, macrophage colony-stimulating factor; CC, cholesterol crystal; MSU, monosodium urate; SOD1, superoxide dismutase-1; AGE-RAGE, advanced glycation endproducts-receptor for advanced glycation endproducts; C/EBP, CCAAT/enhancer binding proteins; IP-10, interferon gamma-induced protein-10; agLDL, aggregated LDL; ETS-1, erythroblast transformation specific-1; KLF-2, Krüppel-like factor-2; ICAM-1, intercellular adhesion molecule-1.

Statin	Model	Summary	Inflammatory Effect		Ref.
			Pro	Anti	
Atorvastatin	Human PBMC derived macrophages	Statins acted as inhibitors of the induction of MHC-II expression by IFN-γ due to suppression of CIITA transcription. Statins repressed MHC-II mediated T-cell activation.		✓	[79]
	Primary macrophages from B10.PL mice	Atorvastatin prevented IFN-γ induced MHC-II, CD40, CD80, and CD86 expression.		✓	[80]
	RAW 264.7 macrophages	Atorvastatin inhibited LPS and IFN-γ-induced NO formation and iNOS induction—thought to be mediated through suppression of NF-κB activation and IFN-γ through STAT1.		✓	[81]
	Murine peritoneal macrophages	Atorvastatin pretreatment enhanced TLR2 and TLR4 ligand-stimulated IL-6 and TNF production.	✓		[82]
	RAW 264.7 macrophages	Enhanced LPS-mediated MMP-9 gene expression.	✓		[83]
	RAW 264.7 macrophages	Atorvastatin pretreatment inhibited oxLDL-induced increase in COX-2, TNFα, and MCP-1 secretion.		✓	[84]
	Murine BMDMs	Atorvastatin pretreatment exacerbated LPS-induced upregulation of Il-1b, IL-6, and NLRP3 transcript levels.	✓		[85]
	Human PBMC derived macrophages	Statin treatment in combination with IL-4 during the macrophage differentiation phase led to increased M2 polarization via PPARγ activation.		✓	[86]
	RAW 264.7 macrophages	Atorvastatin pretreatment inhibited LPS-induced IL-1β and TNFα production in RAW 264.7 macrophages through the enhancement of autophagy. Statin treatment was seen to attenuate NLRP3 inflammasome induction in response to LPS stimulation. Atorvastatin pretreatment inhibited the expression of IL-1β in response to LPS stimulation in peritoneal murine macrophages through autophagy activation, but not that of TNFα.		✓	[87]
	Human PBMC derived macrophages	Atorvastatin reduced matrix degradation capability via reduced MMP-14 activation and uPAR localization to filipodia in LPS and IFN-γ stimulated macrophages.		✓	[88]
	RAW 264.7 macrophages and J774 macrophages	Atorvastatin increased Rac1 GTP-loading in LPS stimulated macrophages, enhancing production of the proinflammatory cytokines IL-1β, TNFα, and IL-6.	✓		[89]

Table 2. *Cont.*

Statin	Model	Summary	Inflammatory Effect		Ref.
			Pro	Anti	
	Human monocyte derived macrophages	Statin treatment during macrophage differentiation phase led to enhanced LPS-induced IL-1β and IL-6 secretion.	✓		[90]
	THP1 derived macrophages	Statin treatment led to increased pro-inflammatory cytokine (IL-1β, TNFα, and IL-6) and AdipoR expression (also seen in combination with oxLDL stimulation); 24 h statin treatment resulted in increased IL-10 mRNA levels, whilst 72 h treatment resulted in decreased expression.	✓		[91]
	Murine BMDMs	Statin-treated macrophages exhibited increased LPS-induced activation of NF-κB and IL-1β protein secretion in response to inflammasome stimulation.	✓	✓	[92]
	Murine BMDMs	Statin pretreatment exacerbated LPS-induced upregulation of IL-1β and NLRP3 transcript levels via p38 and mTOR.	✓		[93]
	THP1 derived macrophages	Impaired MWCNT-elicited IL-1β secretion.		✓	[94]
Cerivastatin	Human PBMC derived macrophages	Cerivastatin treatment suppressed growth of macrophages expressing MMPs and TFs.		✓	[95]
	Rabbit foamy macrophages	Decreased protein expression and activity of MMP-1, MMP-2, and MMP-9.		✓	[96]
	RAW-Blue™ cells and Murine BMDMs	Cerivastatin increased NF-κB/AP-1 activation in unstimulated and LPS-activated macrophages. LPS-induced TNF, IL-1β, and IL-6 expression was amplified. Expression of arginase-1 and GILZ was enhanced in unstimulated, LPS- and IL-4-activated macrophages.	✓	✓	[97]
Fluvastatin	human PBMC derived macrophages	Fluvastatin decreased TF activity in both unstimulated and LPS-, or ac-LDL-stimulated macrophages, but enhanced IL-1β cytokine release.	✓	✓	[98]
	Murine peritoneal macrophages and human PBMC derived macrophages	Simvastatin decreased MMP-9 protein secretion and inhibited TPA-induced enhanced MMP-9 release.		✓	[99]
	RAW 264.7 macrophages	Fluvastatin inhibited LPS and IFN-γ-induced NO formation and iNOS induction.Thought to be mediated through suppression of NF-κB activation and IFN-γ through STAT1.		✓	[81]
	RAW 264.7 macrophages	Fluvastatin upregulated macrophage *Socs3* expression, resulting in low responsiveness to inflammatory signals (IFN-γ, IL-6, and M-CSF) due to lower activation of STAT1, STAT3, and STAT5.		✓	[100]
	THP1 derived macrophages and THP1 derived acLDL loaded macrophages	Fluvastatin reduced both the expression, secretion, and proportion of active MMP-9 in PMA stimulated and acLDL-loaded THP1 derived macrophages.		✓	[101]
	RAW 264.7 macrophages and murine BMDMs	Fluvastatin inhibited LPS-induced suppression of CD9, leading to reduced formation of CD14/TLR4 complexes and TNFα and MMP-9 release.		✓	[102]
	Murine BMDMs	Fluvastatin pre-treatment exacerbated LPS-induced upregulation of IL-1b, IL-6, and NLRP3 transcript levels. Statin and LPS treatment of BMDMs harvested from NLRP3$^{-/-}$ mice synergistically enhanced IL-6 but did not affect IL-1β secretion. Statin treatment alone had no effect on the production of inflammatory mediators.	✓		[85]
	Human monocyte derived macrophages	Statin treatment during macrophage differentiation phase led to enhanced LPS-induced IL-1β and IL-6 secretion.	✓		[90]
	Murine BMDMs	Statin pretreatment exacerbated LPS-induced upregulation of IL-1b and NLRP3 transcript levels via p38 and mTOR.	✓		[93]
	THP1 derived macrophages	Impaired MWCNT-elicited IL-1β secretion.		✓	[94]
	Human PBMC derived macrophages	Decreased the activity of iNOS in M1 macrophages.		✓	[103]

Table 2. *Cont.*

Statin	Model	Summary	Inflammatory Effect		Ref.
			Pro	Anti	
Lovastatin	Rat peritoneal macrophages and microglia	Inhibited LPS-induced production of NO, TNFα, IL-1β, and IL-6 in rat primary microglia and macrophages.		✓	[104]
	Human PBMC derived macrophages	Statins acted as inhibitors of the induction of MHC-II expression by IFN-γ due to suppression of CIITA transcription. Statins repressed MHC-II mediated T-cell activation.		✓	[79]
	RAW 264.7 macrophages	Lovastatin inhibited LPS and IFN-γ-induced NO formation and iNOS induction—thought to be mediated through suppression of NF-κB activation and IFN-γ through STAT1.		✓	[81]
	RAW 264.7 macrophages	Lovastatin upregulated macrophage *Socs3* expression, resulting in low responsiveness to inflammatory signals (IFN-γ, IL-6, and M-CSF) due to lower activation of STAT1, STAT3, and STAT5.		✓	[100]
	Rabbit foamy macrophages	Decreased protein expression and activity of MMP-1, MMP-2, and MMP-9.		✓	[96]
	RAW 264.7 macrophages	Lovastatin increased LPS-induced TNFα production.	✓		[105]
	P388D1 macrophages	Statins increased production of MMP-12 in activated macrophage.	✓		[106]
	RAW 264.7 macrophages	Lovastatin increased CD14 expression and enhanced LPS-induced membrane levels leading to greater TNFα production, but simultaneously suppressed soluble CD14.	✓		[107]
	BMDMs from C57BL/6J mice and RAW 264.7 macrophages	Lovastatin blocked IFN-γ-induced *Citta* gene expression by inhibiting transcriptional events at *Citta* pIV, thereby suppressing MHC-II expression.		✓	[108]
	RAW 264.7 macrophages	Lovastatin treatment induced NO release but did not affect pro-inflammatory cytokine levels in unstimulated cells. However, with LPS it synergistically enhanced IL-6, IL-12p40, IL-1β, and NO release.	✓		[109]
	Murine BMDMs	Lovastatin pretreatment exacerbated LPS-induced upregulation of IL-1b, IL-6, and NLRP3 transcript levels.	✓		[85]
	THP1 derived macrophages	Impaired MWCNT-elicited IL-1β secretion.		✓	[94]
Metavastatin	P388D1 cell line	Statins increased production of MMP-12 in activated macrophages.	✓		[106]
	U937 derived macrophages and RAW 264.7 macrophages	Metavastatin pretreatment significantly increased bacterial clearance, despite reducing oxidative burst and phagocytosis due to increased induction of extracellular traps.	✓	✓	[110]
	J774A.1 mouse macrophages	Increased levels of iNOS and killing of internalized *S. pneumoniae*.	✓		[111]
Pitavastatin	RAW 264.7 macrophages	Suppressed LPS-induced upregulation of MCP-1, iNOS, and IL-6 gene expression.		✓	[112]
	THP1 derived macrophages, and murine peritoneal macrophages and BMDMs (BALB/cCrSlc mice)	Pravastatin repressed mature IL-1β release elicited by MWCNT/CC/MSU exposure in THP1-derived macrophages, and LPS + MWCNT induced mature IL-1β release in peritoneal macrophages. Pravastatin pretreatment strongly enhanced mature IL-1β release in LPS + MWCNT exposed BMDMs.	✓	✓	[94]
Pravastatin	Human PBMC derived macrophages	Statins acted as inhibitors of the induction of MHC-II expression by IFN-γ due to suppression of CIITA transcription. Statins repressed MHC-II mediated T-cell activation.		✓	[79]
	RAW 264.7 macrophages	Pravastatin inhibited LPS and IFN-γ-induced NO formation and iNOS induction—thought to be mediated through suppression of NF-κB activation and IFN-γ through STAT1.		✓	[81]
	RAW 264.7 macrophages	Pravastatin upregulated macrophage *Socs3* expression, resulting in low responsiveness to inflammatory signals (IFN-γ, IL-6, and M-CSF) due to lower activation of STAT1, STAT3, and STAT5.		✓	[100]

Table 2. *Cont.*

Statin	Model	Summary	Inflammatory Effect		Ref.
			Pro	Anti	
	RAW 264.7 macrophages	Suppressed LPS-induced upregulation of MCP-1, iNOS, and IL-6 gene expression.		✓	[112]
Rosuvastatin	Human monocyte derived macrophages	Rosuvastatin reduced MMP-7 and MMP-9 production.		✓	[113]
	oxLDL induced THP1 foam cells	Rosuvastatin inhibited ox-LDL-induced reduction of SOD1 expression.		✓	[114]
	THP1 derived macrophages	Rosuvastatin inhibited the AGE-RAGE axis and ROS production.		✓	[115]
	RAW 264.7 macrophages and J774 macrophages	Rosuvastatin increased Rac1 GTP-loading in LPS-stimulated macrophages, enhancing production of the proinflammatory cytokines IL-1β, TNFα, and IL-6.	✓		[89]
	Human monocyte derived macrophages	Statin treatment during macrophage differentiation phase led to enhanced LPS-induced IL-1β and IL-6 secretion	✓		[90]
	THP1 derived macrophages	Statin treatment led to increased pro-inflammatory cytokine (IL-1β, TNFα, and IL-6) and AdipoR expression (also seen in combination with oxLDL stimulation); 24 h statin treatment resulted in increased IL-10 mRNA levels, whilst 72 h treatment resulted in decreased expression.	✓		[91]
	THP1 derived macrophages	Inhibited foam cell formation and lessened the secretion of inflammatory cytokines (e.g., TNFα, IL-1β, and IL-6) from oxLDL-treated macrophages		✓	[116]
Simvastatin	Human monocyte derived macrophages	Simvastatin decreased superoxide production and therefore LDL oxidation		✓	[117]
	human PBMC derived macrophages	Simvastatin decreased TF activity in both unstimulated and LPS-stimulated/ac-LDL-stimulated macrophages. The suppression of TF activity induced by statin treatment was accompanied by a diminution in TF mRNA expression.		✓	[98]
	Murine peritoneal macrophages	Simvastatin decreased MMP-9 protein secretion and inhibited TPA-induced enhanced MMP-9 release.		✓	[99]
	Rabbit foamy macrophages	Decreased protein expression and activity of MMP-1, MMP-2, and MMP-9.		✓	[96]
	Peritoneal murine macrophages and RAW 264.7 macrophages	Simvastatin pretreatment enhanced both IL-12p40 and TNFα LPS-induced mRNA expression and protein production by a mechanism involving the AP-1 and C/EBP transcription factors, but IP-10 levels were reduced.	✓	✓	[118]
	PBMC derived human macrophages	Simvastatin inhibited IFN-γ-induced upregulated mRNA expression of the chemokines MCP-1, MIP-1a, and MIP-1b and the chemokine receptors *CCR1*, *CCR2*, and *CCR5*. MCP-1 protein expression was also notably reduced.		✓	[119]
	human primary monocyte derived macrophages	Statin administration significantly increased the secretion of IL-1β but had no significant effect on IL-8 or IL-6 and inhibited the secretion of TNFα. In combination with agLDL loading, statin treatment enhanced secretion of IL-1β and IL-8, but had no effect on TNFα or IL-6 secretion.	✓	✓	[120]
	BMDMs from C57BL/6J mice and RAW 264.7 macrophages	Simvastatin blocked IFN-γ-induced *Citta* gene expression by inhibiting transcriptional events at *Citta* pIV, thereby suppressing MHC-II expression.		✓	[108]
	PBMC derived human macrophages and THP1 derived macrophages	Simvastatin treatment led to the downregulation of inflammatory signalling pathways, marked by a reduction in the gene expression of proinflammatory associated chemokines (MCP-1, MIP-1, and tissue factor) and transcription factors (NF-κB and ETS-1). The anti-inflammatory associated transcription factor KLF-2 had upregulated gene and protein expression.		✓	[121]
	Murine peritoneal macrophages	Simvastatin pretreatment enhanced TLR2 and TLR4 ligand-stimulated IL-6 and TNF production.	✓		[82]
	RAW 264.7 macrophages	Enhanced LPS-mediated MMP-9 gene expression.	✓		[83]

Table 2. *Cont.*

Statin	Model	Summary	Inflammatory Effect		Ref.
			Pro	Anti	
	PBMC derived human macrophages, HL-60 derived macrophages and murine peritoneal macrophages (treated with simvastatin in vivo)	Simvastatin reduced phagocytosis and oxidative burst of IgG opsonized bacteria but enhanced the production of inflammatory mediators (TNFα and COX-2). No effect was seen on inflammatory mediators in response to non-opsonized bacteria, but impairment of phagocytosis remained.	✓	✓	[122]
	RAW 264.7 macrophages	Simvastatin pretreatment reduced basal and *S. aureus*-stimulated levels of C5aR and dampened macrophage sensitivity to membrane vesicles released from infected cells, decreasing TNFα production.		✓	[123]
	RAW 264.7 macrophages and murine BMDMs	Simvastatin inhibited LPS induced suppression of CD9, leading to reduced formation of CD14/TLR4 complexes and TNFα and MMP-9 release.		✓	[102]
	RAW 264.7 macrophages and murine BMDMs	Simvastatin pretreatment enhanced IL-12p40 and TNFα production in IFN-γ and *L. monocytogenes* stimulated macrophages. Statins suppressed MHC-II surface expression on IFN-γ-activated macrophages	✓	✓	[124]
	THP1 derived macrophages	Simvastatin pretreatment inhibited IFN-γ induced expression of MCP-1 and ICAM-1.		✓	[125]
	Murine BMDMs and human PBMCs	Simvastatin enhanced LPS-stimulated pro-IL-1β (28 kDa form), which disrupted mature IL-1β inflammatory actions.		✓	[126]
	Murine BMDMs	Simvastatin pretreatment exacerbated LPS-induced upregulation of IL-1b, IL-6, and NLRP3 transcript levels.	✓		[85]
	Murine BMDMs	Simvastatin reduced parasite burden by enhancing oxidative burst and phagosome maturation.	✓		[127]
	Raw 264.7 macrophages	Simvastatin repressed IL-1β secretion in response to *H. pylori* infection and increased autophagy.		✓	[128]
	Human monocyte derived macrophages	Statin treatment during macrophage differentiation phase led to enhanced LPS-induced IL-1β and IL-6 secretion	✓		[90]
	RAW-Blue™ cells and Murine BMDMs	Simvastatin increased NF-κB/AP-1 activation in unstimulated and LPS-activated macrophages. LPS-induced TNF, IL-1β, and IL-6 expression was amplified. Expression of arginase-1 and GILZ was enhanced in unstimulated, LPS-, and IL-4-activated macrophages.	✓	✓	[97]

2. In Vitro Evidence Demonstrating the Direct Effects of Statins on Macrophages

An abundance of in vitro studies have reported paradoxical statin-mediated effects on inflammation (Table 2, Figures 3 and 4), resulting from either blunting or enhancing pro-inflammatory signalling cascades. However, a limited number of studies have also reported that statins may alter the differentiation of macrophages rather than simply acting as regulators of inflammatory signalling pathways.

2.1. Statins Modulate TLR Inflammatory Signalling Pathways

Cell surface TLRs, such as TLR1, TLR2, TLR4, TLR5, and TLR6, are key initiators of innate immune responses. They are predominantly involved in host defence mechanisms through their recognition of a diverse array of stimulatory signals related to microbial membrane components, such as lipids, lipoproteins, proteins, and LPS [129]. TLR engagement triggers a range of antimicrobial responses, including the production of reactive nitrogen and oxygen species, inflammatory cytokines, and matrix metalloproteinases (MMPs). However, alongside their responsiveness to exogenous ligands, TLRs also recognise endogenous ligands (e.g., oxLDL) released from damaged tissues or dead cells, thereby regulating sterile inflammatory processes [130]. Indeed, prolonged TLR activation has been associated with uncontrolled chronic inflammatory diseases, including atherosclerosis [131–133]. TLR4, in particular, is upregulated in atherosclerotic plaques and demonstrates increased expression

as a result of ox-LDL exposure [134,135]. TLR4 signalling is mediated by the adaptor proteins myeloid differentiation primary response 88 (MyD88) and TIR-domain-containing adapter-inducing interferon-β (TRIF), which initiate two separate signal transduction pathways that culminate in the activation of a multitude of transcription factors [136,137], including members of the NF-κB [138] and IRF [139] families. MyD88-dependent signalling cascades include the activation of NF-κB and mitogen-activated protein kinase (MAPK) family members, such as extracellular signal-regulated kinase1/2, p38, and c-Jun N-terminal kinase (JNK), which, in turn, mediate the activation of AP-1 family transcription factors or the stabilization of mRNA to regulate inflammatory responses [129]. In contrast, TRIF-mediated TLR4 signalling occurs through the activation of IFN3 and STAT1, which induce the expression of IFN genes (e.g., *IFN-B*) and are also involved in late-phase NF-κB activation [138,140]. A number of accessory proteins, such as CD14 and CD36, are also suggested to play a role in macrophage inflammation cascades through their association with TLR4 [38].

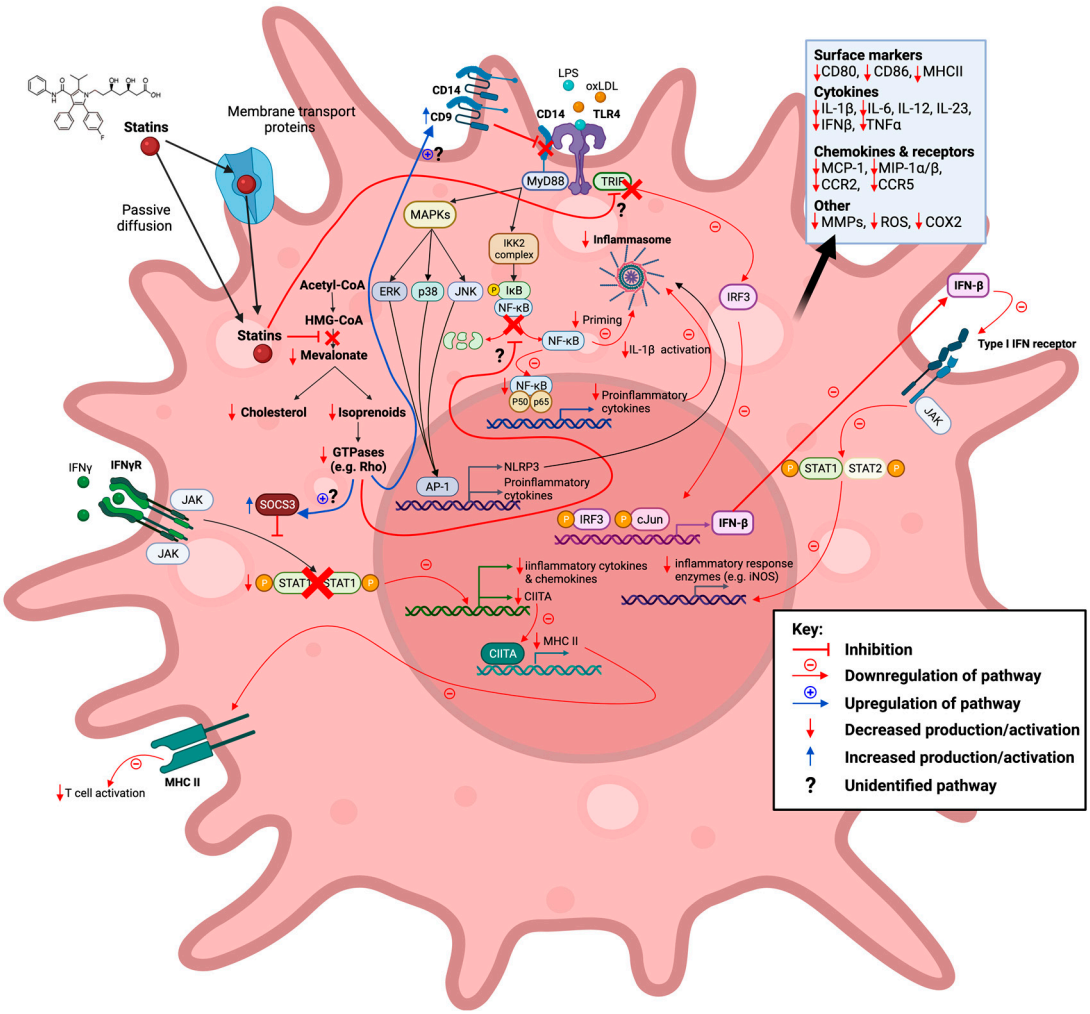

Figure 3. Statins inhibit the mevalonate pathway leading to both reduced cholesterol and isoprenoid biosynthesis, thereby also blocking farnesylation and geranylgeranylation of GTPases. Reduction in these downstream mevalonate intermediates is demonstrated to affect M1-associated macrophage inflammatory signalling pathways in vitro in an anti-inflammatory manner. This action of statins is seen in response to exogenous lipopolysaccharide (LPS), endogenous (interferon gamma (IFN-γ), and oxidized low-density lipoprotein (oxLDL) ligands. Created with BioRender.com, accessed on 7 March 2022.

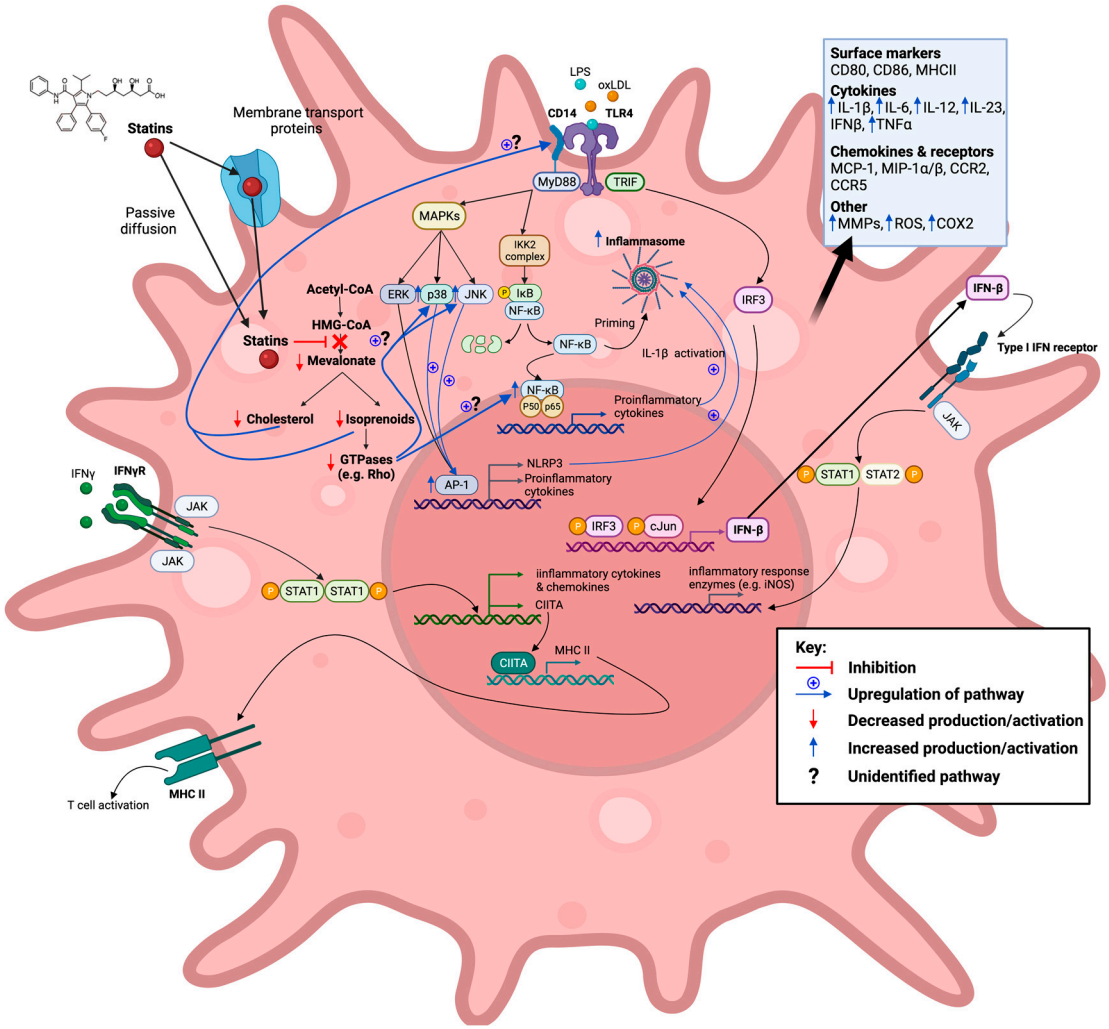

Figure 4. Statins inhibit the mevalonate pathway leading to both reduced cholesterol and isoprenoid biosynthesis, thereby also blocking farnesylation and geranylgeranylation of GTPases. Reduction in these downstream mevalonate intermediates is demonstrated to affect M1-associated macrophage inflammatory signalling pathways in vitro in a pro-inflammatory manner. This action of statins is seen in response to exogenous lipopolysaccharide (LPS) and oxidized low-density lipoprotein (oxLDL) ligands. Created with BioRender.com, accessed on 7 March 2022.

2.1.1. Anti-Inflammatory Modulation of TLR Signalling Pathways

As noted, NF-κB, through its activation in the TLR4 signalling pathways, is a key regulator of both macrophage inflammatory responses to pathogens and their role in sterile inflammatory diseases. Multiple statins (atorvastatin [81], fluvastatin [81,98], lovastatin [81,104], pravastatin [81], and simvastatin [98,121]) have been shown to inhibit NF-κB activation. The effects of statins on NF-κB activation are suggested to be the result of statins' inhibition of the mevalonate pathway, specifically the isoprenoid branch, as various studies have reported that the addition of mevalonate, FPP, and GGPP reverses their action on NF-κB [98,121]. The exact links between statins' inhibitory action on both protein prenylation and NF-κB activation have yet to be fully elucidated, although it has recently been reported that statins attenuate the degradation of the NF- κB inhibitor protein IκB [141]. IκB degradation is reliant on the phosphorylation of the IKK2 complex, which may be regulated by Rac1 in macrophages [142]. The upregulated gene and protein expression of Krüppel-like factor 2 [121] (a potent regulator of pro-inflammatory activation) and SOD1 [114] (associated with increased antioxidant enzyme activity and decreased ROS production [143]) have also been reported to occur in statin-treated macrophages and may

contribute to the suppression of NF-κB-driven signalling pathways. Statin-mediated inhibition of the IκB/NF-κB pathway has been shown to result in a global anti-inflammatory effect on macrophages, with mRNA and protein analysis revealing the attenuated expression of many pro-inflammatory associated mediators, including cytokines (TNFα, IL-1β, and IL-6) [104,121], chemokines (MCP-1 and MIP-1α/β) [121], and tissue factor (a membrane-bound glycoprotein that plays a prominent role in the extrinsic pathway of blood coagulation and fibrin deposition) [98], and NO production [81,103,104]. Importantly, the inhibitory effects of statin treatment on NF-κB-induced cytokine synthesis have also been seen when using the CVD-relevant endogenous ligand oxLDL and are associated with reduced macrophage oxLDL loading and foam cell formation [84,114,116,117].

Interestingly, statin-mediated inhibition of the MyD88/NF-κB pathway has also been implicated in reducing inflammatory responses through enhancing autophagy [87,128,144,145] via the Akt-mTORC1 axis [87,144], but there are conflicting thoughts on whether this results from the inhibition of the cholesterol or isoprenoid biosynthesis branch of the mevalonate pathway [128,144,145]. The increased autophagy resulting from statin treatment has been noted to restrict NLRP3 (NOD-, LRR-, and pyrin domain-containing protein 3) inflammasome activation and thus reduce pro-inflammatory cytokine release [87,128].

In addition to signalling through NF-κB-dependent pathways, which are thought to be induced predominantly by MyD88-signalling, it has been proposed that statins' inhibitory effects on macrophage inflammatory responses result from a downstream suppression of TRIF-mediated signalling [112]. Pravastatin and pitavastatin treatment of TLR4-stimulated RAW264 macrophages have a strong inhibitory effect on the TRIF/IRF3/IFN-β pathway in macrophages. The reduction in IFN-β expression resulting from statin treatment led to decreased STAT1 phosphorylation and the attenuation of pro-inflammatory gene expression in macrophages, evidenced by the reduced secretion of MCP-1, NO, and IL-6. Unlike previous studies, the researchers could not identify whether this action was the result of mevalonate or isoprenoid inhibition by statins, as they noted that mevalonate itself also suppressed LPS-induced expression of IFN-β [112].

Statin treatment has also been reported to reduce the matrix degrading capacity of M1-like polarized macrophages through the modulation of matrix metalloproteinase (MMP) expression [88,96,99,101]. This is particularly relevant to CVD, as atherosclerotic lesions show enhanced MMP expression, and this is thought to contribute to the weakening of the vascular wall, aiding plaque rupture [146]. Atorvastatin co-incubation during the polarization of classically activated macrophages was found to reduce MMP-14 activation [88], which is thought to mediate the expression of other MMPs, such as MMP-9. MMP-9 is one of the most widely investigated MMPs and is known to be involved in inflammation (e.g., extracellular processing of IL-1β [147]) and fibrosis in CVD [148]. In line with this, various studies have reported that statin treatment decreases MMP-9 protein secretion, thereby reducing its activity [99,101]. Importantly, this effect was also seen in in vitro studies of foamy macrophages [96], which are abundant in atherosclerotic plaques. This effect of statins is thought to be dependent on their action as mevalonate inhibitors [88,99], and there is evidence that the uncoupling of JAK/STAT signalling plays a role [101]. However, it should be noted that most of the studies examining statin-mediated effects on MMP expression in macrophages have not investigated the potential underlying mechanisms, and the exact point in the TLR-signalling pathway that is impacted awaits clarification. Macrophage production of MMPs in the absence of statin treatment is regulated via both the NF-κB [149,150] and MAPK [151] pathways.

A final means by which statins are thought to blunt TLR4-induced macrophage inflammation is not via inhibition of its signalling cascade but rather via the enhancement of anti-inflammatory response elements. In this respect, it has been reported that fluvastatin and simvastatin upregulate CD9 expression in both RAW264.7 cells and murine bone-marrow derived macrophages (BMDMs) treated with LPS [102], consequently leading to reduced TNFα and MMP-9 production. CD9 is a recognised anti-inflammatory marker of macrophages [152] and negatively regulates LPS-induced macrophage activation by

preventing the formation of CD14/TLR4 complexes [153]. Indeed, statin treatment no longer resulted in significant inhibition of TNFα and MMP-9 in BMDMs from CD9 knockout mice, suggesting that statins' anti-inflammatory effects are, to a degree, dependent on CD9 [102]. The upregulation of CD9 observed following statin treatment appears to be dependent on their inhibitory action on protein prenylation (Figure 2), specifically geranylgeranylation, as GGTI-298 (a geranylgeranyltransferase inhibitor), but not FTI-277 (a farnesyl transferase inhibitor) increased LPS-treated CD9 levels to a comparable degree. However, the precise mechanism by which decreased isoprenoid synthesis confers CD9 upregulation is currently unknown.

2.1.2. Pro-Inflammatory Modulation of TLR Signalling Pathways

In contrast to the anti-inflammatory properties of statins described above, a growing number of in vitro studies are reporting that statins paradoxically enhance pro-inflammatory signalling in macrophages (Table 2). LPS-triggered TLR4 activation in macrophages activates both NF-κB and AP-1 transcription factors [154], which have both been implicated in statin-induced pro-inflammatory responses [93,118].

In one of the earliest studies [118] reporting pro-inflammatory effects, it was demonstrated that simvastatin pre-treatment enhanced LPS-induced IL-12p40 (a constituent of the bioactive cytokines IL-12 and IL-23) and TNFα mRNA expression and protein production by a mechanism involving AP-1 and C/EBP transcription factors. Specifically, statin treatment decreased c-FOS binding to the AP-1 promoter region (a negative regulator of the signalling system) whilst simultaneously enhancing JNK-mediated c-Jun phosphorylation, thereby stimulating the transcription of inflammatory genes. In keeping with this, atorvastatin and simvastatin pre-treatment is observed to enhance TLR2/TLR4 ligand-stimulated IL-6 and TNFα production [82], and various research groups have found statins to induce the activation of the MyD88 pathway transcription factor NF-κB [92,97] (alongside AP-1). There is evidence that these effects depend on the isoprenoid branch of the mevalonate pathway [118] and on Rho GTPases [92,105]. The molecular mechanisms connecting the effects of statins on GTPases and the increased expression of the AP-1 transcription factor remain poorly understood, but it has been suggested that Rho GTPase inactivation by the suppression of prenylation abolishes an inhibitory feedback loop in this pathway, thereby resulting in an enhanced upregulation of cytokine gene expression.

Statins have also been found to enhance pro-inflammatory macrophage responses by increasing NLRP3 inflammasome activation in a p38-dependent manner [93]. IL-1β is unique compared to most cytokines in that it requires post-translational modification via caspase-1 to reach its mature form, being originally translated as a 33 kDa inactive precursor (pro-IL-1β) [155]. Caspase-1, in turn, requires NLRP3 inflammasome activation to mediate this process [156]. Several studies have found that statins promote caspase-1 and NLRP3 activation and have shown that statin-stimulated IL-1β release is dependent on their enhanced activation [85,89,93]. Statin treatment is proposed to facilitate LPS-induced capase-1 and inflammasome stimulation via its disturbance of isoprenoid biosynthesis, as the effect was reversible with GGPP addition [89]. Furthermore, the deletion of geranylgeranyltransferase type 1 (GGTase-I; responsible for carrying out GTPase geranylgeranylation) in macrophages mimicked the effects of statins. Later studies by the group suggested that Rac1 mediates the hyperactivity to pro-inflammatory stimuli observed in statin-treated and GGTase-I-deficient macrophages because the deletion of Rac1 abolished the enhanced release of pro-inflammatory cytokines, whereas the deletion of other GTPases (RhoA and Cdc42) did not [89]. However, how statin-induced hyperactive Rac1 activation may drive the enhancement of LPS-stimulated p38 activation and thus increase pro-inflammatory IL-1β secretion has yet to be explored.

In consideration of the relevance of statins to atherosclerosis management, various research groups have also investigated the effects of statins on macrophage TLR-mediated cytokine responses using endogenous molecules (e.g., LDL and cholesterol crystals), with mixed findings. Lindholm and Nilsson reported that in combination with aggregated LDL

(agLDL) loading, statin treatment enhanced secretion of IL-1β and IL-8 but had no effect on TNFα or IL-6 secretion in human primary monocyte-derived macrophages isolated from buffy coats [120]. Cui et al. also reported statin treatment to strongly enhance mature IL-1β release in murine BMDMs stimulated with a combination of LPS and cholesterol crystals but noted the opposite to be true in THP-1 derived macrophages [94]. Interestingly, despite the conflicting data between macrophage cell types, these effects were all reported to be isoprenoid dependent [94,120]. At present, it remains unclear which TLR-pathway signalling elements are affected by statin treatment in ox- and agLDL-stimulated macrophages but, given that (for reasons not completely understood) different TLR4 stimuli induce different cellular responses [157,158], future studies may find the involvement of signalling components outside of those noted in the LPS experiments.

It has also been suggested that statin-mediated effects on TLR-inflammatory responses may not solely be the result of their action on its signalling pathway but may also result from an increase in membrane CD14 expression [107]. RAW 264.7 macrophage incubation with lovastatin both alone and in combination with LPS promoted increased CD14 mRNA and protein levels, resulting in greater LPS-induced TNFα secretion. Coincubation of lovastatin-treated macrophages with FPP, GGPP, or water-soluble cholesterol was seen to prevent LPS-induced TNFα levels, suggesting that statin effects on macrophage responses may be regulated at multiple levels.

2.2. Statins Modulate IFN-γR Inflammatory Signalling Pathways

Cytokines are major regulators of macrophage activation, and aberrant secretion is implicated in several disease states, including chronic inflammatory diseases such as atherosclerosis. IFN-γ, particularly, is known to play a role in atherosclerotic development, being highly expressed in lesions [159] and inducing foam cell formation [160] in macrophages via increased LDL uptake. IFN-γ exerts its biological activities by binding to a specific cell surface receptor, IFN-γR, which utilises the Jak-STAT pathway in its signal transduction (a recurring theme amongst members of the cytokine receptor superfamily). Through this mechanism, IFN-γ induces the expression of numerous genes that play a role in macrophage inflammatory responses, such as ROS production and communication between macrophages and other immune cells (e.g., T lymphocytes) via chemokine secretion and surface marker expression [161]. Notably, IFN-γ is also thought to participate in an amplification loop to increase immune system sensitivity, as it has been seen to enhance LPS-induced NF-κB activation and increase TLR expression, whilst in turn, TLR ligands, such as LPS, augment local IFN-γ induction [161].

2.2.1. Anti-Inflammatory Modulation of IFN-γR Signalling Pathways

In both human and mouse-derived macrophages, a variety of statins have been found to reduce IFN-γ-induced MHC-II expression through the downregulation of the class II transactivator (CIITA), thereby interfering with their ability to prompt T cell activation, indicative of an immunosuppressive impact [79,80,108,124]. Further examination of this effect provided some insight into the potential molecular basis, with Kwak et al. and Lee et al. finding that statins specifically decrease the expression of CIITA at the transcriptional level, after noting that CIITA mRNA destabilisation did not occur in the presence of simvastatin. The transcription of IFN-γ-inducible CIITA expression is controlled by a large regulatory region containing three independent promoters pI, pIII, and pIV, which, in turn, are controlled by distinct regulatory elements [162]. As Kwak et al. [79] had noted that constitutive MHC-II expression, which is controlled by pI and pIII, was not affected by statin treatment it was suggested that pIV may be involved. Lee et al. [108] therefore focused their investigation on this particular promoter region, discovering that its transcription factors STAT1 and IRF-1 were both downregulated. In addition to this, the team also documented that the addition of GGPP, but not cholesterol, abolished the statin-mediated reduction in IFN-γ-induced MHC-II expression, signifying again that the effect was likely to be dependent on statins' action as isoprenoid inhibitors. They next tested the effects of two

specific inhibitors of Ras superfamily protein prenylation: GGTI-298 and FTI-277. GGTI-298 was found to mimic the inhibitory actions of simvastatin on CIITA expression, but FTI-277 had no effect, indicating the specific involvement of geranylgeranylation. Furthermore, a Rac1-specific inhibitor was also shown to capture this effect, revealing its contribution to IFN-γ-induced STAT1 activation. Another potential factor leading to STAT1 suppression was suggested by Huang et al., who demonstrated that lovastatin and fluvastatin upregulate mRNA expression of the *Socs-3* gene in macrophages [100]. SOCS proteins are known to negatively regulate cytokine signalling through their binding to the cytoplasmic domain of recognition receptors [163]. Regardless of the precise signalling mechanisms involved, the dampening of IFN-γ inflammatory stimulation via STAT1 inhibition has also been found to affect a number of other pro-inflammatory responses, including reduced mRNA expression of chemokines (monocyte chemotactic protein-1 (MCP-1) and macrophage inflammatory proteins-1 α and β (MIP-1α/β)) [112,119,125], chemokine receptors (CCRs—*CCR1*, *CCR2*, and *CCR5*) [119] and cytokines (IL-6), along with reduced NO production [81].

2.2.2. Pro-Inflammatory Modulation of IFN-γR Signalling Pathways

Interestingly, reports of statins enhancing pro-inflammatory signalling have not cited the involvement of IFN-γR pathways. Indeed, although simvastatin pre-treatment was found to enhance IL-12p40 and TNFα production in murine macrophages stimulated with both IFN-γ and *L. monocytogenes* infection [124], the researchers highlighted that this was most likely to be the result of TLR-mediated signalling pathways as they found that IFN-γ treatment alone in macrophages had no effect on pro-inflammatory cytokine production. Moreover, in agreement with anti-inflammatory reports, they noted a decreased surface expression of MHC-II. Another study by Linnenberger et al. agreed with this finding that statin treatment had no effect on macrophage stimulation by IFN-γ (despite enhancing LPS-induced expression of TNF, IL-1β, and IL-6) [97].

2.3. Statins Play Roles in Macrophage Differentiation

Alongside their effects on inflammatory signalling pathways, more recent studies have suggested that statins may directly alter the differentiation of macrophages in vitro. In one study, atorvastatin enhanced an IL-4-induced M2 phenotype via p38 MAPK-dependent PPARγ activation when added at the start of the differentiation process [86]. However, in other work, macrophages differentiated overnight in the presence of fluvastatin were more reactive to LPS stimulation than those that were not, characterised by a greater secretion of IL-1β and IL-6 and dependent on Rac1-geranylgeranylation [90]. Taken together, these studies suggest that macrophages differentiated in the presence of statins may be more immune-responsive to various stimuli and therefore can enhance either pro or anti-inflammatory functions depending on the particular stimulating agents they are exposed to.

3. In Vivo Studies Investigating the Effects of Statins on Macrophages

In vivo exploration of statins' inflammatory potential (mostly in rodent models) has likewise resulted in paradoxical anti- and pro-inflammatory findings.

A recent study by Wang et al. presented the idea of statins playing a role in macrophage polarization. In their study examining the effects of simvastatin in a rat model of intracerebral haemorrhage, statin treatment was seen to upregulate CD36 expression as well as increasing PPARγ activation, facilitating M2-like phenotype polarization in perihematomal microglia [164]. Similarly, rosuvastatin-loaded nanomicelles were found to stimulate microglia/macrophages to an M2 phenotype in a mouse model of intracerebral haemorrhage, where they also reported reduced tissue levels of IL-1β and TNFα and increased levels of IL-10 [165]. Various other studies in a range of rodent models have also reported atorvastatin and pravastatin to have macrophage-polarizing actions, demonstrated by augmented M1/M2 ratios [103,166–168]. Numerous reports have also demonstrated statin treatment to decrease macrophage infiltration and proliferation within inflamed tissues [95,167,169–172], which are features associated with atherosclerotic lesion regression [173].

Conversely, Kiener et al. reported lipophilic statins to markedly increase leukocyte influx into inflamed tissues in mice [174], and lovastatin treatment was found to both inhibit M2-like polarization in tumour-associated mice macrophages and enhance an M1-like phenotype [175]. Additionally, a recent report in *Apoe*$^{-/-}$ mice demonstrated that 20 weeks of oral atorvastatin therapy resulted in increased calcifications in atherosclerotic plaques and that Rac1 activity was significantly elevated in macrophage-rich plaque areas [92]. In line with this, increased coronary artery calcium scores were seen in high-risk patients taking statins, and Rac1 activity was found to be significantly elevated in patient monocytes. Further in vitro studies by the group revealed that statin administration of BMDMs led to disruption between the Rac1 complex and its inhibitor (RhoGDI), resulting in its increased activation. This process was reversed by FPP and GGPP supplementation but not by the addition of squalene. However, notably, statin treatment of these *Apoe*$^{-/-}$ mice did not lower cholesterol levels or prevent plaque progression, which contrasts with what is observed in human clinical trials [176–179].

Overall, it is important to note that unlike the in vitro scenario, there is scarce evidence of statin-mediated mevalonate pathway inhibition having direct effects on macrophage responses in vivo, and therefore their reported actions on macrophage polarization and accumulation may be the result of the influence of statins on other cell types and the macrophage microenvironment. Indeed, Hardtner et al. noted this was likely to be the case as they failed to detect relevant concentrations of atorvastatin in atherosclerotic plaques in both mice and human patients, despite finding oral statin administration to induce retardation of plaque progression and macrophage proliferation [169].

4. Clinical Evidence for Inflammatory Effects of Statins on Macrophages

Clinical data regarding the immunomodulatory role of statins with specific respect to macrophage function are limited. However, despite the differential effects noted in the in vitro and in vivo research explored, the few studies conducted have only found statins to exhibit immunosuppressive effects on macrophages. In 2011, Pucci et al. demonstrated that intra-plaque macrophage content and circulating CRP levels were lower in statin-treated patients compared to untreated hypercholesterolemic patients, reaching a level comparable to normolipidemic subjects [180]. Additionally, PPARγ expression was notably increased in coronary-plaques and peripheral blood monocytes in statin-treated patients. A similar result was seen in a study conducted by Hothersall et al. where the effect of daily oral atorvastatin treatment was found to reduce the number of macrophages in the sputum, although there was no improvement in the control of asthma symptoms [181]. In opposition to this, John et al. found no significant difference in patients treated with simvastatin [182]. Finally, a recent study by Kauerova et al. investigating the influence of statin treatment on macrophage polarization in human adipose tissue reported statin therapy to increase the proportion of M2-like macrophages compared to M1-like ones [103]. Similarly, to the in vivo reports, researchers were unable to elucidate the underlying mechanisms of these effects. Therefore, it is possible that statins may be indirectly causing the observed macrophage responses in human pathologies.

5. Discussion

Over the years, support has grown for the notion that the efficacy of statins in atherosclerotic CVD treatment results not only from their ability to lower plasma cholesterol but also from their immunomodulatory properties. Macrophages play a crucial role in the immune responses associated with atherosclerosis, and there is evidence that statins can alter their inflammatory profile, potentially lessening their contribution to the progression and development of the disease. This has led to the suggestion that statins may offer potential therapeutics for pathologies beyond CVD, such as cancers [183], autoimmune disorders [184] and infectious diseases [185]. However, paradoxically, an increasing number of in vitro and in vivo studies have also demonstrated that statins can enhance macrophage pro-inflammatory responses, such as the increased secretion of pro-inflammatory cytokines.

These contradictory findings may, in part, result from differences in experimental design, with studies employing various animal and cell models, as well as diverse treatment regimens.

Due to the complex nature of cell culture, cell-based assays can exhibit a high degree of inter- and intra-laboratory heterogeneity. Biological (cell type, seeding density, and medium composition) and technical (edge effect, drug type and dose, incubation conditions, treatment time, and duration time) parameters can introduce variation, and this may contribute to the differential effects observed between the various investigations of statins' effects on macrophage inflammatory responses. Indeed, a recent study that reviewed the effects of pravastatin treatment on various macrophage cell types noted that it acted in synergy with LPS to promote IL-1β expression in BMDMs but markedly repressed its production in both peripheral-blood monocyte and THP1-derived macrophages [94]. Taking this into consideration, it is interesting to note that many of the studies involving the investigation of BMDMs support the idea of statins enhancing a pro-inflammatory macrophage response to some degree and, more specifically, found statins to enhance IL-1b transcription (Table 2). Differing responses to identical stimuli between both murine [186,187] and human [188,189] macrophage lineages have also previously been noted, and this is not surprising considering that they are known to vary in their surface marker expression and plasticity to environmental stimuli [187]. Additionally, the lack of characterisation of macrophage activation states (e.g., M0, M1, or M2) in many investigations both prior to and after statin treatment makes it challenging to compare studies directly and to pinpoint at which stage statins impact macrophage responses.

Macrophages modify their properties in response to their specific microenvironment, and therefore differences in culture conditions can also result in stark variations in functional output. Within the literature summarised in Table 2, variations in assay media (e.g., presence of serum) and cell density are present, both of which are noted to influence macrophage phenotypes and responses heavily [190–192]. Moreover, the inflammatory stimuli differ between studies, and as noted earlier, this can contribute to different inflammatory outcomes. For example, statins appear to confer anti-inflammatory effects more consistently via IFN-γR-mediated signalling pathways compared to TLR-mediated pathways.

Due to differences in their pharmacokinetics and pharmacodynamics, some have suggested that different statins may have distinct pleiotropic actions [13]. Importantly, however, the studies which note the differential effects of statin types on macrophage responses [79,81,83,98] show this property to manifest as differences in the magnitude of their inflammatory capacity rather than the pro- or anti-inflammatory direction of the response. There are no individual investigations that have found one statin to be uniquely pro-inflammatory and another to be uniquely anti-inflammatory, but groups have reported certain statins to elicit a greater effect than others [83,98]. In line with this, various in vitro studies have reported a positive correlation between statins' inflammatory potential and their concentration [81,82,84,91,98,118,119,124,128] and exposure time [91]. Notably, all statins examined to date have been found to promote both pro- and anti-inflammatory features in vitro, with the exception of pravastatin (Table 2). However, further studies are needed to confirm whether pravastatin really does only induce anti-inflammatory features or if this is because there have been only a few investigations evaluating its effects on a limited number of macrophage models.

It is worth noting that even when taking all these factors into consideration, some investigations have found that statins simultaneously promote pro- and anti-inflammatory phenotypes [92,97,110,122]. One example of this is a study by Linnenberger et al. in which, under both unstimulated and LPS-activated conditions, statins enhanced M1-like proinflammatory cytokine release but also increased the expression of arginase, a classical marker of M2 macrophages which antagonises NO production (indeed, NO release was unaltered upon statin treatment). Therefore, it may also be the case that the rigid M1/M2 classification system is limiting the interpretation of statin effects.

Taken together, the current evidence suggests that statins can modulate both pro- and anti-inflammatory macrophage responses depending on the macrophage cell types involved and

on the particular immune stimuli used and their respective signalling pathways. Statin type, concentration and incubation time do not greatly impact whether the response in macrophages is pro- or anti-inflammatory but do influence the magnitude of the effect. Importantly, these suggestions agree with the idea that statins promote macrophage immunomodulatory effects through their actions as HMG-CoA reductase inhibitors, which is supported by the majority of in vitro studies to date (regardless of the particular inflammatory leaning of macrophage responses to statin treatment) [79–82,85,88,89,92,94,95,98–100,102,107,108,118–122]. This has been demonstrated through the addition of various components of the mevalonate pathway (e.g., squalene, FPP, or GGPP) and the evaluation of their ability to reverse the impact of statins. Specifically, most studies have reported the inhibition of protein prenylation to be the predominant factor underlying statin effects, and compelling evidence pinpoint GTPases, such as Rho family members, as key molecular targets [89,92,105,108,122]. However, it is still unclear how dissimilar macrophage cell types or varying inflammatory stimuli may contribute to the differential regulation of the isoprenoid pathway and subsequently result in opposing inflammatory actions.

While cell culture studies have been important in establishing the concept that statins have direct immunomodulatory effects on macrophages, it is important to validate and contextualise these findings through systematic in vivo and clinical research. This approach could also help to answer important questions pertaining to the suitability of statins as immunotherapeutic agents (e.g., do statin effects on macrophages vary depending on their tissue-specific characteristics and does this lead to differential impacts on particular disease processes?). Moreover, it is important to review how statin effects on macrophage responses may vary between individuals due to differences in underlying comorbidities and individual factors such as age. However, as yet, relatively few studies have investigated the specific effects of statins on macrophage responses in whole organisms, and it is difficult to define in those that have whether the results are due to direct or indirect actions. Future in vivo studies may be able to address this, for example, through the use of cell-specific drug targeting strategies, such as nanoparticles [193].

In summary, while multiple studies over the last 25 years have demonstrated statins' direct immunomodulatory effects on macrophages, it is challenging to draw definitive conclusions regarding their specific impact due to the considerable heterogeneity between studies. Additional investigations are therefore needed to fully elucidate the particular molecular targets of statins involved in their immunomodulatory actions and how these impact inflammatory signalling pathways. Clarification of the underlying factors contributing to statins' paradoxical effects on macrophage inflammation may aid the development of novel statin-based immunotherapeutic approaches in the treatment of atherosclerosis and other diseases, such as infections, sepsis, chronic inflammatory diseases (e.g., rheumatoid arthritis), and cancerous tumours.

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