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«B CELLS AND ATHEROSCLEROSIS IN SYSTEMIC LUPUS ERYTHEMATOSUS»

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Abstract

Cardiovascular (CV) events, as a result of accelerated atherosclerosis, are an important cause of mortality in patients with Systemic lupus erythematosus (SLE). The etiology of SLE is multifactorial and still unclear; among other potential culprits, excessive B cell activation seems to play a crucial role. Accumulating evidence supports a contributory role of B cells in pathogenesis of atherosclerosis as well. This article focuses on the contribution of B cells and of several autoantibodies in the pathogenesis of atherosclerosis in both general and lupus populations. Review of the published literature on experimental models has also been performed. Distinct B cell subsets seem to exhibit separate effects on the progression of atherosclerosis, with B2 B cells displaying a mainly atherogenic phenotype, while B1 B cells are mostly viewed as atheroprotective. Selective B2 inhibition by anti-B cell therapies seems a promising therapeutic strategy against atherosclerosis development in the setting of lupus.

Keywords: autoantibodies, atherosclerosis, B cells, B1 cells, B2 cells, BAFF, systemic lupus erythematosus

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1. Introduction

Accelerated atherosclerosis in patients with Systemic lupus erythematosus (SLE) is a well-recognized complication associated with increased mortality rates due to an excess of CV events (1, 2). For years, atherosclerotic plaque formation was regarded as the result of passive accumulation of lipids in the vascular wall. However, accumulating evidence over the last decades points towards a strong inflammatory component in the origin of atherosclerosis, with an emerging role of B cells (3, 4). In contrast to B1 B cells mainly viewed as atheroprotective, B2 B cells are considered atherogenic; yet this distinction is neither absolute nor has it been sufficiently researched in lupus related atherosclerosis (5). The role of antibodies is also rather complex, especially in the setting of autoimmune disease. The objective of this review is to focus on the contribution of B cells and several autoantibodies in the pathogenesis of atherosclerosis in both general and lupus populations, based on data derived from studies on humans and experimental models.

2. Lupus related atherosclerosis

2.1 Epidemiology

The bimodal mortality pattern, proposed by Urowitz and colleagues in 1976, showed that deaths in late SLE were primarily linked to advanced CV disease, mainly manifesting as myocardial infarction (MI) (1), an observation confirmed in a recent study (2). Though the first peak in mortality, related to high disease activity and infection, has been decreased as a result of improved treatment, the same cannot be said for the second peak (2).

It has been established that SLE patients suffer from increased risk for CV disease, which cannot be adequately explained by the traditional CV risk factors (6). The Nurses' Health Study showed that the risk for developing CV events in women suffering from SLE was over 2 times greater than that of the general female population (7). In another study by Manzi et al, the age specific incidence rate of MI in women with SLE aged 35 to 44 years old was found to be 50 times greater compared to the Framingham study database (8). The heightened CV burden reflects the presence of accelerated atherosclerosis in SLE patients, as evidenced by increased carotid intima-media thickness (CIMT) scores and prevalence of carotid plaques in several systematic reviews and meta-analyses (9, 10). Increased atherosclerosis in SLE patients is thought to result from a combination of traditional risk factors together with the inflammatory burden and immune dysregulation characterizing SLE (11).

2.2 Traditional and disease related risk factors

Certain traditional CV risk factors have been found to be increased in patients with SLE, especially hypertension and a "lupus pattern" dyslipidemia (12, 13). The latter is characterized by high levels of very low density lipoprotein (VLDL) cholesterol and triglycerides, high or unchanged levels of low density lipoprotein (LDL) cholesterol and low levels of high density lipoprotein (HDL) cholesterol (14, 15). Smoking, diabetes mellitus, older age and hyperhomocysteinemia have all been linked to subclinical atherosclerosis and/or CV events in these patients (12, 16-18). In a recent report, MTHFR 677 TT polymorphisms - the main genetic determinant for hyperhomocysteinemia- have

been shown to be independent predictors for lupus related subclinical atherosclerosis (19).

A strong indication of the role of inflammation in atherosclerotic process comes from the observation that disease activity in SLE is positively associated with CV risk (17). Moreover, longer disease duration has been linked to coronary artery calcification and presence of atherosclerotic plaques (20, 21). Cumulative dose of corticosteroids has been also shown to contribute to subclinical atherosclerosis development (12), in contrast to hydroxychloroquine, which seems to exert antithrombotic and antilipidemic effect (22, 23).

Other SLE – specific risk factors associated with high CV risk are low complement levels, presence of anti-dsDNA and antiphospholipid antibodies, mainly lupus anticoagulant (24). Interestingly, anxiety in SLE patients has been linked to higher IMT scores, while personality traits of extraversion were negatively associated with atherosclerotic plaque formation (25).

2.3 Pathophysiology of lupus related atherosclerosis

Endothelial dysfunction (impaired bioavailability of nitric oxide – NO) and endothelial cell activation (expression of adhesion molecules and proinflammatory cytokines) seem to be converging processes, characterized by an inability of the endothelium to maintain its homeostatic properties; as a result, a proinflammatory and procoagulant state occurs (26, 27), essential to the development of atherosclerosis (28). Several factors have been associated with compromised endothelial function; traditional CV risk factors such as dyslipidemia, increased oxidative stress, and in the case of SLE, cytokines (mainly type I IFNs), antiendothelial and antiphospholipid antibodies and neutrophil extracellular traps have all been implicated in endothelial damage (27, 29). The ensuing altered endothelial permeability and oxidative stress allows for the accumulation and oxidation of LDL particles in the vascular intima. Subsequently, chemoattracted monocytes migrate to the vascular tissue where they differentiate into macrophages that in turn take up oxidized LDL (oxLDL) and turn into inflammatory foam cells, marking the onset of the

atherosclerotic lesion. Several other immune cells are recruited in this inflammatory process, among which T and B cells, neutrophils, dendritic and mast cells. The formation of the early "fatty streak" mainly consists of T cells along with lipid laden foam cells. A necrotic core is gradually shaped from the accumulation of lipids and apoptotic cells. Activated smooth muscle cells (SMCs) migrate from the media to the vascular intima and proliferate, participating in the creation of the fibrous cap of the atherosclerotic lesion. In the meantime, the continuous release of cytokines results in an ongoing inflammatory process in the vascular wall, promoting the progression of atherosclerosis (30). The involvement of adaptive immunity in the atherosclerotic process is indicated not only by the presence of T and B cells in atherosclerotic plaques (31), but also by the occurrence of adventitial artery tertiary lymphoid organs (ATLOs), that appear to bear an important role in local immune response and atherosclerosis (32). Chronic local inflammation leads to recruitment of several cells, of innate and adaptive immunity, and to their progressive organization in compartments, finally leading to the formation of local lymphoid tissue (33). ATLOs have been shown to regulate diverse B cell responses in local atherosclerotic lesions (34).

In SLE, LDL displays both greater susceptibility to oxidation and ability to increase oxidative stress levels (35). What is more, a dysfunctional form of HDL (piHDL) that arises under inflammatory conditions, present in SLE patients, seems to lack the regular anti-inflammatory and anti-oxidant properties of HDL, does not efficiently participate in cholesterol efflux and allows for the oxidation of LDL. Indeed, the presence of this aberrant form of HDL in SLE patients has been associated with higher IMT scores and plaque occurrence (36). Once is oxidized, LDL becomes immunogenic promoting an immune humoral response against oxLDL resulting in B cell activation, autoantibody production and formation of immune complexes, all of which participate in the atherosclerotic process, in complex and distinct ways (37).

3. B cells and Atherosclerosis

Numerous studies have shown opposing effects of different B cell subsets and several autoantibodies on atherogenesis. Herein, studies exploring the effect of B cells and antibodies on atherosclerosis in both experimental models and human subjects are reviewed.

3.1 B cell subsets

Studies have mainly focused on the effect of B2 cells and B1 cells in animal models. B2 cells consist of follicular B cells and marginal zone B cells (MZBs), but the term is mostly used to describe the former group, responsible for the production of high affinity antibodies during infection. B1 cells are the main source of natural antibodies that are characterized by self-reactivity, poly-reactivity and low affinity, and they are mostly found in peritoneal and pleural spaces. In mice, B1 cells are further categorized into B1a cells, that express CD5 (cluster of differentiation) (CD5+), and B1b cells that do not (CD5-) (38). B1b cells respond to natural infection or vaccination by producing antibodies, in a T cell independent manner. They are capable of inducing memory and maintaining long-term efficacy against several bacteria (39).

3.1.1 B2 cells – Atherogenic effects

The study of B2 cells on atherosclerosis has yielded rather conflicting results, though accumulating evidence points towards a more atherogenic effect. Initially, B cells were credited with atheroprotective properties; splenectomy (40) and induction of B cell deficiency (41) in hyperlipidemic murine models resulted in aggravation of atherosclerosis. Moreover, transfer of splenic B cells to ApoE-/- mice resulted in marked decrease of atherosclerotic lesions (40). In contrast, depletion of B cells using anti-CD20 monoclonal antibodies resulted in amelioration of atherosclerosis (42, 43). Anti-CD20 antibodies affected B cell populations unevenly, affecting mostly splenic rather than peritoneal B cells (44), suggesting that depletion of B2 cells was responsible for the atheroprotective effect. Confirmation of the harmful effect of B2 cells was provided by

transferring separately B2 cells and B1 cells, to lymphocyte deficient mice. Only the adoptive transfer of B2 cells resulted in aggravation of atherosclerosis (43). B2 cell depletion by use of anti-CD23 monoclonal antibody (the receptor for the Fc component of IgE, present on B cells) also led to lower levels of IgG antibodies and reduction of atherosclerotic plaques (45). The atherogenic properties of B2 cells have been attributed to the production of high affinity pathogenic IgG antibodies (46) and proinflammatory cytokines, such as TNFa (47) and the T-cell-originating IFNγ (42). B cell depletion therapy, by anti-CD20 monoclonal antibody (rituximab) in refractory SLE, led to improved lipid levels in association with low disease activity (48). No information on atherosclerosis was however available.

MZBs are also classified as B2 cells, however they do differ significantly from follicular B cells (the main B2 cell subset) in their ability to generate T cell independent antibody responses (38). Splenic MZB deficiency in LDLr-/- mice was recently shown to aggravate atherosclerosis, without significantly affecting levels of lipids or antibody responses to modified LDL. It appears that MZBs' atheroprotective action relies on suppression of the proatherogenic activity of follicular helper T cells in response to a high fat diet (49). Additional research is needed, exploring the differential effects of follicular and MZB cells on atherosclerotic outcomes.

3.1.2 B1 cells - Atheroprotective effects

The atheroprotective role of B1a cells mostly relates to their ability to produce natural IgM antibodies, since modified B1a B cells without secretory IgM ability, lack atheroprotective properties (50). The protection exerted by B1a cells seems to be highly dependent on Toll-Like Receptor 4 (TLR4) and MyD88 signaling, through which atheroprotective polyclonal IgM production is achieved. TLR4 and MyD88 expression in B1a cells also enhances their atheroprotective effect by increasing the anti-inflammatory cytokines IL-10 and TGFβ and decreasing the proatherogenic CD4+ and CD8+ T cells in atherosclerotic lesions (51). Sialic acid binding immunoglobulin like lectin G (Siglec-G) has been found to promote atherosclerosis by suppressing B1a cell action and antibody

production (52). B1b cells (CD5-) have also been found to exert atheroprotective effects through secretion of reactive IgM antibodies to oxLDL both in vitro and in vivo. Adoptive transfer of these cells to lymphopenic ApoE-/- mice also resulted in attenuation of atherosclerotic lesions by IgM production, independently of T or other B cell interaction (53).

In contrast to the atheroprotective role mainly attributed to B1a cells via IgM production, a recent study showed that innate response activator B cells (IRA), a subset of B1a cells, aggravate atherosclerosis (54). Specifically, IRA B cells arise from B1a cells after activation via pattern recognition receptors (PRRs), migrate to the spleen or lung, produce granulocyte macrophage – colony stimulating factor (GM-CSF) and display a significant role in abating bacterial infections (55, 56). IRA B cells aggravate atherosclerosis by promoting classic dendritic cell expansion that consequently leads to T cell IFN-γ production and Th1 mediated $IgG2_c$ anti-oxLDL secretion (54).

3.1.3 B regulatory cells (Bregs)

A small number of studies examined the role of Bregs in atherosclerosis. Adoptive transfer of a Breg subset resulted in marked reduction of atherosclerosis development, mainly via IL-10 production (57), though B-cell-derived IL-10 has also been shown to have no effect on atherosclerosis (58). In patients suffering from coronary artery disease (CAD), a subset of Bregs (Tim1+ B cells; the main IL-10 producing B cells) lack the ability of increased IL-10 production upon proper stimulation and also the ability to suppress IFNγ expression by T cells, unlike Bregs in healthy individuals (59).

3.2 Mediators on B cell function

3.2.1 Impact of hyperlipidemia on B cells

Dyslipidemia has been shown to affect B cell behavior, leading to a more activated B cell phenotype (60). In hyperlipidemic murine models, there is a marked increase in both the germinal center B cell compartment and the number of antibody forming cells in the spleen. Proliferation of MZB cells has been reported to a great extent as well (61). Increased activity of B cells was also observed in lupus hyperlipidemic models along with marked production of anti-oxLDL and antiphospholipid antibodies (62, 63).

3.2.2 B cell activating factor (BAFF)

Recent studies support a significant role for B cell activating factor (BAFF/BLyS) on atherosclerosis, though the currently available data are rather conflicting.

BAFF is a tumor necrosis factor ligand (TNF ligand) that binds to BAFF-R (BAFF receptor), TACI (transmembrane activator and calcium modulator and cyclophiline ligand interactor) and BCMA (B cell maturation antigen), all of which are expressed on B cells. BAFF acts as a survival/developmental factor for B cells, but is not essential for B1 cell survival (64). Knockout of BAFF-R in mice led to decreased B2 cell numbers, unaffected B1a and non B cell populations and significant reduction in atherosclerosis (65, 66). Targeting BAFF-R with a monoclonal antibody also resulted in attenuation of atherosclerotic lesions (67). Apart from B2 cell depletion, reduction of CD4+ and CD8+ T cells (66, 67) and proinflammatory cytokines in atherosclerotic lesions (67) possibly contributed in amelioration of atherosclerosis in these models.

In a recent study in a human lupus cohort, increased BAFF levels in the 75^{th} quartile together with genetic variants of the BAFF gene were found to be associated with increased levels of subclinical atherosclerosis (68). In a small study consisting of 7 patients with active SLE, Spinelli et al showed that anti-BAFF treatment resulted in higher numbers of endothelial progenitor cells, 4 weeks after the initiation of therapy, suggesting a possible short-term atheroprotective action, through enhancement in

endothelial repair mechanisms (69). Moreover, in patients with acute MI, high BAFF levels also associated with high risk of death or MI recurrence (70).

Conversely, hyperlipidemic mice with BAFF overexpression showed signs of substantial reduction of atherosclerosis and marked decrease in lipids; this atheroprotective effect of BAFF was found to depend on its interaction with TACI on B cells that consequently led to increased levels of protective IgM anti-oxLDL (71). Moreover, BAFF, in collaboration with angiotensin II, displays atheroprotective properties by promoting IL-10 production by Bregs (72). Towards the same direction, BAFF depletion by a monoclonal antibody, worsened atherosclerosis, despite competent depletion of B2 cells. Indeed, the results of anti-BAFF treatment on B cell populations were very similar to those of anti-CD20 treatment, but the outcome on atherosclerosis was reversed (73); in this experiment, the atheroprotective effect of BAFF was B cell independent and relied on BAFF-TACI interaction on myeloid cells (73) (Figure 1).

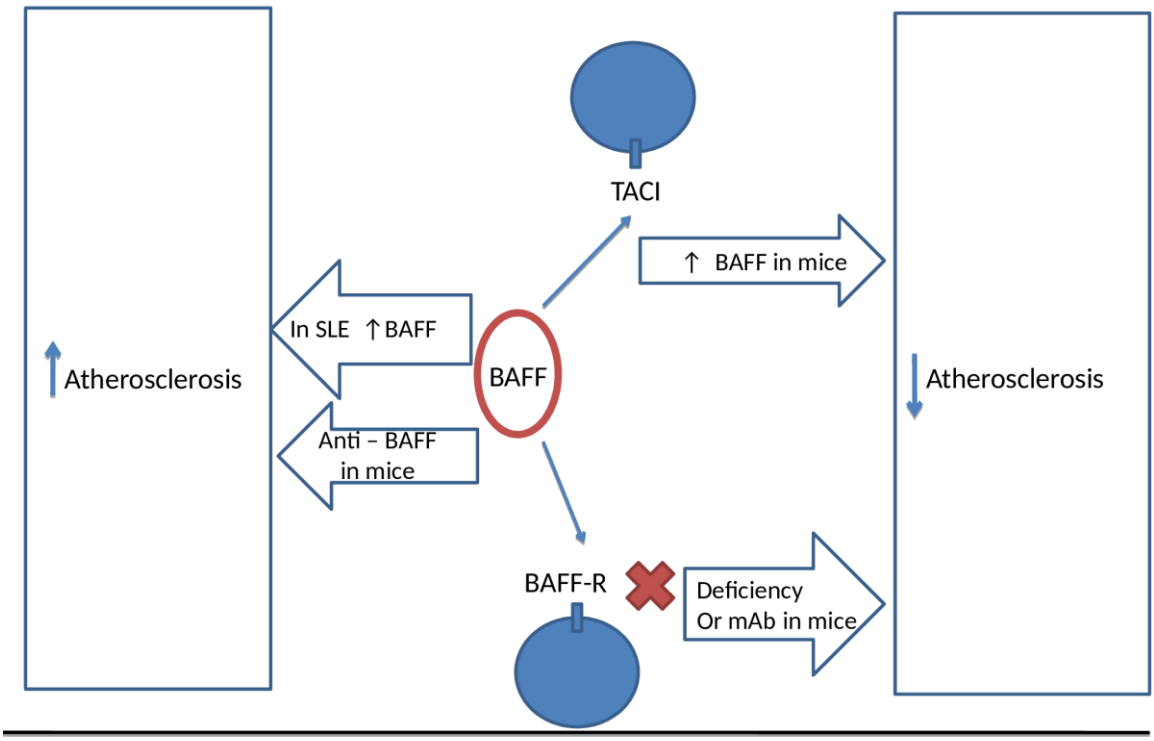

Figure 1. Schematic representation of the effect of BAFF manipulation in atherosclerosis
BAFF: B cell activating factor **BAFF-R:** BAFF receptor
TACI: transmembrane activator and calcium modulator and cyclophiline ligand interactor

APRIL (A proliferating inducing Ligand), an additional factor that contributes to B cell development and binds to TACI, has not yet been studied thoroughly in atherosclerosis. APRIL overexpression in hyperlipidemic mice did not seem to affect atherosclerotic lesions even though it led to increased levels of IgM and B1a cells and increased SMC content (74). A recent study examined the interaction of the macrophage migration inhibitory factor (MIF) - a known proinflammatory and proatherogenic cytokine - and B cells in atherosclerosis. Global MIF deficiency in ApoE-/- mice resulted in attenuation of atherosclerosis that was associated with an increase in IgM anti-oxLDL antibodies. On the other hand, bone marrow specific deletion of MIF led to equal increase in IgM and IgG anti-oxLDL antibodies and had no effect on atherosclerosis (75).

3.3 Antibodies

The effect of antibodies on atherosclerosis is even more complex. Substantial research was focused on antibodies against oxidized forms of LDL in atherosclerosis, due to its important role in atheroma formation. Several issues arising in the study of these antibodies include the lack of antigen standardization as well as the contradictory results among healthy individuals, patients with CV or autoimmune diseases, implying the presence of diverse immune responses among different groups. Antibodies in human populations are mostly studied as predictive markers rather than causative agents of atherosclerosis. Differences between animal models and humans hinder safe translation to human pathophysiology.

3.3.1 Animal models

3.3.1.1 IgM antibodies

Natural IgM antibodies seem to have an overall protective function against atherosclerosis formation; they recognize oxidation specific epitopes (OSEs), such as oxidized phospholipids, both on apoptotic cells and oxLDL (76, 77), facilitate clearance of apoptotic cells (77) inhibit oxLDL uptake by macrophages (77, 78), thus preventing foam cell formation, and lastly, prevent expression of proinflammatory genes caused by certain OSEs (79).

In hyperlipidemic mouse models, levels of antibodies against oxidized epitopes of LDL were found to be increased (80, 81). IgM deficiency alone, in mice with hyperlipidemia, caused significant increase in atherosclerotic lesions, verifying the atheroprotective effect of IgM antibodies (82). Several immunization experiments proved atheroprotection by IgM antibodies, relying on the molecular similarity of oxidized phospholipids on oxLDL and apoptotic cells and the phosphorylcholine (PC) of the streptococcal membrane, and recognition of these patterns by natural antibodies (76). Immunization of LDLr-/- mice with Streptococcus pneumoniae ameliorated atherosclerosis development, resulted in high levels of IgM anti-oxLDL antibodies and in increase of IgM – T15 secreting cells in

the spleen of the immunized mice (83). Treatment of ApoE-/- mice with injections of apoptotic cells or phosphatidylserine liposomes (a molecule exposed on apoptotic cell membranes) resulted in amelioration of atherosclerosis attributed to B1a B cell expansion and IgM production (84). Even passive immunization with monoclonal IgM anti-PC showed to protect from progression of atherosclerosis (85).

3.3.1.2 IgG antibodies

The role of IgG antibodies in the pathogenesis of atherosclerosis has been also conflicting. As discussed previously, some IgG antibodies have been credited with atherogenic properties; reduction of the B2 B cell population and thus of IgG titers has led to amelioration of atherosclerosis (43, 45, 46). IgG antibodies against heat shock proteins (HSP60/65) have been shown to aggravate atherosclerotic lesions (86, 87). However, use of polyclonal IgG treatment in ApoE-/- mice led to reduction of atherosclerosis (88), as did the injections of human IgG1 against malondialdehyde (MDA) modified apoB100 (89). Immunization of ApoE-/- mice with PC or MDA-LDL had similar atheroprotective results attributed to high IgG antibody titers parallel to IgM antibody increase (90) and elevated T cell dependent IgG antibody production against MDA-LDL (91, 92). Recently, it was shown that IgG anti-LDL promoted clearance of LDL via immune complexes and significantly associated with atheroprotection (93).

3.3.2 General population

The use of total levels of several classes of immunoglobulins as predictive markers for CV events revealed contradicting results (94, 95). Overall, antibodies against oxidized epitopes of LDL have been associated with CV disease and atherosclerosis, though these associations did not always reach statistical significance in multivariable analyses. IgM antibodies against oxidized epitopes of LDL negatively associated with CV events and CAD, contrary to IgG antibodies (96, 97). Similar associations have been observed in atherosclerosis studies; inverse association with subclinical atherosclerosis has been observed for IgM anti-MDA-LDL and MDA modified apoB100 (98, 99), whereas IgG antibodies against MDA-LDL have been associated with accelerated atherosclerosis,

independently of other measured risk factors (100). Furthermore, in symptomatic patients that underwent carotid endarterectomy, high levels of IgM antibodies against oxidized epitopes of LDL were associated with plaques enriched in fibrous tissue, with fewer macrophages and lipids -low risk rupture plaques- while the opposite applied for high IgG titers (101). In contrast, IgG titers against oxLDL in a healthy population were found to be inversely associated with CIMT scores (102). Finally, IgM anti-PC antibodies have been negatively associated with accelerated atherosclerosis and high CV risk (103), while high antibody titers against HSP60/65 have shown positive correlation with accelerated atherosclerosis (104, 105) (Table 1).

Table 1. Association of IgG and IgM autoantibodies with atherosclerosis and CV morbidity in the general population

Antigen	Population	Antibodies	Evidence of Atherosclerosis	Association	Ref.
-	♂ with dyslipidemia	Total serum IgM/IgG/IgA/IgE	MI SCD	Positive: IgG, IgE, IgA	(94)
MDA-LDL	Hypertensive ♂ & ♀	IgM/IgG Total IgM/IgG	CV events	Negative: Total IgM & IgG	(95)
MDA-LDL, Cu-oxLDL, apoB-IC	♂ & ♀	IgM/IgG	Risk for CV events	Negative: IgM MDA-LDL Positive: IgG Cu-oxLDL	(96)
MDA-LDL, Cu-oxLDL, oxCL, apoB-IC	♂ & ♀ (clinical indication for coronary angiography)	IgM/IgG	Angiographically determined CAD (>50% diameter stenosis)	Negative: All IgM Positive: IgG Cu-oxLDL (ns in multivariable analysis)	(97)
Natural & MDA apoB100 peptide p210	♂ & ♀ Healthy High Risk	IgM p210mda IgG p210natural	CIMT	Negative: IgM p210mda & IgG p210nat (ns in multivariable analysis)	(98)
MDA-LDL, Cu-oxLDL	♂ & ♀	IgM/IgG/IgG2	CIMT	Negative: IgM MDL-LDL	(99)
Native LDL, MDA-LDL, Cu-oxLDL	♂	IgG/IgM/IgA	CIMT	Positive: IgG MDA-LDL	(100)
MDA-LDL, aldehyde modified apoB-100 peptides p45,p210,p240	♂ & ♀ (carotid endarterectomy)	IgM/IgG	Plaque vulnerability	Negative: IgM p240 & IgM p210 Positive: IgG p210	(101)
oxLDL	♂ & ♀ Healthy	IgG	CIMT	Negative	(102)
PC	♂ & ♀ High Risk	IgM	CIMT progression Risk for CV events	Negative (in ♂ only)	(103)
HSP65	♂ & ♀	IgG	Carotid plaques	Positive	(104)
HSP65	♂ Routine coronary angiography	IgG	Angiographically determined CAD	Positive	(105)

ns: no statistical significance **MDA**: Malondialdehyde **LDL**: Low density lipoprotein
apoB100: apolipoprotein B100 **Cu-oxLDL**: copper oxidized LDL **oxLDL**: oxidized LDL
PC: Phosphorylcholine **oxCL**: oxidized cholesterol linoleate **apoB-IC**: apoB100 immune complexes
HSP65: Heat shock protein 65 **PC**: phosphorylcholine **CV**: Cardiovascular
CIMT: Carotid intima media thickness **MI**: Myocardial Infarction **SCD**: Sudden cardiac death

3.3.3 SLE patients

Patients with SLE, even in the absence of antiphospholipid syndrome, display increased levels of antiphospholipid antibodies compared to controls (106), as well as occurrence of an immunogenic β2GPI-oxLDL complex (107). Even though both antiphospholipid and anti- β2GPI-oxLDL antibodies highly correlate with thrombotic events (107), statistically significant association with accelerated atherosclerosis has not yet been confirmed (108, 109).

Furthermore, levels of antibodies against HDL, paraoxonase 1 (PON1) and apoprotein A1 (apoA1) are also higher in SLE patients compared to controls (110, 111), suggesting a proatherogenic profile. Anti-HDL antibodies positively correlate with CV disease and anti-PON1 antibodies associate with high IMT scores (110). However, anti-apoA1 antibodies do not seem to correlate with CV events (111).

IgM anti-PC antibodies -mainly viewed as atheroprotective- display negative correlation with subclinical atherosclerosis in SLE (112, 113), similarly to the general population (103). The titers of these antibodies are very low in SLE patients compared to healthy controls (113). High IgM anti-PC titers have also been associated with lower disease activity and severity as well as lower risk for CV events in lupus patients (114). Similar observations came from a study by Rahman et al regarding both IgM anti-PC and anti-MDA antibodies (115). Up-regulation of T regulatory cells and reduced in vitro production of TNFa and IL-17 has been proposed as a potential atheroprotective mechanism (116).

IgG anti-PC antibodies in lupus patients were also lower compared to healthy controls and associated negatively with disease activity and organ damage. Moreover, they were shown to block the expression of adhesion molecules on endothelial cells (117), implying a protective role in atherosclerosis as well.

In a recent study from our group, IgG anti-oxLDL antibodies in SLE patients did not demonstrate association with IMT scores or atherosclerotic plaques, though these antibodies proved to be inversely correlated with markers of atherosclerosis in a group of patients with Sjogren's syndrome (118). Conversely, in an older cohort of lupus patients by Doria et al, IgG antibodies against oxidised palmitoyl arachidonoyl phosphocholine (oxPAPC), a main antigenic determinant of oxLDL, were associated with high IMT scores (12). Moreover, it has been observed that, in SLE patients, antibodies against peptides of apoB100 are significantly lower compared to healthy population. Specifically, IgG p210 and IgM p45 (both natural and MDA) were found to be considerably lower in SLE patients and were proved to be inversely associated with CV disease (119).

Although anti-HSP antibodies have been implicated in atherogenesis in animal models (87) and have been associated with subclinical atherosclerosis in general populations (104), no such correlations have been established in lupus patients (12). Likewise, ACEAs have been implicated in endothelial damage (120) but no association with proof of endothelial dysfunction was found in SLE patients (121).

In search of possible novel markers relating to disease features of SLE, among 77 studied protein fragments, Frostegard et al observed significant IgG reactivity against zinc finger protein 688 (ZNF688), compared to healthy controls, in association with atherosclerotic plaque formation and plaque vulnerability (122). Finally, in SLE, microparticles containing IgG (microparticle immune complexes) and exposing phosphatidylserine, were found to positively correlate with high CIMT scores (123) (Table 2).

Table 2. Association of autoantibodies and subclinical atherosclerosis and CV morbidity in SLE patients (results refer only to SLE patients)

Antigen	Population	Antibodies	Evidence of ATH	Association	Reference
oxPAPC, HSP65, β2GPI	SLE	IgG	CIMT – carotid plaques	Positive: IgG oxPAPC	(12)
Phospholipids	♂ > ♀	aCL LAC	MI CIMT Carotid plaques	Positive with MI: LAC	(108)
Apo-A1	♀ > ♂	IgG	CV disease	None	(111)
HDL **PON1**	SLE	IgG	CV disease CIMT	Positive with CV disease: IgG HDL Positive with CIMT: IgG PON1	(110)
β2GPI/oxLDL **β2GPI**	APS SLE (+/- APS)	IgG	Arterial thrombosis	Positive	(107)
PC	SLE	IgM	CIMT - carotid plaques – CV events	Negative	(112-114)
PC, MDA	SLE	IgM	CIMT - carotid plaques	Negative	(115)
oxLDL	SLE, RA, SS	IgG	IMT – carotid plaques	None	(118)
Native and MDA p45 , p210	SLE	IgM/IgG	CV Disease	Negative: IgM p45 & IgG p210	(119)
77 protein fragments	SLE - controls	IgG	CIMT – carotid plaques	Positive: IgG ZNF 688	(122)
MPIC	SLE women		CIMT	Positive	(123)

oxLDL: oxidized LDL **MDA**: Malondialdehyde **HSP65**: Heat shock protein 65 **PC**: phosphorylcholine **apoA1**: apolipoprotein A1 **oxPAPC**: Oxidised palmitoyl arachidonoyl phosphocholine

β2GPI: β2 glycoprotein I **MPIC**: Microparticles containing IgG immune complexes
SS: Sjogren Syndrome **RA**: Rheumatoid Arthritis **SSc**: Systemic Sclerosis
APS: Antiphospholipid Syndrome **MI**: Myocardial Infarction **LAC:** Lupus anticoagulant
CIMT: Carotid intima media thickness **aCL:** anticardiolipin antibodies **PON1**: Paraoxonase 1
HDL: High density lipoprotein

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4. Conclusion

B cell subpopulations and distinct antibodies seem to have opposing effects on atherosclerosis. Manipulation of these subpopulations, without directly affecting other components of the immune system, could result in aggravation or amelioration of atherosclerotic risk. While B1 B cells seem to bear an atheroprotective role through IgM antibody production, B2 B cells appear to worsen atherosclerosis through pathogenic IgG antibodies against oxLDL epitopes. SLE presents a suitable environment for LDL oxidation, while aberrant B cell function may promote immune reaction skewed towards more prominent B2 cell and/or dampened B1 cell activation, as evidenced by the relative shortage of protective antibodies in lupus patients. More studies in SLE patients and lupus prone mice are needed to examine B cells on atherosclerosis, since autoimmune conditions may alter the results displayed in general healthy populations.

The contribution of B cell activation in the pathogenesis of lupus and atherosclerosis points towards a potentially significant role of B cell mediated effects on lupus related atherosclerosis, a major disease burden related to increased mortality. Due to physiological differences between animal models and humans, translation of the experimental findings to human disease is challenging. For instance, both excess of BAFF and blocking the BAFF receptor is shown to ameliorate atherosclerosis in mice (67, 71). This discrepancy possibly implies that BAFF-BAFF-R interaction results in atherogenic events, while interaction of BAFF with other receptors is atheroprotective, as

was observed in several experimental models (71, 73). In humans, high levels of BAFF along with BAFF gene variants were found to be associated with increased risk for lupus related atherosclerosis (68). This observation, could imply a dominant role for BAFF-BAFF-R interactions in SLE resulting in increased auto-reactive B2 cell survival, with deleterious effects both on disease burden and atherosclerotic risk, though these data need to be confirmed in further studies.

In regard to the role of B cells in atherosclerosis, it seems that B2 cells are atherogenic, while B1 cells are atheroprotective. Thus, selective inhibition of atherogenic B2 cells and preservation of atheroprotective B1 cells seems to be a reasonable goal in the treatment of lupus related atherosclerosis (Figure 2). Towards this direction, commercially available anti-B cell therapies such as anti-CD20 and anti-BAFF monoclonal antibodies already implemented in the treatment of lupus patients could serve as potential therapeutic regimens against lupus atherosclerosis as well. Indeed, anti-CD20 mediated B cell depletion was shown to reduce atherosclerosis in animal models through predominant reduction of pathogenic IgG oxLDL compared to atheroprotective IgM antibodies, given the rather selective resistance of peritoneal B1 cells (42). Likewise, since BAFF is essential for the survival of B2 cells but not B1 cells (64, 124), BAFF inhibition seems a promising therapeutic strategy for the prevention of accelerated atherosclerotic events in the setting of lupus.

Aside from a very small study (69), no data so far is available on the effect of BAFF inhibition on lupus related atherosclerosis. Therefore, close observation of the atherosclerotic and CV status of SLE patients under anti-BAFF therapy is of great importance.

The role of distinct B cell subsets in human atherosclerosis both in general and lupus populations warrants further research efforts in order to clarify current contradicting outcomes. For instance, B2 cells refer to both follicular and MZB cells that are quite different in terms of function, suggesting potential diverse effects in atherosclerosis as well. Aside from the apparent atheroprotective role of IgM antibodies, the effect of IgG antibodies in atherosclerosis is still unclear. Distinction of the IgG subclasses and of the targeted antigen is of major significance in the clarification of IgG participation in

atherosclerosis. Implementation of mice immunization protocols resulting in attenuation of atherosclerotic lesions in human populations is also of great interest. Prospective cohort studies investigating the role of B cells, antibody levels, disease activity, atherosclerosis evaluation and CV events in response to therapeutic regimens could shed light on the underlying mechanisms. Overall, the involvement of B cells in lupus related atherosclerosis is a field of great interest in need of further and elaborate investigation.

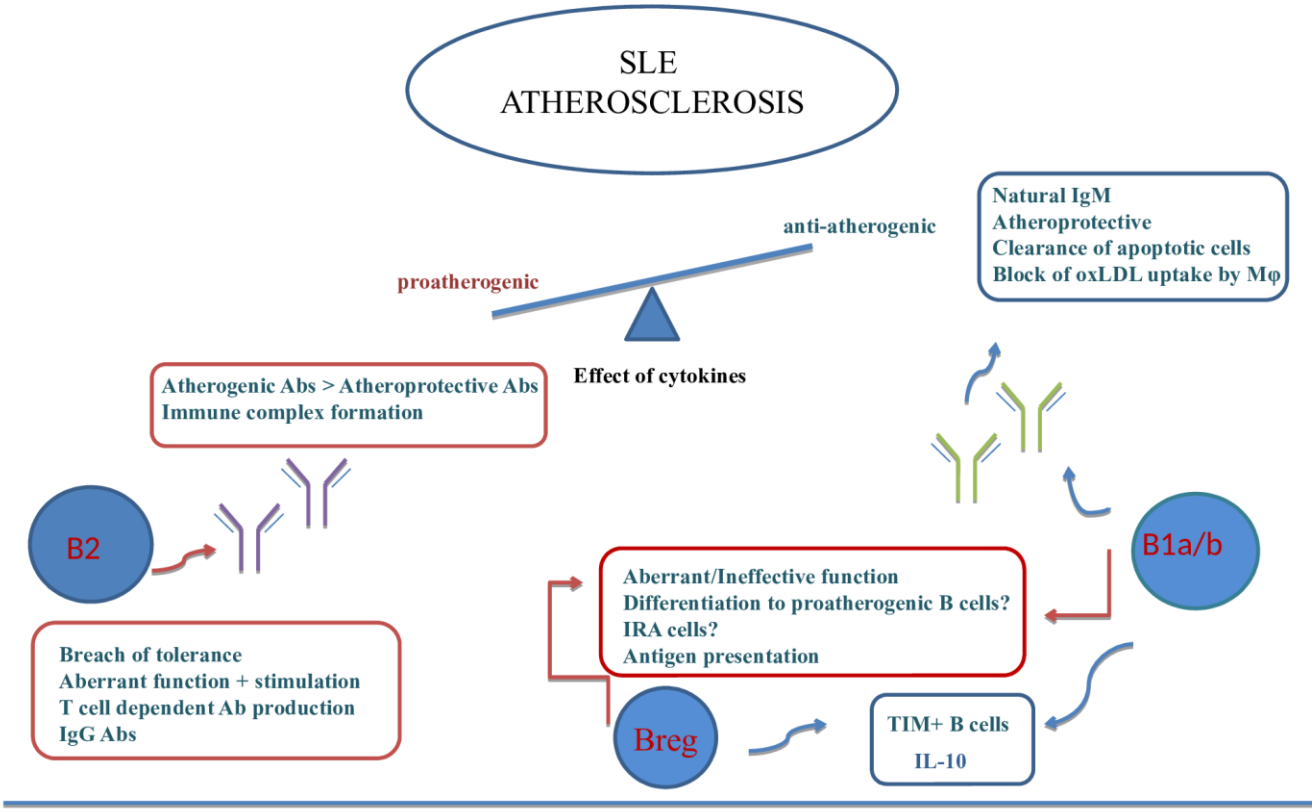

Figure 2. Potential mechanism of B cell behavior in lupus related atherosclerosis

Blue lines: Atheroprotective action **Abs**: antibodies

Red lines: Atherogenic action **Mφ:** Macrophages

Highlights box:

- B2 cells are mostly seen as atherogenic, while B1 cells as atheroprotective
- Natural IgM antibodies display mostly atheroprotective properties- IgM anti-PC prevalence is lower in SLE patients compared to healthy populations
- BAFF-R inhibition results in atheroprotection in experimental models
- BAFF excess in murine models is atheroprotective, while the opposite associations have been observed in general and lupus human populations
- B cell targeted therapy is already in use in lupus patients, though its effects on lupus related atherosclerosis are still unidentified

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