

T Lymphocytes as Targets of Statins: Molecular Mechanisms and Therapeutic Perspectives

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Abstract: Statins are cholesterol-lowering drugs extensively used for primary and secondary prevention of cardiovascular events related to hypercholesterolemia. Because of their capacity to inhibit HMG-CoA reductase, statins also block the production of isoprenoids required for post-translational modification of proteins such as Ras superfamily GTPases, which are master regulators in signaling pathways triggered by surface receptors. As such, statins have pleiotropic effects on many cell types. In the immune system, statins harbor strong anti-inflammatory properties, which result from their capacity to interfere with the activation of proinflammatory cells, including macrophages and endothelial cells. More recently, T-lymphocytes have been identified as cellular targets of statins. Here we shall review recent findings, which document an inhibitory activity of statins on T-cell activation, proliferation, differentiation to Th1 cells and migration across the blood-brain barrier. The therapeutic perspectives of these findings, based on animal models and ongoing clinical trials, will also be discussed.

Keywords: HMG-CoA reductase, prenylation, GTPase, Th1/Th2, autoimmune disease, allograft rejection, animal model, clinical trial.

Statins are a family of chemically related small molecules, which inhibit hydroxy-methyl-glutaryl Coenzyme A (HMG-CoA) reductase, a rate-limiting enzyme that catalyzes the conversion of HMG-CoA to mevalonate, a key intermediate in the cholesterol biosynthetic pathway. These molecules, first identified in a fungal extract in 1976, are structurally very similar to HMG-CoA, but have a higher affinity for HMG-CoA reductase. By binding to the active site of HMG-CoA reductase, statins competitively inhibit the enzyme and hence block cholesterol biosynthesis [1]. As such, statins have become the most widely used drugs in the treatment and prevention of hypercholesterolemia [2, 3].

In recent years, a large body of evidence has been generated documenting pleiotropic effects of statins beyond their blood cholesterol-lowering properties. Of particular relevance are the results of large-scale clinical trials, which have demonstrated that the benefits of statin therapy in the prevention of cardiovascular events are greater than would be expected from the bare lowering of cholesterol levels [4, 5]. Accumulating evidence supports the notion that statins have anti-inflammatory and immunomodulatory properties and may thus regulate important processes in both immune and vascular biology [6, 7]. We refer the reader to excellent reviews on statins for in-depth information on their anti-inflammatory activities [8, 9]. Here we shall briefly discuss the molecular targets of statins and focus on the immunomodulatory activities of these drugs on T lymphocytes.

1. MOLECULAR TARGETS OF STATINS

1.1. Statins and Protein Prenylation

Mevalonic acid is not only an intermediate in the biosynthesis of cholesterol, but is also the precursor of isoprenoids (Fig. 1), a class of lipids which are post-translationally added to cellular proteins, resulting in their docking to the inner leaflet of biological membranes. Among these proteins of paramount importance are the Ras superfamily GTPases which are master regulators in signaling pathways triggered by surface receptors and control key processes including cell proliferation, cell motility and intracellular membrane trafficking [10-12]. The principal members of this family are Ras (H-Ras, K-Ras, N-Ras), Rho (Rho, Cdc42, Rac) and Rab proteins. Although some promiscuity can occur, the lipid moiety is normally farnesyl pyrophosphate (FPP) for Ras proteins and geranylgeranyl pyrophosphate (GGPP) for Rho and Rab proteins [13]. Membrane targeting of these proteins is essential for their function, as in that location they can effectively interact with their upstream activators and downstream targets [11]. Because of their capacity to inhibit mevalonate biosynthesis, statins block protein isoprenylation, thereby impairing membrane localization and function of Ras and Ras-like proteins [14-16].

1.2. Statins and Lipid Rafts

Cholesterol is an essential component of biological membranes. By blocking cholesterol biosynthesis, statins may be expected to impair the integrity of cell membranes and in particular, affect the cholesterol-enriched membrane microdomains, which are known as lipid rafts fundamental for signal transduction. Lipid rafts are assemblies of sphingolipids and cholesterol that form tightly packed gel-like

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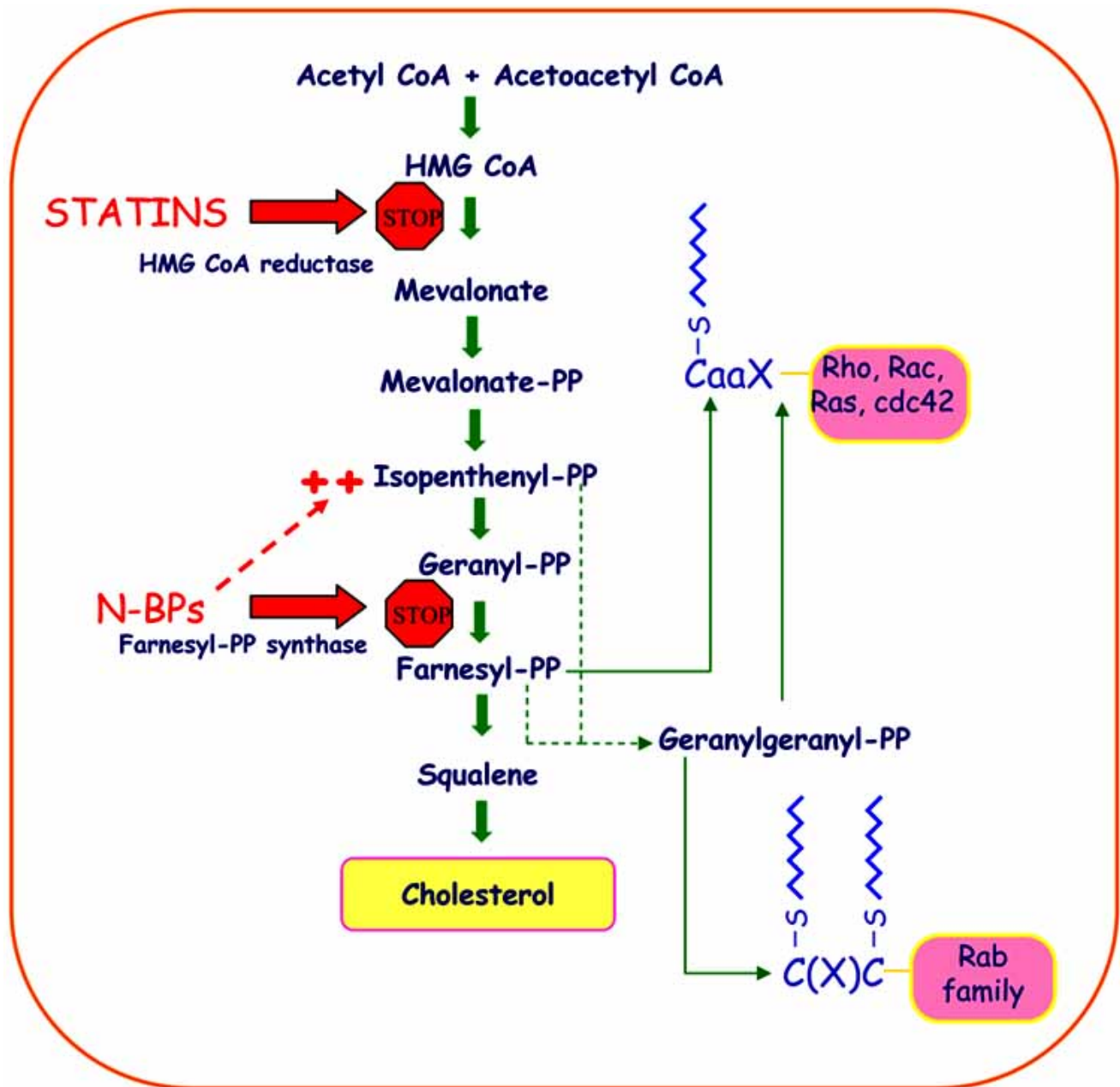


Fig. (1). Cholesterol biosynthetic pathway. Inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase by statins decreases the synthesis of isoprenoids and cholesterol. Nitrogen-containing bisphosphonates (N-BPs) inhibit farnesyl diphosphate (FPP) synthase, thereby also inhibiting cholesterol and isoprenoid biosynthesis, which results in accumulation of isopentenyl pyrophosphate (IPP), a non peptide phosphoantigen able to activate $\gamma\delta$ T-cells. PP, pyrophosphate.

regions with a diameter of about 70 nm, floating in the liquid-disordered glycerophospholipid-rich environment of cell membranes. These membrane microdomains are able to recruit specific sets of membrane proteins, such as GPI-anchored proteins, to the outer membrane leaflet and palmitoylated proteins (*e.g.* Src family protein tyrosine kinases) to the inner membrane leaflet, while excluding others [17]. As a result of this highly specific lipid and protein composition, lipid rafts are involved in key cellular functions, such as vesicular transport, membrane trafficking and initiation of signal transduction pathways [18]. Although statins can alter lipid raft dependent functions by decreasing the levels of

membrane cholesterol in some cell types [19-25], the effect of statin-induced raft on immune cells is controversial [15, 16, 26-29].

2. T LYMPHOCYTES AS CELLULAR TARGETS OF STATINS

The finding that statins attenuate autoimmunity in mouse disease models, as well as evidence emerging from clinical trials on the beneficial effects of statins on pathologies such as solid organ transplant rejection and multiple sclerosis, have directed significant research effort to the characterization of the effects of statins on T lymphocyte-mediated im-

munity. *In vitro* and *in vivo* studies have demonstrated that statins, by acting directly or indirectly on T-cells, can affect their migration, activation, proliferation and differentiation. Fig. 2 summarizes the activities of statins on T-cells.

2.1. Indirect Effects of Statins on T Lymphocytes

2.1.1. Inhibition by Statins of T-Cell Homing and Migration by Targeting Leukocytes and Endothelial Cells

An intense cross-talk between leukocytes and endothelial cells, mediated by receptor surface expression, cytokine production and chemokine gradients, is required for T-cell recruitment to the site of inflammation. In this complex process, leukocytes and phagocytes are attracted to the site of infection by chemokines produced by the endothelium, while a number of adhesion molecules sequentially control leukocyte rolling, adhesion and transvascular migration. Following activation in the inflamed tissue, leukocytes upregulate surface molecules and produce cytokines, which amplify inflammation and promote lymphocyte recruitment.

Statins have been shown to inhibit T lymphocyte migration by interfering with chemokine expression by leukocytes and endothelial cells [6, 9, 30]. Bustos *et al.* [31] have indeed demonstrated that atorvastatin inhibits the expression of IL-8, a chemokine produced by endothelial cells and responsible for T-cell migration towards the site of infection. In a more recent study, Veillard *et al.* [32] have shown that simvastatin blocks both chemokine (MCP-1, MIP-1 α , MIP-1 β) and chemokine receptor (CCR1, CCR2, CCR4, CCR5) expression in human vascular endothelial cells and macrophages independently of its capacity to inhibit cholesterol production. Of note, this effect could be mimicked by a pharmacological geranylgeranyl transferase inhibitor, suggesting that this activity of statins is dependent on inhibition of protein prenylation.

T-cell extravasation to the site of infection requires rolling and adhesion to the endothelium. Fluvastatin has been reported to interfere with the expression of the adhesion molecules, E- and P-selectin on endothelial cells. This effect was found to be dependent on the inhibition of small Ras-like GTPases [33, 34]. In agreement with this finding, *in vivo* experiments showed that atorvastatin administration to C57 BL/6J mice results in decreased endothelial expression of VCAM-1 and ICAM-1 independently of the reduction in circulating cholesterol [35]. Hence, by affecting the activation of leukocytes and endothelial cells, statins can indirectly impair T-cell migration and homing (Fig. 2A).

2.1.2. Inhibition of T-Cell Activation by Targeting Antigen Presenting Cells

The primary signal in the initiation of T-cell activation is delivered by the T-cell antigen receptor (TCR) engaged by peptide antigen presented by MHC. Following encounter with foreign antigen, antigen presenting cells (APC) upregulate MHC expression, which results in enhanced capacity to present antigen to T-cells [36]. Although constitutive MHC expression by mature professional APCs is largely unaffected by statins [16, 37], Kwak *et al.* [27] have demonstrated that atorvastatin and, to a lesser extent, pravastatin and lovastatin, inhibit IFN γ -inducible upregulation of MHCII molecules on APCs, thereby attenuating antigen presentation to CD4⁺ T-cells. Interestingly, this suppressive

effect can be reversed by both mevalonate and GGPP, but not squalene, indicating this activity of statins involves the inhibition of Ras-like GTPases through impaired prenylation. Inhibition by statins of inducible HLA-DR expression on professional human APCs has also been documented [38].

In addition to TCR engagement, T-cell activation requires engagement of a number of adhesion and costimulatory molecules at the immunological synapse. The counter-receptors of these accessory molecules are inducibly expressed on activated APCs. Statins have been shown to prevent cytokine-induced APC maturation, as demonstrated by the inhibition of expression of CCR7, CD40, CD83 and CD86 on cytokine-stimulated DC. As a result, T-cell proliferation is greatly reduced when cells are primed by statin-treated DCs [38]. Similar results have been observed in microglia and vascular endothelial cells, where expression of costimulatory molecules (CD40, CD80, CD86) was found to be suppressed by atorvastatin treatment [39].

Statins have been recently reported to inhibit not only APC maturation, but also antigen processing and presentation by APC, a process involving the coordinated activities of Rho and Rab GTPases. Simvastatin was indeed found to potently suppress tetanus toxoid processing and presentation to CD4⁺ T-cells by inhibiting protein antigen uptake both through receptor-mediated endocytosis and through macropinocytosis. This effect could be accounted for by defective prenylation of Rho and Rab GTPases, in the absence of any measurable perturbation of lipid rafts [16]. Inhibition of fluid-phase endocytosis by DC was also reported for both simvastatin and atorvastatin [40]. Interestingly, simvastatin preferentially affected the invariant chain (Ii)-dependent MHCII pathway [16].

Collectively, these data indicate that, by interfering both with APC maturation and with antigen processing and presentation, statins can indirectly inhibit T-cell activation (Fig. 2B).

2.2. Direct Effects of Statins on T Lymphocytes

2.2.1. Inhibition of T-Cell Activation by Statins

The first evidence that T lymphocytes are direct cellular targets of statins was reported by Weitz-Schmidt *et al.* [41], who observed that a subset of statins, which includes lovastatin and simvastatin, but not mevastatin and pravastatin, can inhibit T-cell activation. Interestingly, this activity was found to be independent of the capacity of these statins to inhibit HMG-CoA reductase, but resulted from statin binding to LFA-1, an heterodimeric glycoprotein belonging to the β -2 integrin family and constitutively expressed in an inactive state on the surface of T-cells. LFA-1 not only promotes leukocyte adhesion and migration, but also delivers a strong costimulatory signal essential for T-cell activation [42]. TCR engagement results in a conformational change in the extracellular domain of LFA-1, which increases its affinity for its counter-receptor ICAM-1 on the APC [42]. Statin binding to the regulatory site prevents LFA-1 interaction with ICAM-1 *via* an allosteric mechanism, which locks the receptor in an inactive conformation, thereby suppressing T-cell activation [41] (Fig. 2C).

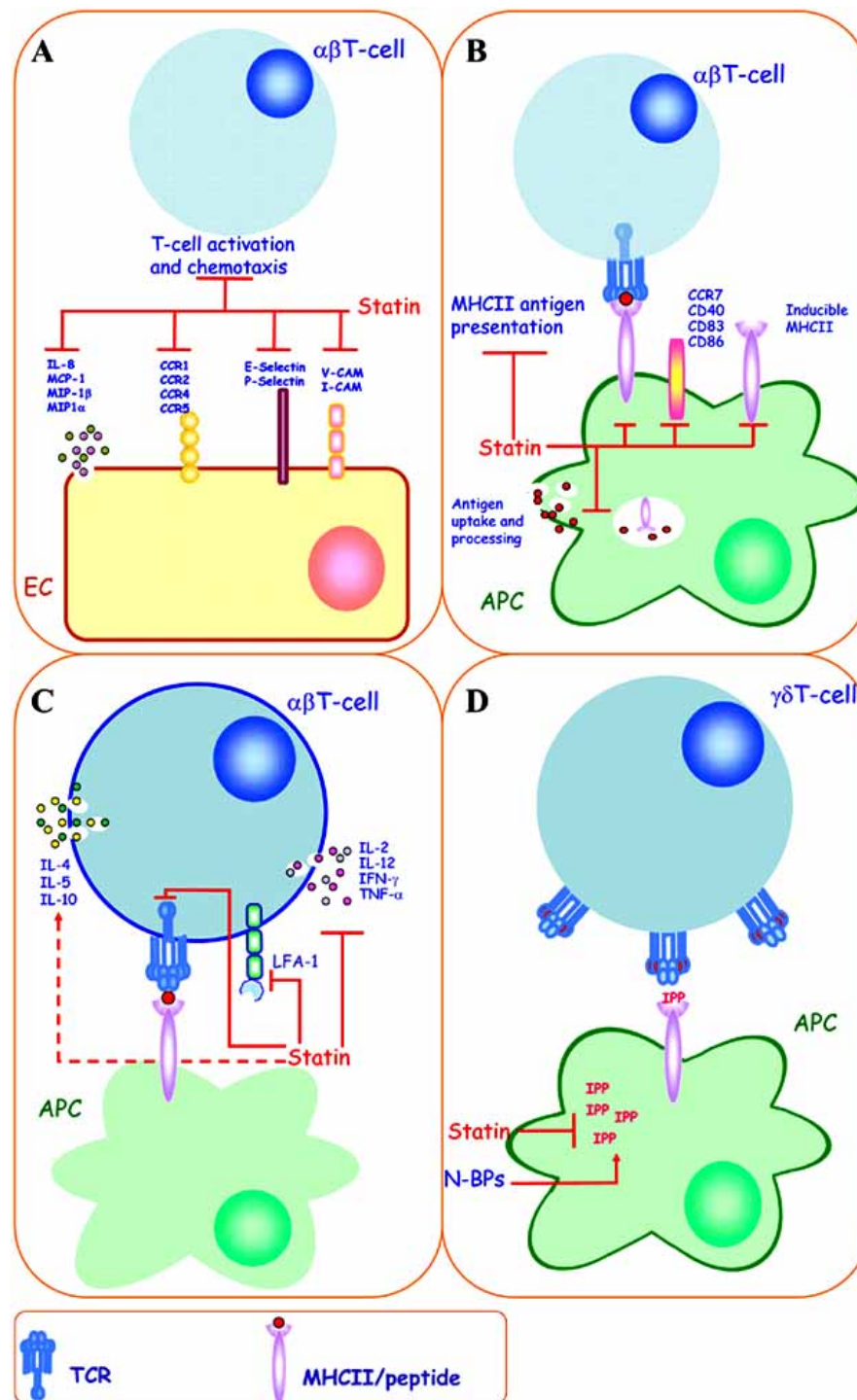


Fig. (2). Direct and indirect effects of statins on T-cell activation and differentiation. **A.** By inhibiting the expression of several chemokines, chemokine receptors and adhesion molecules on endothelial cells (EC), statins block T-cell activation and migration. This activity results from defective protein prenylation. **B.** Antigen uptake, processing and presentation, as well as expression of MHCII and of several costimulatory receptors, are inhibited in antigen presenting cells (APC) treated with statins as the result of defective prenylation of small GTPases, thereby impairing T-cell activation and proliferation. **C.** Direct effects of statins on T-cells. Statins block T-cell activation and proliferation by inhibiting TCR signaling. These effects result from defective prenylation of small GTPases. Furthermore, a subset of statins can lock in an inactive form the adhesion molecule LFA-1, preventing T-cell activation. Statins also affect helper T-cell differentiation by modulating expression of specific cytokines and skewing polarization towards a Th2 phenotype, an activity which results from impaired small GTPase prenylation. Moreover, cholesterol depletion and lipid raft disruption by statins increase shedding of CD30, of which the soluble form has been shown to polarize T-cells towards a Th2 phenotype. **D.** Statins act as suppressors of $\gamma\delta$ T-cells activated by the isoprenoid intermediate, isopentenyl-pyrophosphate (IPP), which is generated as the result of cell treatment with amino-bisphosphonates (N-BPs). By inhibiting the production of the N-BP substrate in the isoprenoid biosynthetic pathway, statins block IPP accumulation, thereby reducing IPP-specific TCR triggering.

In addition to this HMG-CoA reductase-independent mechanism, statins have been more recently reported to inhibit T-cell activation through a HMG-CoA reductase-dependent mechanism [15]. In this study, simvastatin was shown to block the expression of the activation markers, CD69 and CD25, as well as T-cell proliferation, by disrupting the TCR signaling cascade at the critical steps regulated by small Ras-like GTPases, including the Ras/MAP kinase, the Rac/stress kinase pathways and the Rab-dependent pathway of receptor endocytosis. These effects were primarily ascribed to the capacity of statins to inhibit prenylation of these GTPases (Fig. 2C). Consistent with these findings, neither lipid raft integrity, nor early steps in TCR signaling, such as CD3 ζ , ZAP-70 and LAT phosphorylation, as well as Ca²⁺ mobilization, were affected by simvastatin [15]. A further contribution of statins to the suppression of T-cell activation, which is also dependent on impaired protein prenylation, has been identified in their capacity to inhibit the movement of CD43 away from the site of TCR engagement, a process associated with T-cell activation and chemokine induction of T-cell motility [26].

2.2.2. Modulation of Th1/Th2 Differentiation by Statins

One of the most exciting findings on the immunomodulatory properties of statins is their capacity to affect Th1 polarization. Studies in experimental autoimmune encephalomyelitis (EAE) and other animal models of Th1-related diseases have indeed revealed that statins interfere with autoimmune destruction of target tissues by inhibiting multiple arms of the immune response and, more importantly, that statins can modulate disease progression. In EAE, lovastatin has been shown to prevent the production by astrocytes and glial cells of the inflammatory mediators, TNF- α , INF- γ and iNOS, as well as to inhibit MHC upregulation and expression of costimulatory molecules [43-45]. Furthermore, statins have been found to affect T-cells that are primarily responsible for the autoimmune process, by preventing their expansion, Th1 differentiation and migration across the blood-brain barrier [45]. The unexpected inhibitory effects of statins on Th1 polarization were first inferred from a bias in the pattern of T-cell cytokine secretion in EAE mice following oral administration of atorvastatin. Indeed, secretion of Th2-type cytokines, such as IL-4, IL-5 and IL-10 was observed in these animals, together with the presence in T-cells of phosphorylated STAT6, which is involved in IL-4 dependent Th2 differentiation. Conversely, secretion of IL-2, IL-12, INF- γ and TNF- α , as well as activation of STAT4, which is required for IL-2-dependent Th1 cell differentiation, were reduced. All these effects could be ascribed to the inhibitory effects of statins on protein prenylation [39, 46, 47]. The capacity of statins to cause a shift in T-cell phenotype from Th1 to Th2 has also been reported in other animal Th1-related disease models such as autoimmune myocarditis [48] and atherosclerosis associated with systemic lupus erythematosus [49].

Two recent studies provided an additional mechanism to explain the statin-dependent Th2 bias. Lovastatin was found to enhance membrane shedding of the lymphoid activation marker CD30. This effect was dependent on lipid raft impairment by cholesterol depletion and involved membrane redistribution of CD30, resulting in its interaction with TACE, the enzyme responsible for CD30 shedding [29]. The

suppressive effect of soluble CD30 on Th1-type immune responses [50] may contribute to the negative effects of statins on Th1 cell differentiation. Arora *et al.* [51] have shown that simvastatin instructs DCs to drive T-cell differentiation toward the Th2 lineage *via* up regulation and secretion of Ym1, a chitinase homolog with a specific binding affinity for heparin/heparan sulfate which has been recently reported to be associated with the development of allergic airways disease. Statins may therefore inhibit Th1 cell differentiation both directly and indirectly by modulating DC function (Fig. 2).

2.2.3. Modulation of $\gamma\delta$ T-Cells by Statins

An emerging field of research relates to the antagonist effects that statins exert on $\gamma\delta$ T-cell activation by a related class of drugs, the amino-bisphosphonates (N-BPs). By inhibiting FPP synthase, an enzyme in the mevalonate pathway (Fig. 1), N-BPs block isoprenoid biosynthesis, thereby preventing isoprenylation of the small GTPases that are necessary for osteoclast function [52]. Because of their capacity to inhibit bone resorption, N-BPs are commonly prescribed in the treatment of metabolic bone disease. The major adverse side effect of intravenous administration of N-BPs is the development of an acute phase response. In their recent work, Thompson and Rogers [53] have attributed this N-BP-mediated effect to the activation and expansion of V γ 9V δ 2 T-cells, the major $\gamma\delta$ T-cell subset, with resulting massive release of proinflammatory cytokines. The authors identified the trigger to V γ 9V δ 2 T-cell activation as an accumulation of intermediates upstream of FPP synthase in the mevalonate pathway, which include isopentenyl diphosphate (IPP) and dimethyl allyl diphosphate, two known non-peptide microbial V γ 9V δ 2 T-cell agonists [54, 55]. Interestingly, mevastatin and lovastatin, which inhibit HMG-CoA reductase upstream of FPP synthase (Fig. 1), prevent IPP and dimethyl allyl diphosphate synthesis, and hence V γ 9V δ 2 T-cell activation (Fig. 2D), thereby overriding the stimulatory effect of N-BPs [53, 56]. These results suggest that coadministration of statins could be a valid approach to prevent the acute phase response to N-BPs.

3. STATINS IN THE TREATMENT OF T-CELL RELATED DISEASES

Work on rodent models, as well as initial evidence from clinical trials, underscores the potential application of statin-based therapies to the treatment of diseases characterized by excessive T-cell responses, either to self-antigens (autoimmune disorders) or to alloantigens (allograft rejection, bacterial infections).

3.1. Autoimmune Disorders

The compelling evidence of the beneficial effects of statins on the clinical manifestations of Th1-based diseases in mouse models has prompted a number of clinical trials aimed at assessing the efficacy of statins in the treatment of autoimmune disorders. The reader is referred to two excellent recent reviews for details on the animal model data [14, 30]. The results obtained in the current clinical trials are summarized in Table 1. Here we shall focus on the results obtained on autoimmune diseases, which represent a paradigm of the "bench-to-bedside" concept of how research on experimental animal models can be translated to the clinic.

Table 1. Clinical Studies in Autoimmune Disorders

	Study	Drug	Patients (n)	Follow-Up	Results	
Multiple Sclerosis						
	Sena <i>et al.</i> [66]	open-label	lovastatin 40 mg/day	7	12 months	no clinical changes; slight MRI ^d improvement
	Vollmer <i>et al.</i> [67]	open-label	simvastatin 80 mg/day	28	6 months	over 40% reduction in MRI lesions
Rheumatoid Arthritis						
	Kanda <i>et al.</i> [75]	open-label	simvastatin 10 mg/day	24	12 weeks	ACR50 ^b in 39%
	Abud-Mendoza <i>et al.</i> [76]	open-label	simvastatin 40 mg/day	10	8 weeks	ACR70 ^b in 70%
	McCarey <i>et al.</i> [77]	double-blind, randomized, placebo-controlled	atorvastatin 40 mg/day	58	6 months	DAS28 ^c improvement (-0.5); no improvement in subjective parameters
	Tikiz <i>et al.</i> [78]	open-label, placebo-controlled	simvastatin 20 mg/day	15	8 weeks	decrease in sCRP ^d and sTNF α , with improvement in endothelial function
Systemic Lupus Erythematosus						
	Abud-Mendoza <i>et al.</i> [76]	open-label	simvastatin 80 mg/day	3	8 days	proteinuria reduction in all patients
Inflammatory Dilated Cardiomyopathy						
	Wojnicz <i>et al.</i> [84]	open-label, randomized, controlled	atorvastatin 40 mg/day	34	6 months	decrease NYHA class ^e , increase LVEF ^f , reduction in lymphoid infiltrates and class II HLA expression
Giant Cell Arteritis						
	Garcia-Martinez <i>et al.</i> [94]	retrospective, open label, controlled	various	17	2.7 years (mean)	no-steroid sparing effect
Ankylosing Spondylitis						
	Van Denderen <i>et al.</i> [96]	open-label	rosuvastatin 10-20 mg/day	15	12 weeks	reduction in CRP and ESR ^g
Autoimmune Thyroiditis						
	Gullu <i>et al.</i> [95]	open-label, controlled	simvastatin 20 mg/day	11	8 weeks	thyroid function improvement; reduction in NK and activated T-cells

^aMagnetic resonance imaging; ^bAmerican College of Rheumatology ACR 50-70% response criteria; ^c28-joint disease activity score; ^dC-reactive protein; ^eNew York Heart Association, classification of clinical severity of heart failure; ^fLeft ventricular ejection fraction; ^gErythrocyte sedimentation rate.

3.1.1. Multiple Sclerosis

Multiple sclerosis (MS) is an inflammatory autoimmune demyelinating disease of the central nervous system that is thought to be mediated, at least in part, by myelin-specific lymphocytes. The activation of CD4⁺ autoreactive T-cells recognizing self-peptides of the myelin sheath, such as myelin basic protein and their differentiation into a Th1 phenotype are crucial events both in the initial steps and in the long-term evolution of the disease [57]. Such mechanisms also characterize experimental autoimmune encephalomyelitis (EAE), which has become a widely accepted animal model of MS [58]. The evaluation of the effects of HMG-CoA reductase inhibitors on the immune processes underlying MS onset and progression is largely derived from investigations performed on the EAE model, where concordant results have been reported for lovastatin or atorvastatin, *i.e.* a relevant attenuation of the disease associated with a decreased leukocyte infiltration in the central nervous system

and a profound interference on CD4⁺ T-cell functions [39, 44, 59-64]. Very recently, two studies evaluated the effects of a combination therapy, including a statin plus an immunomodulatory agent, in EAE [45, 65]. In the first study, atorvastatin was associated to glatiramer acetate, a drug approved for the treatment of relapsing-remitting MS in man, whereas in the second, lovastatin was administered together with 5-aminoimidazole-4-carboxamide-1- β -D-ribofuranoside, an immunomodulator, which activates AMP-activated protein kinase. In both studies, a marked improvement of the neurological and histological findings of the disease was demonstrated, associated with a shift from a Th1 to a Th2 response.

In contrast to the relevant amount of data obtained in the experimental animal model, only a limited number of studies is available documenting the effects of statins in MS patients (Table 1). In a 12-month open-label study with lovastatin, Sena *et al.* [66] found by magnetic resonance imaging (MRI)

evidence of decreased inflammation in the absence of any clinical change. In a subsequent, similarly designed trial, treatment with simvastatin significantly reduced the number and volume of gadolinium-enhancing lesions, as assessed by MRI [67]. Although these reports are encouraging, they need to be interpreted with caution because of the absence of a placebo arm in both trials. This issue is currently being addressed in a large, multicenter, randomized, double-blind, placebo-controlled trial conducted on 152 MS patients treated with atorvastatin or placebo [68]. On the basis of *in vitro* results showing an additive inhibitory effect of statins on T-cell activation when used in combination with IFN β [69], a small clinical trial is currently being conducted to evaluate the combination of high dose IFN β with atorvastatin in patients with relapsing-remitting MS [68].

3.1.2. Rheumatoid Arthritis

Autoimmune rheumatic diseases encompass a heterogeneous group of disorders characterized by systemic involvement and production of auto-antibodies, in which a Th1-polarized immune response appears to play a crucial role [70]. To date, the majority of studies have focused on rheumatoid arthritis (RA). Interesting, but partially conflicting data have been obtained on rodent animal models of RA. In a murine collagen-induced arthritis model, simvastatin markedly inhibited not only developing but also clinically evident disease. *Ex vivo* analysis demonstrated significant suppression of collagen-specific Th1 responses, as well as a reduction of T-cell proliferation and IFN γ release from mononuclear cells derived from peripheral blood and synovial fluid [71]. In contrast, no effects on clinical, immunological and biochemical parameters of inflammation and autoimmunity were observed in the same animal model treated with two different statins (atorvastatin and rosuvastatin) [72]. A marked amelioration in the histopathological findings of joints obtained from rats with adjuvant-induced arthritis was instead observed following atorvastatin treatment. Moreover, a reduction in pro-inflammatory cytokines (IL-1, IL-6, TNF β) and chemokines (CCL2, CCL5), associated with an increase in the anti-inflammatory Th2-dependent cytokine IL-10, was detected in the serum of atorvastatin-treated arthritic rats [73].

The first indication of the therapeutic potential of statins in RA management has emerged from a study on a small cohort of hypercholesterolemic RA patients treated with simvastatin, which at the end of the follow-up showed a significant improvement in several clinical and laboratory parameters [74]. These encouraging results were confirmed in a subsequent extension of this study [75]. Concordant results were obtained by Abud-Mendoza *et al.* [76], who documented a considerable and rapid disease improvement in simvastatin-treated RA patients. While promising, these are open-label, single-arm studies involving a small number of patients. A large randomized placebo-controlled trial was more recently carried out by McCarey *et al.* [77], where the entity of disease improvement, albeit significant, appeared less relevant than that observed in the previous reports. In fact, in a study involving 116 RA patients subjected to a combination therapy with disease-modifying antirheumatic drugs and atorvastatin or placebo, both clinical objective signs of disease activity (28-joint disease activity score, DAS28, and DAS28 European League Against Rheumatism,

EULAR, response) and hematic bio-markers of inflammation (C-reactive protein and erythrocyte sedimentation rate) significantly improved in statin-treated patients compared to controls. Clinical subjective parameters failed however to change. Furthermore, as a confounding element, more patients in the atorvastatin group were taking metotrexate *vs* the placebo group. Potentially beneficial effects of statin therapy, as evaluated by serum C-reactive protein and TNF α , were also demonstrated in a small trial involving 15 RA patients receiving simvastatin, compared to 15 RA control patients receiving either the ACE-inhibitor quinapril or placebo, as well as disease-modifying antirheumatic drugs [78].

3.1.3. Systemic Lupus Erythematosus

At present, only limited information is available regarding the effects of HMG-CoA reductase inhibitors in systemic lupus erythematosus (SLE), mainly derived from animal models. Administration of atorvastatin to lupus-prone NZB/W F₁ mice produced a significant reduction in serum anti-double stranded DNA antibodies, associated with a decrease in glomerular immunoglobulin deposition and injury, leading to a reduction in proteinuria. Disease improvement was paralleled by a decreased expression of MHCII on monocytes, B lymphocytes and glomerular cells together with a strong impairment in T-cell proliferation [79]. Concordant results have been obtained on *gld/apoE^{-/-}* mice, which exhibit accelerated atherosclerosis and aggravated lupus-like features. Simvastatin-treated mice showed not only reduction in atherosclerotic lesions, but also amelioration of lymphadenopathy and renal damage. These effects correlated with a reduction in serum TNF α and IFN γ levels and an increase in IL-4 and IL-10 transcripts in lymph nodes, which were associated with enhanced STAT6 and decreased STAT4 activity, suggesting that simvastatin affects atherosclerotic lesions and lupus-like autoimmunity *via* a shift from a Th1 to a Th2 phenotype [49].

Although the data obtained in the animal models are very promising, to date the efficacy of statins in patients with SLE has not been assessed, with the exception of one study involving 3 SLE patients refractory to conventional therapy, for whom a rapid and significant reduction in proteinuria was reported following simvastatin administration [76].

3.1.4. Autoimmune Myocarditis

It is likely that the majority of cases of myocarditis in humans result from a viral infection, which may progress to an autoimmune phase after the resolution of the initial lesions, eventually leading to progressive dilation of the heart [80]. The evolution rate from myocarditis to dilated cardiomyopathy (DCM) is about 30%, and the successive progression to heart failure represents a major cause of morbidity and mortality among young adults [80]. The immunopathogenesis of myocarditis and DCM involves cross-reacting antibodies and cytokine activation, but the pivotal role in myocyte damage is thought to be exerted by autoreactive CD4⁺ Th1 cells [80].

Rat immunization with myosin results in the development of experimental autoimmune myocarditis (EAM), a Th1-driven animal model of human myocarditis and post-myocarditis DCM [81]. Three recent studies evaluated the efficacy of statin administration in counteracting the immune mechanisms operating in EAM. In the first, oral fluvastatin

administration to EAM rats resulted in improved cardiac function and a reduction in CD4⁺ T-cell infiltration, Th1-type cytokine (IFN γ and IL-2) production and NF κ B activation in the myocardium [82]. Similar conclusions emerged from the other two studies, in which atorvastatin was used [48, 83]. In both investigations, the authors reported a significant amelioration of myocardial functional parameters, an attenuation of the histopathological severity of myocarditis and a shift from a Th1 to a Th2 cytokine pattern in treated rats. Li *et al.* [83] also demonstrated a decrease of MHCII molecules on the “non professional” APC cardiomyocytes and a downregulation of type IV CIITA transcripts.

At present, the only available clinical transposition of these models is a very recently randomized study performed in 74 patients affected with heart failure secondary to inflammatory DCM, diagnosed on the basis of endomyocardial biopsies. After 6 months of therapy, the atorvastatin-treated subjects showed a significant improvement both in clinical and ecocardiographic parameters compared to patients under conventional treatment. Interestingly, a significant reduction in the frequency of T-cells and macrophages in myocardial infiltrates, paralleled with a decrease in the expression of the class II HLA antigens, was detected in repeated biopsies in the atorvastatin group [84].

3.1.5. Type 1 Diabetes Mellitus

Conflicting results have emerged from the few experimental studies aimed at establishing the efficacy of statins in delaying or even preventing diabetes onset in animal models of type 1 diabetes mellitus, a chronic autoimmune disease resulting from T-cell-mediated destruction of pancreatic β -cells [85], in which disease susceptibility is conferred by expression of specific MHCII and non-MHCII alleles [86]. Two studies performed in different mouse disease models (multiple low-dose streptozocin and spontaneous non-obese diabetic mice) reported neither a significant reduction in the incidence of diabetes nor a delay in disease onset following atorvastatin treatment [87, 88]. In agreement with these findings, no effects of atorvastatin were detected on islet inflammatory infiltration or MHC II expression [87].

In contrast with these results, a recent investigation in a different murine model of type 1 diabetes mellitus demonstrated the ability of atorvastatin to prevent disease onset when administered in the neonatal period, and to stabilize glucose levels when administered in mice with a mild form of diabetes. The pancreas of protected animals, analyzed 1 week after interruption of treatment, was found to be free of lymphocyte infiltration. Furthermore, *ex vivo* analysis of splenic cells demonstrated a prominent Th2 phenotype both in the pattern of secreted cytokines (increase of IL-4, reduction of IL-2 and IFN γ) and in transcription factor expression (increased levels of STAT6, c-Maf and GATA3 mRNA) [89].

3.1.6. Other Autoimmune Diseases

Very preliminary, but potentially interesting results documenting the capacity of statins to interfere with basic immunological mechanisms operating in T cell-mediated autoimmune disorders have been obtained both in animal models (autoimmune uveoretinitis and inflammatory bowel disease) [90-93] and in very small number of patients (giant cell arteritis, ankylosing spondylitis, autoimmune thyroiditis)

[94-96] (Table 1). A notable exception to the beneficial effects of statins summarized above is represented by myasthenia gravis (MG), for which the current evidence indicates a detrimental effect of these drugs. Several authors reported indeed cases of statin-associated exacerbation or *de novo* onset of MG [97-99]. A patient described by Cartwright *et al.* [97] appeared paradigmatic, as dysarthria occurred in four separate occasions following treatment with four different statins and consistently improved after discontinuation of the medication. Several hypotheses have been proposed to explain such phenomenon, including mitochondrial dysfunction in synaptic junctions by endogenous coenzyme Q10 depletion [100] or statin-associated myopathy onset exacerbating the underlying MG. These hypotheses do not account however for the cases with *de novo* antibody formation. An attractive explanation may directly involve the immunomodulatory properties of statins and, specifically, the well-documented ability of these drugs to induce a Th2 shift, since Th2 cytokines contribute to MG development [101]. It is possible that, by upregulating these molecules, statins could worsen pre-existing MG or trigger *de novo* disease development in predisposed subjects.

3.2. Allograft Rejection

T-cells play a key role in the induction and maintenance of the allo-immune response underlying acute and chronic rejection of transplanted organs. Based on preliminary data obtained both *in vitro* [102, 103] and in animal models [104], which suggested a suppressive activity of HMGCoA-reductase inhibitors on T-cells and NK cells, Kobashigawa *et al.* [105] evaluated the effect of an early use of pravastatin on outcomes after cardiac transplantation. After 12 months, pravastatin-treated patients showed a decrease in the incidence of major acute rejection, a lower incidence of chronic rejection and better survival compared to the control group. Moreover, the cytotoxicity of NK cells was found to be lower in statin-treated patients than in controls. In the wake of this pioneering study, performed over 10 years ago, the protective effect of statins in allograft rejection has been widely investigated. These studies have highlighted a major role of statin-mediated T-cell suppression in the beneficial effect of statins on transplant rejection. The clinical studies on the effects on statins on allograft rejection are summarized in Table 2.

3.2.1. Acute Rejection

The capacity of statins to counteract the development of acute heart rejection, first suggested by Kobashigawa *et al.* [105], has been amply confirmed. A recent meta-analysis of three randomized controlled studies demonstrated a significant reduction in cardiac rejection events with hemodynamic compromise and in mortality in transplanted patients treated with pravastatin or simvastatin [106]. Similar results were obtained in an observational study of 91 heart transplants patients treated with pravastatin [107]. A beneficial effect of statins on acute rejection has also been reported for kidney transplants. A reduction in the incidence of biopsy-proven acute rejection episodes and in the level of immunosuppression needed for rescue from severe acute rejection has indeed been documented in a prospective randomized pilot study performed in pravastatin-treated vs pravastatin-untreated patients undergoing kidney transplantation [108]. Similar

Table 2. Clinical Studies in Organ Transplantation

	Study	Drug	Patients (n)	Follow-Up	Results	
Heart Transplantation						
	Kobashigawa <i>et al.</i> [105]	open-label, randomized, controlled	pravastatin 20-40 mg/day	47	12 months	reduction of severe AR ^a and CAV ^b with better survival
	Wenke <i>et al.</i> [130]	open-label, randomized, controlled	simvastatin 5-20 mg/day	35	4 years	lower incidence of CAV and better survival
	Mehra <i>et al.</i> [106]	open-label, controlled	simvastatin 10 mg/day or pravastatin 20 mg/day	26 24	12 months	lower mean biopsy score and better survival
	Wenke <i>et al.</i> [129]	open-label, randomized, controlled	simvastatin 5-20 mg/day	35	8 years	lower incidence of CAV and better survival
	Stojanovic <i>et al.</i> [107]	open-label, retrospective, controlled	pravastatin 20-40 mg/day	91	5 years	lower incidence of severe AR ^a and CAV with better survival
	Kobashigawa <i>et al.</i> [126]	open-label, randomized, controlled	pravastatin (dosage not available)	47	10 years	lower incidence of CAV and better survival
	Mahle <i>et al.</i> [127]	open-label, retrospective, controlled	pravastatin 0.1-0.3 mg/kg/day	90 (pediatric)	6.1 years (mean)	lower incidence of CAV
	Wenke <i>et al.</i> [131]	open-label, randomized, controlled	simvastatin 5-20 mg/day	35	11 years	lower incidence of CAV and better survival
Kidney Transplantation						
	Katznelson <i>et al.</i> [108]	open-label, randomized, controlled	pravastatin 20 mg/day	24	4 months	reduction of AR incidence
	Tuncer <i>et al.</i> [109]	open-label, randomized, controlled	simvastatin 10 mg/day or pravastatin 20 mg/day	16 16	12 months	reduction of AR incidence
	Sahu <i>et al.</i> [118]	double-blind, randomized, controlled	lovastatin 20 mg/day	33	3 months	no effect on AR incidence
	Holdaas <i>et al.</i> [116]	double-blind, randomized, controlled	fluvastatin 40 mg/day	182	3 months	no effect on AR incidence
	Kasiske <i>et al.</i> [117]	open-label, randomized, controlled	simvastatin 10 mg/day	53	3 months	no effect on AR incidence
	Fellstrom <i>et al.</i> [115]	double-blind, randomized, placebo-controlled	fluvastatin 40-80 mg/day	1050	5-6 years	no effect on the incidence of renal graft loss
Lung Transplantation						
	Johnson <i>et al.</i> [110]	open-label, controlled	various	39	2.0 years (mean)	reduction of AR and CR ^c incidence; spirometry improvement; lymphocyte reduction in BAL ^d

^aAcute rejection; ^bCardiac allograft vasculopathy; ^cChronic rejection; ^dBronchoalveolar lavage.

conclusions were obtained in a study where the effects of simvastatin and pravastatin on acute rejection episodes in renal transplant patients were compared [109]. Substantial clinical benefits deriving from statin use were also reported after lung transplantation. Statin-treated patients developed acute rejection less frequently, had significantly better spirometry and a lower proportion of lymphocytes in bronchoalveolar lavage than control subjects, which correlated with longer survival [110].

Supporting evidence has been obtained in animal models, where the interference of statins with specific targets of the immune response in relation to the development of acute rejection has been investigated. In rodents, pravastatin improved survival rates in liver transplantation [111] and prolonged graft survival with a reduction of acute rejection epi-

sodes both in lung [112] and in islet transplantation [113]. In lung transplanted rats, the longer graft survival in the statin-treated group was associated with lower CD4 and MHCII expression on PBMC, also after IFN- γ stimulation *in vitro* [112]. Moreover, Horimoto *et al.* [114] demonstrated an effective suppression of acute rejection by cerivastatin in rat cardiac allografts, which was associated with a lower frequency of CD4⁺ cells in the context of the infiltrating mononuclear cells, a suppression of the proliferative response of alloreactive T-cells and lower IL-2 concentrations in mixed lymphocyte reaction cultures.

Unfortunately, the evidence obtained from the more recent and larger trials performed on kidney transplanted patients is not in agreement with the results of early studies. In fact, three different statins (fluvastatin, simvastatin and lo-

vastatin) failed to show any measurable effect either on the incidence and severity of acute rejection episodes, or on graft loss [115-118]. Globally considered, the available data suggest that the T-cell modulating activities of statins may be of use in the prevention of acute rejection after heart and, perhaps, lung transplantation. On the contrary, notwithstanding the promising results of *in vitro* studies, the efficacy of statins in reducing acute rejection rate in renal allograft recipients appears more questionable.

3.2.2. Chronic Rejection

Accelerated vascular disease is a characteristic feature of chronic graft dysfunction in organ transplantation. The pathogenesis of chronic rejection involves both immunological and non-immunological risk factors. T-cells have been found in the arterial lesions characterizing cardiac allograft vasculopathy [119] and CD4⁺ cell depletion prevented the formation of such lesions in an experimental animal model [120]. Th1 cells appear to play a crucial role in the genesis of cardiac allograft vasculopathy, as demonstrated using mice deficient in IFN- γ (mediating a Th1-type response) or IL-10 (mediating a Th2-type response). Cardiac allografts in IFN- γ deficient recipients harbored indeed decreased graft vasculopathy, whereas those in IL-10 deficient recipients displayed increased vascular occlusion [121]. In agreement with the data obtained in the animal models, peripheral expansion of circulating Th1 cells predicted allograft coronary endothelial dysfunction, which represents an early clinical indicator of transplant vasculopathy, in a study performed on 32 heart transplanted patients [122].

On this basis and taking into account the inhibitory effect of statins on T-cell activation and Th1 cell differentiation, the outcome of statin treatment on chronic rejection has been investigated. Solid evidence supports the effectiveness of HMG-CoA reductase inhibitors in counteracting the development of chronic rejection. Pravastatin has indeed been shown to prevent the progression of accelerated coronary disease after heart transplantation in different animal models [121, 123, 124], as well as of chronic rejection in rat liver and renal allografts [111, 125]. Similar results have been obtained in the clinical setting, particularly in heart transplanted patients. In fact, several studies demonstrated the beneficial effects of pravastatin on the incidence of heart transplant vasculopathy both in the short-term and after protracted follow-up periods [105, 107, 126], also in pediatric patients [127]. A significant reduction in transcatheter IL-6 and TNF α gradients was associated with better endothelial function, lower intimal thickness and plaque areas in coronary arteries of heart transplanted patients under statin treatment [128]. Similar clinical results have been obtained with simvastatin [129-131], which resulted in a reduction of cardiac allograft vasculopathy incidence of such entity that the authors concluded that heart transplant patients should receive early treatment with a statin as a matter of routine. Statin administration (pravastatin, atorvastatin and simvastatin) was also associated with a lower incidence of chronic rejection after lung transplantation [110]. The clinical studies on the effects on statins on chronic allograft rejection are summarized in Table 2.

The relationship between the statin-associated reduction in chronic rejection development and cellular immune response has been recently assessed. Inhibition of NK cell-

enhancement factor, associated with milder histological signs of chronic rejection, has been observed in simvastatin-treated liver transplants [111]. Moreover, a scantier T-cell infiltration in the graft, associated with an intragraft cytokine pattern indicative of Th2 polarization, has been documented in a rat kidney transplant model of chronic rejection following pravastatin treatment [125]. It should be pointed out that the development of chronic allograft rejection is dependent not only on immunological mechanisms, but also on metabolic factors, which include hyperlipidemia and hyperhomocysteinemia [119, 132]. Since statins exert a positive influence also on these parameters in organ transplantation [133, 134], it is likely that the antagonistic activity of these drugs on chronic rejection may not be solely dependent on their immunosuppressive properties.

3.3. Bacterial Infections

Based on the consideration that severe sepsis is the result of an exacerbated systemic immuno-inflammatory response to bacterial antigens, several studies have evaluated the possible application of the immunomodulatory properties of statins to the prophylaxis and treatment of this condition. Both cerivastatin and simvastatin were found to dramatically improve survival in two different experimental mouse models of sepsis [135, 136]. Several clinical investigations confirmed these preliminary -and unexpectedly positive- data [137-141] (Table 3). The mechanisms underlying the anti-septic activity of statins are probably multiple, including interference with proinflammatory cytokine production [135], reduction in monocyte adhesion to the endothelium [136] and enhancement in endotoxin clearance [142]. Of relevance to the topic of this review, a recent report has highlighted a contribution of the T-cell immunomodulatory properties of statins to this phenomenon. The authors demonstrated indeed that simvastatin inhibits CD4⁺ T lymphocyte activation by the superantigen staphylococcal enterotoxin B [143]. Superantigens are known to induce T-cell activation by binding to MHCII and TCR β chain in a peptide-independent manner, thereby activating a significant proportion of CD4⁺ cells. Since this process is dependent on LFA-1 interaction with ICAM-1, the inhibitory effect of simvastatin may result from its previously reported binding to the adhesion site of LFA-1 [41].

Obviously, the immune response induced by bacterial infections depends principally on the activation of the innate immune system. Since statins also significantly affect the functions of macrophages and neutrophils in animal models of sepsis [135, 136], further studies are required to identify in the clinical setting the specific contribution of statin-mediated inhibition of T-cell activation by superantigens to the beneficial effects of these drugs on sepsis.

3.4. Allergic Asthma

Asthma pathogenesis is driven by a mixed Th1/Th2 response. Compelling evidence from mice and humans pinpoints Th2 cytokines as major contributors to allergy and asthma. It is now acknowledged that allergen-specific Th1 responses are responsible for the pathogenetic effects observed in patients affected with the more severe chronic forms of the disease [144]. The potential application of HMG-CoA reductase inhibitors to the treatment of allergic asthma has been to date investigated primarily in animal

Table 3. Clinical Studies in Bacterial Infections

	Study	Drug	Patients (n)	Follow-Up	Results
Hackam <i>et al.</i> [155]	retrospective cohort analysis	various	34584	-	decreased sepsis, severe sepsis and mortality
Liappis <i>et al.</i> [139]	retrospective case-records analysis	various	35	-	lower overall and bacteremia attributable mortality
Kruger <i>et al.</i> [138]	retrospective cohort analysis	various	66	-	reduction in all-cause hospital mortality and death attributable to bacteremia
Thomsen <i>et al.</i> [140]	prospective observational cohort study	various	176	180 days	no effect on short-term mortality due to bacteremia. Decreased between 31 and 180 days after bacteremia
Almog <i>et al.</i> [137]	prospective observational cohort study	various	82	28 days or until death	lower rate of severe sepsis and intensive care unit admission

models. A reduction in total inflammatory cell infiltrate and eosinophilia, together with a decrease in IL-4 and IL-5 levels, have been observed in bronchoalveolar lavages of simvastatin-treated mice previously sensitized to ovalbumin and subsequently challenged by ovalbumin inhalation. Additionally, both Th1 (IFN γ) and Th2-related (IL-4, IL-5) cytokines were reduced in thoracic lymph node cultures from simvastatin-treated mice [145]. Similarly, pravastatin treatment resulted in a significant inhibition of eosinophil infiltration and in a reduction in the levels of IL-5, MCP-1 and PGE₂ in lavage fluid [146]. In agreement with the data obtained in the mouse, fluvastatin was found to prevent the migration of Th1 and Th2 cells *ex vivo* and to decrease the production of IL-5, IFN γ and chemokines in PBMC from patients with allergic asthma [147]. While awaiting clinical confirmation, these data suggest that, by regulating Th1/Th2 cell activity, statins could become a valuable tool in the management of allergic asthma.

The effect on allergic asthma appears conflicting with the Th2-biasing effect of statins, and no clear explanation for this phenomenon can be proposed at present. However, based on the consideration that the Th1 response may also contribute to the disease [144] and this response is affected by statins, the intriguing hypothesis that statins may exert a rebalancing activity on the Th1/Th2 equilibrium rather than a Th2 shift may be tentatively proposed, at least for allergic asthma.

3.5. Adverse Effects

Statins are a class of safe and well-tolerated drugs. However, some side effects occasionally occur in treated patients, particularly hepatotoxicity (<3%), manifested as a reversible elevation of transaminases, and myopathy (<0.2%). The latter effect rarely (<0.05%) evolves in rhabdomyolysis with myoglobinuria, potentially resulting in kidney failure [148]. The larger clinical studies evaluating the role of statins as a therapy of autoimmune disorders [65, 77] reported a similar risk profile, without apparent differences compared to the results from the trials performed in patients with hyperlipidemia and cardiovascular diseases. However, a dosage reduction is required when statins are used in organ transplanted patients. In fact, it has been demonstrated that cyclosporin A, a therapeutic tool for the prevention of acute graft rejection, increases the plasma levels of the majority of statins [5] and, concomitantly, the risk of myopathy [149].

As an exception, fluvastatin exhibits much less interaction with cyclosporin A [5] and the ALERT study, performed on 2100 renal transplanted patients, revealed no difference from placebo in the incidence of adverse effects [150].

In relation to the topic of the present review, the issue of whether the inhibitory activity exerted by statins on T-cells could result in an increased risk of immunosuppression-related adverse effects, *i.e.* infections and tumors, must be addressed. At the best of our knowledge, based on the results of large clinical trials, statin use is not associated with a higher incidence of cancer and/or infections [151, 152]. On the contrary, several studies reported data consistent with putative antineoplastic [153] and anti-infective [154, 155] properties of these drugs. This could suggest that statins may exert an immunomodulating activity rather than a mere immunosuppressive effect.

CONCLUDING REMARKS

The anti-inflammatory and immunomodulatory properties of statins are by now well established both *in vitro* and *in vivo*. These effects appear to be largely dependent on their capacity to interfere with protein prenylation and compelling evidence pinpoints small Ras-like GTPases as key molecular targets of statins. Of note, the immunomodulatory effects of statins are probably comparable within the class, independently of their specific pharmacodynamic or pharmacokinetic properties. Although some effects appear specific to individual statins (*e.g.* the interaction with the adhesion site of LFA-1), the relevance of such mechanisms remains to be understood.

The effectiveness of statins in reversing clinical features and histological damage in animal models of Th1-driven auto- and allo-immune disorders has highlighted T-lymphocytes as particularly promising cellular targets of statins in the treatment of these diseases in humans. Encouraging data are indeed emerging from the available clinical trials, although the picture is as yet fragmentary as to the relative efficacy of individual statins, as the majority of clinical trials on autoimmune diseases have assessed the effects of simvastatin or atorvastatin, while pravastatin represents the most widely tested statin as immunomodulating agent in allo-immune responses. The translation of these results to the clinical setting is so far in a preliminary phase. Regarding the applications of statins to autoimmune disorders, there is a significant lack of large randomized, con-

trolled clinical trials and, in many cases, no clinical study has been performed. The results of clinical trials on the treatment of alloimmune responses are more significant. Nevertheless, while the beneficial effect of statins in the prevention of chronic rejections appears significant, particularly regarding heart transplantation, the results on acute allograft rejection are more conflicting. Whichever the disease, the consensus is that statin-based therapies should be planned in the context of a combined, synergistic approach rather than as a monotherapy.

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