Accelerated atherosclerosis in systemic lupus erythematosus: mechanisms and prevention approaches

Systemic lupus erythematosus (SLE) is a multi-organ autoimmune disease characterized by increased serum autoantibody levels and tissue damage. With improved diagnosis and more effective treatment of the resultant kidney disease, accelerated atherosclerosis has become a major cause of morbidity in patients suffering from SLE. Although the exact mechanisms for SLE-accelerated atherosclerosis are unknown, multiple factors have been established as potential players in this process. Among these potential players are dysregulation of T- and B-cell populations and increased circulating levels of inflammatory cytokines. In addition, SLE patients exhibit a proatherogenic lipid profile characterized by low high-density lipoproteins and high low-density lipoproteins and triglycerides. Recent therapeutic approaches have focused on targeting B cells, the producers of autoantibodies, but most studies do not consider the effects of these treatments on atherosclerosis. Evidence suggests that T cells play a major role in SLE-accelerated atherosclerosis. Therefore, therapies targeted at T cells may also prove invaluable in treating SLE and atherosclerosis.

KEYWORDS: atherosclerosis ■ B cells ■ belimumab ■ HDL ■ regulatory B cells ■ regulatory T cells ■ rituximab ■ systemic lupus erythematosus ■ T cells

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Atherosclerosis & systemic lupus erythematosus

Atherosclerosis is a chronic inflammatory disorder that typically occurs when excess lipoproteins deposit in the intima and are oxidized [1], leading to recruitment of monocytes/macrophages and T cells to the artery wall [2]. These cells then produce inflammatory cytokines, increasing recruitment of T cells and macrophages to the lesion as part of an inflammatory cascade [3]. Dendritic cells (DCs) and B cells are also present in the plaque and adventitia and play important roles in the disease process [4]. Given the strong involvement of the immune system and that recognition of oxLDL is involved in the initiation of atherosclerosis, the idea that atherosclerosis is an autoimmune disease has gained support [5,6]. Systemic lupus erythematosus (SLE) is a systemic autoimmune disease involving multiple organs and is characterized by increased serum autoantibody levels and tissue damage [7]. T cells in SLE exhibit impaired function and increased apoptosis, while B cells are hyperactivated and immune cell production of proinflammatory cytokines is increased [8]. Both SLE and cardiovascular disease (CVD) pose threats to human health, with CVD being the leading cause of death in the USA [9], and SLE currently affecting more than 1 million Americans [10]. Evidence suggests interplay between the two diseases as both mouse and human studies have shown increased atherosclerosis in SLE [11–14]. With the increased life expectancy resulting from more effective treatments that address common complications such as nephritis, atherosclerosis now poses a significant health threat to SLE patients [14]. This review will summarize potential mechanisms for SLE-accelerated atherosclerosis and therapies aimed at targeting SLE and atherosclerosis.

■ Accelerated atherosclerosis in SLE

In 1976, Urowitz *et al.* first reported increased risk of CVD in SLE patients [13]. This study outlined a bimodal curve of mortality for SLE, where early deaths (<1 year after diagnosis) were attributable to kidney disease and infection, while later deaths were associated with CVD. Since that time, reports have shown that, on average, the risk of CVD is two- to ten-fold higher in SLE patients compared with healthy controls [15–18]. Due to their lower risk at baseline, the relative risk for CVD is highest in premenopausal women with SLE, as they have been shown to be 50-times more likely to experience myocardial infarction than their age-matched counterparts [14]. With treatments for SLE disease activity becoming more effective, CVD is now a leading cause of death in SLE patients [19,20]. However, the mechanisms contributing

Future Medicine part of fsg

to increased risk of CVD in SLE are not fully understood. We will begin this review by summarizing the contribution of immune cells to accelerated atherosclerosis in SLE.

Role of T cells in SLE & atherosclerosis

T cells can be found in atherosclerotic plaques in both humans and mice [21–23], making up approximately 10% of the cells in the lesion [23]. Of the T cells present in atherosclerotic lesions, $CD4^+$ T cells with a Th1 phenotype are the primary T-cell subset [22,24]. These cells are thought to play a pathogenic role in atherosclerosis, demonstrated by the fact that in immune-deficient *scid/scid*, $ApoE^{-/-}$ mice, transfer of $CD4^+$ T cells resulted in increased atherosclerosis [25]. Importantly, these T cells infiltrated the atherosclerotic plaques and T-cell transfer was accompanied by an increase in circulating IFN-γ [25], which has been established as an atherogenic cytokine [26]. Furthermore, in hypercholesterolemic mice lacking $CD4^+$ T cells ($CD4^{-/-}$, $ApoE^{-/-}$ mice), atherosclerosis was reduced [27]. These studies and others highlight a pathogenic role for $CD4^+$ T cells in atherosclerosis. This pathogenesis is thought to occur through their production of inflammatory cytokines, such as IFN-γ, which activate macrophages and other cell types in the atherosclerotic lesion [21]. These reports, however, did not take into account the potential divergent effects of various T-cell subsets. For example, maintenance of the Th1/Th2 balance has been shown to be important in atherosclerosis. Schulte *et al.* demonstrated that mice skewed more towards a Th1 phenotype demonstrated increased atherosclerosis, while a bias towards a Th2 phenotype resulted in less atherosclerosis [28], indicating differential effects of each of these subsets on atherosclerosis. In addition, more recent atherosclerosis studies have focused on the role of Treg in this process, identifying these cells as having atheroprotective properties (discussed in more detail below). Thus, while the majority of T cells in atherosclerotic lesions are of the atherogenic Th1 phenotype, the presence of Th2 and Treg can be important for controlling the atherosclerotic response.

Just as T cells play an important role in atherosclerosis, they also have a significant role in SLE pathogenesis. The idea that they contribute to disease pathogenesis is highlighted by mouse studies demonstrating that depletion of $CD4^+$ cells through use of a monoclonal antibody was beneficial in the prevention and treatment of SLE [29]. Furthermore, T cells were shown to be required for the development of SLE in NZB/W mice, as thymectomized mice failed to develop the disease [30]. Since that time, much focus has been placed on the pathogenic properties of T cells in SLE. Overall, SLE T cells have been shown to be hyperactive, with a reduced threshold of activation [31]. Upon activation, these cells secrete inflammatory cytokines such as IFN-γ and IL-17 [32]. In addition, SLE T cells exhibit increased survival and are resistant to apoptosis [33]. These properties of SLE T cells contribute to disease pathogenesis and result in activation of DCs and B cells [34].

Given that T cells play such important roles in both atherosclerosis and SLE, it is reasonable to think that dysregulated T cells might contribute to the increased risk of CVD observed in SLE patients. In fact, studies from our laboratory have demonstrated increased infiltration of $CD4^+$ T cells into the atherosclerotic lesions of $LDLr^{-/-}$ mice following transfer of bone marrow from lupus-susceptible mice [12]. This increase was independent of high-fat diet feeding [35], and unpublished data suggest that trafficking or accumulation of T cells in the lesions is required for the acceleration of atherosclerosis in this model.

In contrast to the pathogenic role of T cells, Tregs maintain self-tolerance through suppression of T-cell activation or by preventing effector T-cell (T_{eff}) responses and have important regulatory roles in both SLE and atherosclerosis. Tregs can also act to induce apoptosis or inhibit function of DCs, macrophages and B cells [36]. Suppression by Tregs occurs in a contact-dependent manner or through their secretion of the anti-inflammatory cytokines IL-10 and TGFβ [36,37]. Atherosclerosis studies have demonstrated a protective role for Tregs, as a reduction of Tregs in $LDLr^{-/-}$ mice and neutralization or deficiency of TGFβ in $ApoE^{-/-}$ mice led to increased atherosclerosis [38,39]. Tregs are equally important in preventing autoimmune disease, as experiments have shown that severe autoimmune disease is a consequence of Treg deficiency [40,41]. Reports in SLE patients and mouse models have indicated a reduction in the number of $CD4^+CD25^+$ and $FoxP3^+$ Tregs [42,43]. However, in both mice and humans, reports as to their suppressive activity differ, with some experiments showing normal suppressive activity [42,44] and others demonstrating reduced suppressive capacity of SLE Tregs [43,45]. It has also been suggested that T_{eff} in SLE may be able to overcome suppression by Tregs [44,46,47]. The observation of the possible

impaired suppression by Tregs and/or resistance of T_{eff} to suppression provides a potential explanation for the increased CVD in SLE.

Another T-cell subset with a potential role in SLE-accelerated atherosclerosis is the Th17 subset. Th17 cells produce IL-17, a cytokine known to play a role in both lupus and atherosclerosis. Th17 cells are present in atherosclerotic plaques of both mice and humans [48,49]. However, due to conflicting results, their role in atherosclerosis is unclear. It appears that IL-17 contributes to vascular inflammation. However, its effect on plaque size is less defined. Studies involving deficiency or neutralization of IL-17A have shown beneficial effects on atherosclerosis, with lesion size reduced by as much as 50% [50,51]. However, a recent report by Taleb *et al.* indicated a protective effect of IL-17 on atherosclerosis [48]. In this study, mice treated with anti-IL-17A had increased atherosclerotic lesions and treatment with rIL-17A reduced lesion size. The reason for these divergent findings on the role of IL-17 in atherosclerosis is not yet clear. In SLE, Th17 cells are thought to contribute to disease pathogenesis. Th17 cells and IL-17 are increased in SLE patients and mouse models [52,53]. In SLE patients, levels of circulating IL-17 correlate with disease activity [54] and tend to be higher in individuals with active nephritis [52]. The ratio of Th17:Th1 appears to be dysregulated in SLE patients, with an increase in Th17 in SLE [55]. In addition, the ratio of Treg/Th17 is reduced in SLE patients [56]. Given their potentially pathogenic role in atherosclerosis, the fact that Th17 cells are increased in SLE and that Tregs are reduced suggests that the dysregulation of these cell types may be a factor contributing to accelerated atherosclerosis in SLE. Refer to TABLE 1 for a summary of studies discussed in this section.

Role of B cells in SLE & atherosclerosis

Another major immune cell playing a role in both SLE and atherosclerosis is the B cell. Previous data have assigned an atheroprotective role to B cells [57,58]. Studies showing increased atherosclerosis as a result of B-cell deficiency support the idea that B cells are protective in atherosclerosis [57,58]. However, recent reports have shown that depletion of mature B cells results in reduced atherosclerosis [59,60], suggesting that the role of B cells in atherosclerosis is more complex. In a normal immune response, B cells have multiple functions, including antigen presentation [61], antibody production [62] and regulation of $CD4^+$ T-cell responses to antigens [63]. In autoimmunity, B cells are hyperactivated [8] and can produce antibodies to self, termed 'autoantibodies'. In addition to autoantibody production, SLE B-cell dysregulation can lead to T-cell activation, DC recruitment, induction of Th1 and Th17 cells and inhibition of Tregs, solidifying B cells as a major player in SLE pathogenesis [64].

In contrast to the potentially pathogenic nature of B cells, regulatory B-cell (Breg) populations are present and can act to suppress T-cell-mediated inflammation through mechanisms that are either dependent on or independent of IL-10 [65–68]. Breg subsets have been shown to suppress autoimmune diseases including experimental autoimmune encephalomyelitis (EAE) [69], SLE [65,70] and collagen-induced arthritis (CIA) [71]. However, recent studies have shown that Bregs from SLE patients have reduced regulatory capacity [70], warranting further examination of the function of Bregs in a lupus model. Given their production of IL-10, a cytokine with atheroprotective effects, these cells may also play a role in atherosclerosis. With current SLE therapies focused on B cells, the potential effects of these therapies on the Breg population are important to consider. Moreover, given the controversial role of B cells in atherosclerosis, the effects of these therapies on atherosclerosis are important to consider given that CVD is a leading cause of mortality in SLE. This point will be discussed in more detail in a later section of this review. Refer to TABLE 2 for a summary of B-cell studies discussed in this section.

Contribution of cytokines to SLE pathogenesis

A number of cytokines are involved in the pathogenesis of SLE, with serum levels of many proinflammatory cytokines increased in SLE patients. Potential roles for IL-17, TGF-β and IL-10 in SLE-accelerated atherosclerosis were discussed earlier in this review as related to Th17 cells, Tregs and Bregs. Thus, they will not be discussed here. Instead, we will focus on IFN-γ and IFNα, two inflammatory cytokines that probably contribute to SLE-accelerated atherosclerosis.

IFN-γ has been shown to be proatherogenic, with mice lacking IFN-γ exhibiting reduced atherosclerosis [26]. Produced by activated T cells in atherosclerosis, IFN γ-contributes to plaque instability [72]. In SLE, IFN-γ levels are increased [73], potentially contributing to the accelerated atherosclerosis. Studies have shown

Table 1. The role of T cells in systemic lupus erythematosus and atherosclerosis.

Statement	Experimental evidence	Clinical evidence
T cells contribute to atherosclerosis	Evidence supporting: • T cells are present in atherosclerotic plaques of LDLr$^{-/-}$ and ApoE$^{-/-}$ mice [22] • CD4^{+} T cells transferred into *scid/scid*, ApoE$^{-/-}$ mice increased atherosclerosis [25] • Atherosclerosis is reduced in CD4$^{-/-}$, ApoE$^{-/-}$ mice [27] • Th1 bias results in increased atherosclerosis [28] • Th17 cells are present in atherosclerotic plaques [48] • Deficiency or neutralization of IL-17 results in up to 50% reduction in atherosclerosis [50,51] Evidence opposing: • Bias towards Th2 reduces atherosclerosis [28] • Anti-IL17A treatment increased atherosclerosis, rIL-17A treatment decreased atherosclerosis [48] • Reduced Treg or neutralization of TGFβ increased atherosclerosis [38,39]	T cells are present in human atherosclerotic plaques [21,23] Th17 cells are present in human atherosclerotic plaques [49]
T cells play a role in SLE pathogenesis	Evidence supporting: • Depletion of CD4^{+} T cells beneficial to SLE [29] • Thymectomized NZB/W mice did not develop SLE [30] • Activated SLE T cells secrete inflammatory cytokines [32] • Th17 cells and IL-17 are increased in SLE [53] • T_{eff} in SLE may become resistant to Treg suppression [46] Evidence opposing: • Treg deficiency results in severe autoimmune disease [40,41] • Tregs are reduced in SLE [42]	SLE T cells are hyperactive and have reduced threshold of activation [31] Increased survival, resistance to apoptosis by SLE T cells [33] Th17 cells and IL-17 are increased in SLE [52] Levels of circulating IL-17 correlate with disease activity [54] IL-17 is increased during active nephritis [52] Th17:Th1 ratio is dysregulated, with increased Th17 [55] T_{eff} in SLE may become resistant to Treg suppression [44,47] Treg deficiency results in severe autoimmune disease [40,41] Tregs are reduced in SLE [43]
T cells contribute to increased CVD risk in SLE	In LDLr.*Sle* mice, atherosclerosis was increased, accompanied by infiltration of CD4^{+} T cells into atherosclerotic lesions [12,35]	

T-cell subsets can have divergent effects on both atherosclerosis and SLE, with Th1 and Th17 contributing to disease pathogenesis and Treg protecting against disease.
CVD: Cardiovascular disease; SLE: Systemic lupus erythematosus. T_{eff}: Effector T cell.

a role for type I IFN, particularly IFN-α, in this process. IFN-α levels are increased in both adult and pediatric SLE patients, and are associated with SLE disease activity [74,75]. This cytokine contributes to endothelial cell damage and depletion of endothelial progenitor cells [76]. Because of these actions, vascular repair is reduced. In endothelial progenitor cells from SLE patients, neutralization of type I IFN led to these cells expressing a phenotype similar to that observed in healthy cells. Conversely, when healthy cells were treated with IFN-α, they exhibited a phenotype characteristic of cells from SLE patients [77]. Furthermore, a recent study by Li *et al.* demonstrated that, through upregulation of SR-A, IFN-α enhances the formation of foam cells [78]. Importantly, SR-A mRNA was increased in cells from SLE patients, indicating that this process is probably occurring *in vivo*. Thus, these reports establish IFN-α as an important player in SLE-accelerated atherosclerosis.

SLE-associated risk factors for atherosclerosis

Risk factors associated with atherosclerosis have been extensively studied. Traditional Framingham risk factors for atherosclerosis include hyperlipidemia, hypertension, age, hyperglycemia, smoking and genetic factors. The increased risk of CVD observed in SLE cannot

be completely explained by these traditional risk factors. Instead, SLE-related risk factors for accelerated atherosclerosis include dyslipidemia, disease activity and duration, autoantibodies, nephritis and circulating immune complexes.

SLE patients also exhibit signs of traditional dyslipidemia observed in CVD, with a pro-atherogenic lipid profile consisting of low levels of high-density lipoproteins (HDLs) and increased levels of low-density lipoproteins (LDLs) and triglycerides [79]. HDL protects against atherosclerosis by facilitating reverse cholesterol transport and exerting antioxidant effects. Levels of HDL are inversely correlated to risk of developing chronic inflammatory disorders such as CVD. In fact, in terms of traditional CVD, HDL is the strongest lipid risk factor, independent of LDL levels [80]. HDL can inhibit the production of inflammatory cytokines such as IL-1β and TNF-α by binding to activated T cells and preventing them from contacting monocytes in the circulation [81]. Therefore, through this inhibition of contact under normal conditions, HDL may be preventing the occurrence of inflammatory disorders. Apolipoprotein A-I is the main protein component of HDL and is required for the formation of HDL particles. In a study of hyperlipidemic mice lacking apolipoprotein A-I and, consequently, HDL, an autoimmune-like phenotype was observed [82]. These mice exhibited increased atherosclerosis, enlarged spleen and lymph nodes, skin lesions and autoantibody production. Results from this study support the idea that HDL is important in maintaining immune homeostasis. As mentioned previously, however, HDL levels are significantly decreased in SLE patients compared with healthy controls. Studies have demonstrated that HDL function is just as important as the amount of HDL present. Under conditions of chronic inflammation, HDL can lose its anti-inflammatory properties and instead become proinflammatory (piHDL) [83]. Reports have indicated the presence of piHDL in 45% of women affected with SLE [83]. Rather than acting to prevent oxidation of LDL as normal HDL particles do, piHDL can instead lead to oxidation of LDL and impairment of reverse cholesterol transport. McMahon *et al.* demonstrated that the presence of piHDL in SLE patients can increase the likelihood of developing CVD by up to 17-fold [83], establishing HDL function as an important factor to consider when discussing atherosclerosis risk in SLE.

SLE patients have increased concentrations of circulating autoantibodies, which may contribute to atherosclerosis. Antiphospholipid syndrome (APS), characterized by increased circulating levels of antiphospholipid antibodies (aPLs), has been associated with increased risk of both CVD and stroke. For example, increased intima–media thickening (IMT) was observed in APS patients in both the internal carotid artery and carotid bifurcation [84]. In addition, increased levels of anticardiolipin antibodies in middle-aged men were predictive of myocardial

Table 2. The role of B cells in systemic lupus erythematosus and atherosclerosis.

Statement	Experimental evidence	Clinical evidence
B cells are atheroprotective	Evidence supporting: • Increased atherosclerosis in B-cell-deficient mice [57,58] • Bregs make IL-10, an atheroprotective cytokine [98,99] • Tregs are reduced in B-cell-deficient mice [67] Evidence opposing: • Depletion of mature B cells decreased atherosclerosis [59,60] • BAFF-R$^{-/-}$, ApoE$^{-/-}$ mice have reduced atherosclerosis [96,97]	
B cells contribute to SLE pathogenesis	Evidence supporting: • B cells in SLE are hyperactivated and produce autoantibodies [8] • B-cell dysregulation in SLE leads to T-cell activation, DC recruitment, induction of Th1 and Th17 cells, and inhibition of Tregs [64] Evidence opposing: • Bregs suppress T-cell-mediated inflammation [65–68] • Bregs reduce EAE [69], SLE [65,70] and CIA [71]	B cells in SLE are hyperactivated and produce autoantibodies [8] Bregs have reduced regulatory capacity [70]

B cells are a major player in SLE pathogenesis, but the Breg subset can be important for suppression of autoimmunity and may explain differing results concerning the role of B cells in atherosclerosis.
CIA: Collagen-induced arthritis; DC: Dendritic cell; EAE: Experimental autoimmune encephalomyelitis; SLE: Systemic lupus erythematosus.

infarction risk [85,86], suggesting a role for aPL in contributing to CVD. However, the role of aPL in SLE-accelerated atherosclerosis is unclear, as data are available supporting a positive correlation between aPL and atherosclerosis [87], while other studies indicate no correlation [17,83] or even that aPL levels, specifically anticardiolipin antibodies, are reduced in SLE patients with atherosclerosis compared with those with no plaque [88]. Although the role of aPL in contributing to SLE-accelerated atherosclerosis remains uncertain, more is known regarding the contributions of specific anti-oxLDL antibodies. SLE patients also have increased levels of these antibodies. While IgM anti-oxLDL antibodies are generally thought to be atheroprotective, IgG anti-oxLDL antibodies have been shown to contribute to disease pathogenesis [89]. Thus, increased autoantibodies in SLE patients probably contribute to SLE-accelerated atherosclerosis.

Prevention approaches

Given that accelerated CVD is now a leading cause of death in SLE, it is only logical that future therapies would target this problem. Thus far, we have outlined the role of T cells, B cells and dyslipidemia in acceleration of atherosclerosis in SLE. Now, we focus on therapies and prevention approaches. Table 3 provides a summary of the prevention approaches described below.

B-cell-targeted therapies & implications in SLE

While therapies for SLE are limited, the majority of the effort towards development of therapeutics is focused on B cells. Initial therapies focused on complete depletion of B cells using an anti-CD20 antibody, rituximab. However, this therapy failed to reach treatment goals in two recent clinical trials, the EXPLORER and LUNAR trials [90,91]. In some cases, depletion of B cells led to development of additional autoimmune diseases including colitis and psoriasis [92,93]. Therefore, the use of rituximab is currently reserved for those SLE patients with advanced disease (life-threatening) who fail to respond to other treatments. The role of anti-CD20 antibodies in atherosclerosis have been examined, with administration of this antibody resulting in reduced atherosclerosis in apoE$^{-/-}$ and LDLr$^{-/-}$mice [59,60]. To date, no studies have assessed the effects of anti-CD20 antibodies on SLE-accelerated atherosclerosis. Given that accelerated CVD is now the leading cause of death in SLE, this is an important point to consider, especially since the role of B cells in CVD is not clear but subsets of B cells have been shown to be atheroprotective. In 2010, belimumab (Benlysta®) became the first therapy for lupus to be approved by the US FDA in 50 years. As opposed to complete B-cell depletion, belimumab binds to BLyS/BAFF, preventing it from binding to its receptor, BAFF-R. In turn, this prevents B-cell stimulation and differentiation. This therapy has been shown to be moderately effective in treating SLE disease activity [94]. Unfortunately, however, belimumab was not effective in treating African–American women with SLE [95]. This is an important point, as African–Americans make up a large proportion of the SLE patient demographic. To date, no studies have directly assessed the effects of belimumab on accelerated atherosclerosis in SLE. Experiments in apoE$^{-/-}$ mice have examined the effects of BAFF-R deficiency on atherosclerosis. BAFF-R$^{-/-}$ mice exhibit selective depletion of B2 B cells, accompanied by a reduction in atherosclerosis [96,97]. These results suggest that belimumab inhibition of BAFF might also prove to be beneficial for SLE-accelerated atherosclerosis. However, additional investigation is needed to confirm this hypothesis.

An additional SLE therapy to be considered is expansion of Breg. As discussed previously, Breg exert anti-inflammatory effects through mechanisms that are IL-10 dependent and some that are IL-10 independent. IL-10 has been shown to have beneficial effects on atherosclerosis, with overexpression inhibiting both fatty streak formation [98] and advanced lesions [99]. Although increased IL-10 has been shown to correlate with disease activity in SLE, the role of this cytokine in SLE is not clear. Data have suggested that IL-10 has both immunosuppressive [100] and immunostimulatory [101] roles in the SLE disease process. The fact that IL-10 has opposing functions, including the ability to activate B cells for antibody production and to inhibit T-cell-mediated inflammation, highlights the importance of this cytokine in autoimmunity. Mouse studies have demonstrated the beneficial effects of Breg on SLE disease activity. Interestingly, expansion of IL-10-producing Bregs through CD40 was effective in reducing autoimmunity [65]. Although small in number, Bregs are a potent regulatory population [68]. Thus, through their production of IL-10, Bregs may have antiatherogenic properties. Therefore, Breg expansion might prove to be an important treatment for both SLE and accelerated atherosclerosis in SLE. Also of potential benefit is

Table 3. Preventative approaches for systemic lupus erythematosus-accelerated atherosclerosis.

Preventive approach	Experimental data	Clinical data
Rituximab	Reduced atherosclerosis in ApoE$^{-/-}$ and LDLr$^{-/-}$ mice [59,60]	EXPLORER and LUNAR trials did not reach treatment goals [90,91] Development of ulcerative colitis [92] or psoriasis [93] following treatment
Belimumab	BAFF-R$^{-/-}$, ApoE$^{-/-}$ mice have reduced atherosclerosis [96,97]	Moderately effective for SLE [94] Not effective in African–American women [95]
CD40	Expansion of Bregs through CD40 reduces autoimmunity [65]	
atRA	atRA and TGFβ increase Treg suppressive function [102] atRA inhibits Th17 development [103]	Reduced induction of Tregs in SLE T cells by atRA [104]
Mycophenolate mofetil	Reduced atherosclerosis [107] Atherosclerosis, CD4$^+$ T cells in atherosclerotic lesions and peripheral CD4$^+$ T-cell activation reduced in LDLr.*Sle* mice [107]	Effective in treating SLE [106] No effects on CIMT or CAC [109]
Statins	Reduced atherosclerosis in gld.apoE$^{-/-}$ mice [11] No effects on atherosclerosis in apoE$^{-/-}$, Fas$^{-/-}$ mice [110]	Improved endothelial-dependent vasodilation in SLE [112] LAPS: no effects on CAC, IMT or endothelial activation in SLE [113] APPLE: no effect on CIMT in SLE
Hydroxychloroquine		Reduced accumulation of damage in SLE [115] Reduced inflammatory cytokines, including IFN-α [116] Reduced serum cholesterol, reduced LDL cholesterol [117–120] Reduced aortic stiffness [121] Negative correlation between hydroxychloroquine treatment and atherosclerosis in SLE [88]
ApoA-I and mimetic peptides	Injection of ApoA-I led to decrease in autoimmune-like symptoms and improved Treg function in LDLr$^{-/-}$, ApoA-I$^{-/-}$ mice [122] L-4F reduced piHDL and circulating autoantibodies in apoE$^{-/-}$, Fas$^{-/-}$ mice [110] L-4F and pravastatin increased atherosclerosis, but also increased lesion smooth muscle cell content and reduced macrophages in atherosclerotic lesions [110]	

*atRA: All-*trans *retinoic acid; CAC: Coronary artery calcification; CIMT: Cartoid intima media thickness; IMT: Intima–media thickening; SLE: Systemic lupus erythematosus.*

the IL-10-independent modulation of autoimmunity by Bregs. In a mouse model of EAE, Ray *et al.* demonstrated that B cells suppressed autoimmunity by maintaining Treg populations through glucocorticoid-induced TNF (GITR) ligand [67]. The fact that Tregs were reduced in B-cell-deficient mice provides one possible explanation as to why depletion of B cells with rituximab resulted in additional autoimmune disease in some cases. The importance of Tregs in atherosclerosis and SLE, as discussed earlier, is well documented. The idea that B cells can work to maintain Treg populations adds importance to these cells as a potential therapeutic target in SLE-accelerated atherosclerosis and warrants further investigation. Likewise, GITR ligand may also be an attractive therapeutic target in SLE and atherosclerosis.

T-cell-targeted therapies for SLE

Given their importance in lupus and atherosclerosis, therapies targeting T cells might also be a viable option for treatment of these diseases. In particular, therapies aimed at targeting the Treg/Th17 balance are attractive given the potential of both cell types to affect atherosclerosis and lupus. All-trans retinoic acid (atRA) induces Tregs and can act to maintain FoxP3 expression on Tregs. Lu *et al.* demonstrated that addition of atRA and TGF-β to cultures resulted in increased suppressive function of Tregs *in vitro* and *in vivo* [102]. atRA also inhibits Th17 cell development by inhibiting IRF-4, IL-6Rα and IL-23R [103]. Currently, there are no *in vivo* reports examining the effects of atRA on SLE. However, *in vitro* experiments have suggested that T cells from SLE patients have a defective response to atRA,

with the induction of Tregs reduced compared with cells from healthy controls [104]. Therefore, more investigation, including *in vivo* studies, is needed to determine whether this might be an effective therapy for SLE and atherosclerosis. As the role of T cells in SLE and SLE-accelerated atherosclerosis becomes more apparent, it will be important to shift some effort into developing more T-cell-targeted approaches.

Mycophenolate mofetil therapy in SLE

Mycophenolate mofetil (MMF) is an immunosuppressant that inhibits development of T and B cells through inhibition of inosine monophosphate dehydrogenase (IMPDH) [105]. MMF has been shown to be effective in both SLE [106] and atherosclerosis [107], but few studies have examined its effects on SLE-accelerated atherosclerosis. One study from our laboratory demonstrated effectiveness of MMF in reducing atherosclerotic lesion burden in $LDLr^{-/-}$ mice reconstituted with bone marrow from SLE-susceptible mice. The reduction of atherosclerotic lesion size in the aortic sinus was accompanied by reduced numbers of $CD4^+$ T cells in the atherosclerotic lesions and reduced $CD4^+$ T-cell activation in the periphery [108]. A recent study in SLE patients reported no effects of MMF on carotid IMT or coronary artery calcium, with measurements taken at baseline and after 2 years of treatment with MMF. However, given the small number of patients receiving MMF in this study (n = 25), studies with larger cohorts are needed to more closely examine the effects of MMF on SLE-accelerated atherosclerosis [109].

Efficacy of statins in SLE-accelerated atherosclerosis

Statins, which inhibit 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA), are the most widely used lipid-lowering agents, with well-established beneficial effects on atherosclerosis. Given the dyslipidemia observed in SLE patients, statins have been suggested as a potentially effective therapy for SLE. Studies in animal models have demonstrated conflicting results, with some indicating efficacy of statins in treating SLE-accelerated atherosclerosis [11] and others reporting no differences between study groups [110]. These divergent results are probably owing to variability in study designs including differences in mouse models and statin dosages, among others. A more comprehensive review of statins in animal studies can be found in van Leuven *et al.* [111].

Thus far, few studies have evaluated the efficacy of statins on atherosclerosis in SLE patients. Ferreira *et al.* examined the effects of atorvastatin (20 mg/day) on endothelial function over an 8-week period, reporting improvement in endothelial-dependent vasodilation [112]. The LAPS followed patients receiving atorvastatin (40 mg/day) for 2 years. At the conclusion of the study, no effects of atorvastatin on coronary artery calcium, IMT or endothelial activation were observed [113]. Another multicenter trial, the APPLE study, focused on the effects of atorvastatin in pediatric patients [114]. After 36 months of follow-up, atorvastatin had no effects on carotid IMT. Thus, with conflicting results in both mouse and human studies, it is unclear what the effects of statins are on SLE-accelerated atherosclerosis.

Role of antimalarial agents in SLE-accelerated atherosclerosis

Antimalarial agents have been utilized to treat SLE and other rheumatic diseases for many years. Of these, the most commonly used is hydroxychloroquine. Hydroxychloroquine has both anti-inflammatory and immunomodulatory properties that make it an effective agent for the treatment of SLE. In addition to reducing the accumulation of damage in SLE [115], hydroxychloroquine has been shown to reduce levels of inflammatory cytokines, including IFN-α [116], mentioned above as a potential contributor to SLE-accelerated atherosclerosis. Also important to its antiatherosclerotic potential, hydroxychloroquine treatment has been shown to reduce total serum cholesterol [117–120]. Specifically, LDL cholesterol levels, which are positively correlated with CVD risk, were reduced in SLE patients after hydroxychloroquine treatment [120]. Along with its cholesterol-lowering effects, a reduction in aortic stiffness has been associated with hydroxychloroquine treatment [121]. Importantly, a negative correlation between hydroxychloroquine treatment and atherosclerosis (as assessed by carotid ultrasound) was observed [88]. Collectively, these studies point towards hydroxychloroquine as a potentially beneficial therapy for SLE-accelerated atherosclerosis.

ApoA-I mimetic peptides as treatment for SLE-accelerated atherosclerosis

As discussed previously, the presence of piHDL in SLE patients confers increased CVD risk. Along with the presence of piHDL, SLE patients have

reduced HDL levels. HDL is well documented as having beneficial effects on atherosclerosis and may have beneficial effects on autoimmunity. In a study of hyperlipidemic mice lacking apoA-I, injection of apoA-I resulted in formation of HDL particles and, importantly, reduction of autoimmune-like symptoms and improved Treg function [122]. Woo *et al.* evaluated the effects of an apoA-I mimetic peptide, L-4F, in an apoE$^{-/-}$, Fas$^{-/-}$ mouse model [110]. After 27 weeks of treatment, piHDL was reduced, along with a reduction in circulating autoantibodies to dsDNA and oxidized phospholipids. Interestingly, the study also included a group receiving both L-4F and pravastatin. Despite an increase in atherosclerotic lesion area, plaques in mice receiving combination therapy showed signs of increased stability, including an increase in smooth muscle cell content and a reduction in macrophages in the lesion. Thus, treatments focusing on HDL may prove to be beneficial in SLE and atherosclerosis and, perhaps, a combination of statins and HDL therapy should be considered.

Future perspective

Considerable advances have been made in the field of SLE research in the last 10 years. An important development came in 2010, when belimumab became the first therapy approved for SLE since the 1950s. However, the effects of B-cell therapies on SLE-accelerated atherosclerosis have not been considered. Likewise, according to the Clinical Trials Database [201], there are currently several T-cell-focused clinical trials underway, with none assessing CVD outcomes. As evidence grows for the role of T cells in SLE-accelerated atherosclerosis, we might expect this to change. It is probable that, in the near future, T-cell-focused therapies will be developed with the goal of treating both SLE and the resultant acceleration of atherosclerosis. Regulatory cell populations are emerging as important factors in the control of autoimmunity and atherosclerosis, also making them attractive therapeutic targets. This is an exciting time for SLE and atherosclerosis research, with important discoveries being made every day. As research continues, it is likely that the next 10 years will bring increased understanding of the mechanisms behind accelerated atherosclerosis in SLE and, along with that, new therapies that are able to address both SLE and SLE-accelerated atherosclerosis, improving the prognosis for these patients. With our understanding of these disease processes growing, it is evident that SLE patients will not be left waiting another 60 years before new therapies become available.

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Executive summary

Accelerated atherosclerosis & systemic lupus erythematosus

- The risk of atherosclerosis is increased, on average, two- to ten-fold in systemic lupus erythematosus (SLE) patients.
- T cells are an important player in atherosclerosis and have been shown to accumulate in atherosclerotic lesions of lupus-susceptible mice.
- Tregs play an important role in inhibiting atherosclerosis, but these cells may be dysfunctional in SLE.
- Increased numbers of Th17 cells and increased circulating levels of IL-17 may contribute to SLE-accelerated atherosclerosis.
- Dysregulation of B cells contributes to SLE and may be important in SLE-accelerated atherosclerosis. Bregs may be an important population to consider when examining SLE-accelerated atherosclerosis.
- IFN-α levels are increased in SLE, and activity of this cytokine contributes to accelerated atherosclerosis in SLE.

Risk factors

- SLE patients have a proatherogenic lipid profile, with reduced high-density lipoprotein and increased low-density lipoprotein and triglycerides. A dysfunctional form of high-density lipoprotein, proinflammatory high-density lipoprotein, is found in SLE patients, contributing to low-density lipoprotein oxidation.

Prevention approaches

- In 2010, belimumab became the first therapy approved for SLE since the 1950s.
- Given their role in atherosclerosis and SLE, T cells might also be an attractive therapeutic target. Particularly, strategies to alter the Treg/Th17 balance may prove effective.
- Other potential therapies for SLE-accelerated atherosclerosis include statins and apoA-I mimetics. Although the effects of statins on SLE-accelerated atherosclerosis have yielded conflicting results thus far, apoA-I mimetic peptides have not been studied in humans.

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