The Interplay of Lipoproteins and Innate Immune Responses in Systemic Lupus Erythematosus and its Role in Premature Cardiovascular Disease.

by

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Dedication

Thank you to all my family for filling my life with love and support. Without you, this would not have been possible.

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List of Abbreviations

1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC)

22 amino acid sequence in ETC-642 (22A)

3-amino-1,2,4-triazole (3-AT)

3-chlorotyrosine (3-ClY)

3-nitrotyrosine (3-NY)

Activating Transcription Factor 3 (ATF3)

Apolipoprotein (apo)

ATP Binding Cassette A1 (ABCA1)

ATP Binding Cassette G1 (ABCG1)

Body Mass Index (BMI)

Cardiovascular Disease (CVD)

Chloroquine (CQ)

Cholesterol Efflux Capacity (CEC)

Cholesteryl Esters (CE)

Cl-Amidine (Cl-Am)

Control (Ctrl)

Coronary Artery Disease (CAD)

Diphenylene Iodonium (DPI)

Endothelial Nitric Oxide Synthase (eNOS)

Endothelial Progenitor Cell (EPC)

Erythrocyte Sedimentation Rate (ESR)

First Apoptosis Signal (Fas) ligand (FasL)

Genome-wide Association Study (GWAS)

Granular Antimicrobial Peptide (gAMP)

High-density Lipoprotein (HDL)

Histone Deacetylase 1 (HDAC1)

Inducible Nitric Oxide Synthase (iNOS)

Interleukin (IL)

Intermediate-density Lipoprotein (IDL)

Krebs Ringer's Phosphate Glucose (KRPG)

Lectin-like Oxidized Low-density Lipoprotein Receptor 1 (LOX1R)

Lipopolysaccharide (LPS)

L-NG-monomethyl-L-arginine (L-NMMA)

Low-density Granulocytes (LDG)

Low-density Lipoprotein (LDL)

Myeloperoxidase (MPO)

Nicotinamide Adenine Dinucleotide Phosphate (NADPH) Oxidase (NOX)

Neutrophil Elastase (NE)

Neutrophil Extracellular Trap (NET)

Nitric Oxide (NO)

Normal-density Granulocytes (NDG)

Nuclear Factor Kappa B (NFκB)

Oxidized High-density Lipoprotein (oxHDL)

Oxidized Low-density Lipoprotein (oxLDL)

Paraoxonase (PON)

Pattern Recognition Receptors (PRRs)

Peptidylarginine Deiminase 4 (PAD-4)

Phorbol 12-myristate 13-acetate (PMA)

Plasmacytoid Dendritic Cell (pDC)

Reactive Nitrogen Species (RNS)

Reactive Oxygen Species (ROS)

Reverse Cholesterol Transport (RCT)

Scavenger Receptor (SR)

Single Nucleotide Polymorphism (SNP)

Sphingomyelin (SM)

Sphingosine 1-phosphate (S1P)

Superoxide Dismutase (SOD)

Systemic Lupus Erythematosus (SLE)

Systemic Lupus Erythematosus Disease Activity Index (SLEDAI)

Toll-like Receptor (TLR)

Transforming Growth Factor Beta (TGF-β)

Triglycerides (TG)

Tumor Necrosis Factor (TNF)

Type I Interferon (IFN-I)

Tyrosine (Y)

Vascular Smooth Muscle Cells (VSMC)

Very Low-density Lipoprotein (VLDL)

Abstract

Systemic lupus erythematosus (SLE) is an autoimmune syndrome that primarily affects women and leads to significant internal organ damage. Patients with SLE exhibit a high risk for premature atherosclerotic cardiovascular disease (CVD) which is not fully explained by traditional risk factors (e.g. smoking, age, and hyperlipidemia). As both SLE and CVD are chronic inflammatory diseases associated with abnormal lipoprotein and myeloid cell responses, certain overlapping, not yet elucidated mechanisms may explain SLE susceptibility to CVD. It is therefore necessary to establish specific biomarkers for CVD risk in SLE patients and further elucidate the interplay between the aberrant innate immune system and lipoprotein biology present in SLE atherosclerosis. Recent evidence implicates abnormal lipoprotein activity as an important factor of accelerated atherosclerosis in SLE, but the mechanisms leading to this alteration and the impact that it has on immune responses in the plaque remains to be determined. Oxidation of high-density lipoprotein (oxHDL) could explain this altered activity and act as a biomarker of SLE CVD as it is already associated with CVD in the general population and linked to deviant pro-atherogenic innate immune responses.

We hypothesized that the chronic oxidative environment characteristic of SLE promotes pro-atherogenic alterations to HDL. We further posited that neutrophil extracellular trap (NET) formation, which is enhanced in SLE, significantly contributes to HDL oxidation. Finally, we predicted that oxidized SLE HDL skews macrophages toward pro-inflammatory pathways, thereby promoting vascular damage.

We demonstrated that lupus patients had high levels of oxHDL and impaired HDL-associated vasoprotective activity. The pattern of oxidation was consistent with alterations previously reported to be pro-atherogenic in the general population and with dysfunctional HDL activity. Because SLE plasma was also highly oxidized, this indicated that the site of oxidation was in the circulation, rather than in tissue-resident atherosclerotic plaques as is typically reported in the literature. Indeed, we demonstrated that the oxidative machinery externalized in NETs induces a pattern of HDL oxidation associated with CVD. Furthermore, when we suppressed NET formation in lupus-prone mice *in vivo*, levels of HDL oxidation significantly decreased. We then assessed the pro-inflammatory effects of lupus HDL on macrophages.

Control macrophages exposed to SLE HDL displayed induction of nuclear factor kappa B (NFκB) activation and synthesis of pro-inflammatory cytokines. While healthy, control HDL blocked toll-like receptor (TLR)-induced inflammatory cytokine production, SLE HDL failed to abrogate this inflammation. This was linked to an impaired ability of SLE HDL to activate the anti-inflammatory, transcriptional repressor activating transcription factor 3 (ATF3), compared to healthy HDL. This effect was dependent upon SLE HDL binding the scavenger receptor lectin-like oxidized low-density lipoprotein receptor (LOX1R). However, when macrophages were cultured with SLE HDL in the presence of the HDL mimetic ETC-642, the anti-inflammatory status of the macrophages was restored. Administration of ETC-642 to lupus-prone mice, *in vivo*, decreased their inflammatory cytokine profile and enhanced ATF3 mRNA levels.

This novel identification of a specific oxidation pattern in SLE HDL and plasma should be examined as a non-traditional, CVD risk factor in a larger lupus cohort to monitor its association with CVD risk. These studies have identified two innate immune response pathways that may contribute to cardiovascular risk and amplification of inflammation in SLE. Enhanced NET

formation generates the oxidative species required for HDL oxidation. The oxHDL, in turn, stimulates pro-inflammatory responses in macrophages and blocks anti-inflammatory pathways. As such, therapies that target the high NET formation in SLE or that block the interaction of oxHDL with macrophages should be explored.

Chapter 1

Introduction

Systemic lupus erythematosus:

A. Definition, Demographics and Clinical Features:

SLE is an autoimmune syndrome of unclear etiology. It affects approximately 5 million people worldwide, primarily women and people of African, Hispanic and Asian descent [1, 2]. It is a multi-systemic condition that can cause significant tissue damage, primarily to the kidneys, skin, brain, vasculature and joints [3-5].

One of the key features of SLE is the presence of auto-antibodies that recognize nuclear components. Indeed, anti-nuclear antibodies are observed in more than 95% of SLE patients [6]. These include antibodies against double-stranded (ds)DNA, and against RNA-binding proteins such as Sm, Ro, La, and ribonucleoprotein (RNP) [6-12]. Because these auto-antibodies target intracellular components, it is considered that aberrant cell death and/or defective clearance of debris promotes the modification and externalization of auto-antigens that, in a predisposed individual, leads to loss of tolerance and unregulated inflammation. Indeed, lupus patients exhibit a number of clinical and subclinical markers indicative of a chronic inflammatory environment including elevated levels of various key cytokines, particularly type I interferons (IFN-I) [4, 13-19].

Patients with SLE have an increased risk of premature CVD. This is particularly striking in young SLE patients (35-44 years old), where the cardiovascular risk is approximately 50-fold [20, 21]. While new medications have improved the quality of life for SLE patients and increase

overall survival rates, cardiovascular disease (CVD) is still one of the major causes of death in SLE, and its incidence and prevalence have not decreased [22-24]. Importantly, this enhanced CVD risk cannot be explained by traditional risk factors (e.g. dyslipidemia, smoking, obesity), suggesting that other factors characteristic of lupus play a prominent role in CVD and need to be elucidated [21, 25-27].

B. Mouse Models of Lupus:

Both spontaneous and genetically modified mouse strains exist which develop a lupus-like phenotype. However, these mice do not completely reflect lupus pathogenesis as mice in general show different antibody isotypes, low levels of peripheral blood neutrophils compared to humans, and a relative protection from CVD [28, 29]. Nevertheless, mice are useful to explore several putative mechanisms associated with human lupus, with some of the most utilized strains summarized below:

New Zealand Black/New Zealand White F1 [(NZB×NZW) F1] Hybrid

The New Zealand Black and New Zealand White mice were one of the first strains to be studied as a spontaneous autoimmunity model [30]. When these strains were back-crossed, the resulting (NZB×NZW) F1 progeny showed an even stronger lupus-like phenotype. (NZB×NZW) F1 mice develop nephritis, anti-dsDNA antibodies, IFN-I response, with a strong sex-bias [30]. The disease progresses relatively slowly in these mice, with an average life span of 35 weeks for females and 58 weeks for males.

NZM2328 (NZM) Mice:

NZM2328 (NZM) mice, developed from numerous (NZB×NZW) F1 back-crosses, show a prominent lupus phenotype [31]. NZM mice develop nephritis, anti-DNA antibodies, accelerated

NETosis, a strong sex-bias and IFN-I signature [16, 32-34]. Proteinuria appears at 24 weeks and most mice die of renal disease by 30 weeks [32, 35].

Other Models:

First apoptosis signal (Fas) signaling is important for the negative selection of auto-reactive lymphocytes during their development. Mice deficient in *Fas* (MRL/lpr) or *FasL* (generalized lymphadenopathy [gld]) are also present in the lupus literature. Though they do possess auto-reactive B and T cells, they are considered less physiologically relevant models [30, 36-39]. These mice can demonstrate accelerated NETosis and an IFN-I signature, but do not show a strong sex-bias [40]. Other mouse strains with reported lupus-like phenotypes are abundant in the literature, but the mice mentioned above are the most commonly used for SLE-related studies.

C. Pathogenesis:

I. Genetic and Gender Links

Certain genetic factors appear to predispose people to SLE. Indeed, individuals are approximately 24 times more likely to have lupus if they possess a sibling with lupus [41]. A number of genome-wide association studies (GWAS) have been performed on SLE patients of various ethnic backgrounds. Single nucleotide polymorphisms (SNPs) linked to SLE can be found in genes related to antigen presentation, B and T cell activation, pattern recognition receptor (PRR) signaling, DNA repair/digestion mechanisms, NFκB and IFN-I signaling and cell debris clearance [42-78]. This suggests that both aberrant adaptive and innate immune response pathways are important in SLE pathogenesis.

As with many autoimmune disorders, gender also appears to play a significant role in SLE. While male lupus patients exhibit increased organ damage and mortality compared to lupus

females, women are particularly prone to SLE [79, 80]. This may be due to aberrant immune cell responses downstream of estrogen signaling [81-88]. Additionally, a number of SLE susceptibility genes are present on the X chromosome, which is more transcriptionally active in lupus females compared to lupus males and control females [89, 90].

II. Adaptive Immune Responses in Systemic Lupus Erythematosus

Because of the syndrome's high association with auto-antibodies, lupus has classically been attributed to abnormal adaptive immune responses. The bone marrow, as a site of B cell genesis and maturation, appears to be significant to lupus pathogenesis. In lupus bone marrow, neutrophils generate high levels of IFN-I, B-cell activating factor (BAFF), and a proliferation-inducing ligand (APRIL) [16, 91, 92]. These cytokines promote the survival, activation and longevity of auto-reactive immature and memory B cells [93, 94]. Despite enhanced B cell survival, lupus bone marrow nonetheless shows higher levels of apoptotic cells than healthy bone marrow [95, 96]. This is due to impaired debris clearance pathways in lupus [97-101]. As such, lupus B cells are reared in an environment which promotes their survival and exposes them, and other marrow-resident leukocytes, at an early stage to cell debris-derived self-antigens. Once outside the bone marrow, B cells are further exposed to self-antigens and induced, with the help of T cells, to differentiate into pathogenic auto-antibody-producing plasma cells. In addition to B cells, there is ample evidence that T cell subsets are aberrant in SLE and important contributors to lupus pathogenesis [102, 103].

Various cytokines enhance T cell survival and activation in SLE [104, 105]. Estrogen signaling also appears to promote aberrant B and T cell survival in SLE [84-86]. Lupus T cells demonstrate an altered T cell receptor physiology, leading to stronger T cell signaling [106-109]. High plasma levels of soluble co-stimulatory factors may also strengthen T cell activity in SLE [110]. These responses trend towards inflammatory rather than anti-inflammatory pathways due to the high ratio of

interleukin (IL)-17 producing T helper (Th) cells to regulatory Th cells in SLE [111-114]. On the other hand, there is evidence that lupus pathogenesis is linked to T cell anergy and decreased IL-2 production [115-118]. More recent evidence implicates abnormalities in other T cell subsets, including T follicular helper cells [102, 103]. Abnormal B and T cell responses seen in SLE not only contribute to idiopathic SLE pathogenesis, but may contribute to the generation of the inflammatory cytokines and auto-antibodies linked to lupus-related atherogenesis, as discussed below.

III. Innate Immune Responses in Systemic Lupus Erythematosus

The contributions of the innate immune system to SLE pathogenesis were long underappreciated, but have surfaced as significant mediators of disease inflammation and promoters of autoimmunity. Most research on SLE innate immune signaling revolves around the aberrant processing and detection of self-nucleic acids within dead cell debris [42, 47-51, 119, 120]. Numerous mechanisms exist in healthy individuals in order to stimulate the quick, anti-inflammatory phagocytosis and digestion of cellular remnants [121-126]. This keeps self-components from interacting with PRRs. In lupus patients, however, these pathways are defective. Lupus serum factors and aberrant phagocyte cell surface marker expression is linked to the impaired removal of dead cell debris [59, 76, 77, 100, 101, 127-130]. Because of this, lupus plasma and serum show high levels of cell fragments [131-136]. Not only do lupus patients have high levels of exposed self-antigens in the circulation, they also appear to have aberrant means of detecting this material. Immune complexes, for example, form when auto-reactive antibodies bind to complement proteins and undigested nucleic acids. These structures are recognized by highly expressed lupus activating Fc gamma receptors, are internalized, and, rather than being digested, are transported to endosomal, nucleic acid-sensing TLRs [137-144]. This process initiates a series of highly inflammatory responses that results in tissue damage.

The activation of nucleic acid-binding PRRs, in particular, will induce an anti-viral response typified by strong IFN-I signaling. Indeed, the risk for SLE is enhanced by SNPs in interferon response factor genes, and increased IFN-I signatures are associated with lupus flares and organ damage [15, 45, 52-54, 56, 145-150]. When IFN-I is used as a therapy for cancer and chronic infections it can induce a lupus-like disease [151-155]. Furthermore, NZM lupus-prone mice that lack IFN-I receptor are protected from many lupus characteristics including vascular pathologies, abnormal B cell populations, renal disease, and auto-antibody levels [16, 34, 35]. Lupus innate immune cells appear to be the main producers of this IFN-I and significant to SLE pathogenesis [156].

Lupus monocytes demonstrate an inflammatory phenotype ($CD14^+$, $CD16^+$), are strong responders to immune complexes, and produce large amounts of oxidative species, IL-6, TNF, and IFN-I [157-166]. This enhanced cytokine production may be due to the increased histone H4 acetylation within these genes in lupus [162, 167]. SLE monocytes appear to accelerate adaptive immune activity through the enhanced production of CD40 and BAFF [168, 169]. Their capacity as antigen presenting cells, however, increases when cultured with IFN-I or SLE serum, pushing them towards dendritic cell differentiation [170-172].

Lupus dendritic cells produce high levels of IFN-I, TNF and IL-6 [159, 170, 173-176]. Non-myeloid, plasmacytoid dendritic cells (pDCs) are particularly inflammatory, especially when stimulated with immune complexes and NETs (described below) [175-178]. Exaggerated pDC activity has been linked to both lupus nephritis and skin lesions [179-181]. Lupus dendritic cells may be potent lymphocyte activators as they express higher levels of MHC II, CD86, APRIL and BAFF [168, 171, 174, 180, 182-185]. Lupus monocytes which differentiate instead into macrophages also appear to have a pathogenic signature, though by which mechanisms is not clearly understood.

Lupus macrophages have primarily been linked to kidney disease [186, 187]. When the kidneys of MRL/lpr mice were injured via an ischemia/reperfusion model, the mice failed to promote the production of repair-associated macrophages (M2, described below) and instead an inflammatory, damaging macrophage phenotype (M1, discussed below) persisted [188]. The pro-inflammatory nature of lupus macrophages may be linked to their increased inflammasome activity [189, 190]. Additionally, mutations in the exonuclease TREX1 are associated with lupus and TREX1 deficient macrophages exhibit inflammatory cytokine production and enhanced antigen presentation activity [191, 192]. Though not well characterized, many of these macrophage inflammatory pathways may also promote SLE atherogenesis [193-198].

While aberrant nucleic acid-sensing PRR signals are significant to lupus pathogenesis, not a lot of work has demonstrated how the SLE innate immune cells mentioned above respond to lipoproteins. What is known is that of the receptors that appear to interact with lipoproteins, SLE shows altered activity and expression of TLR-2 and TLR-4, and patients possess auto-antibodies against certain scavenger receptors [119, 199-209]. This gap in SLE research is not insignificant given the high association of SLE with CVD.

Due to the apparent relationship between SLE pathogenesis and dead cell debris, extensive research has been conducted on cell death pathways in lupus. Leukocytes and non-immune cells do show a propensity for cell death in SLE, which was initially only attributed to apoptosis [210-214]. However, as apoptosis is normally considered an anti-inflammatory response, and as not all lupus patients exhibit impaired clearance activity, it was necessary to identify additional mechanisms by which modified self-epitopes became readily exposed to the periphery. Within the last decade, neutrophil extracellular trap (NET) formation was identified as one of the putative mechanisms.

a. Neutrophil Extracellular Trap Formation

NET formation was initially described as an innate immune response performed by granulocytes to kill or inactivate bacteria [215]. The process is distinct from both apoptosis and necrosis, is caspase-independent, and is characterized by the decondensation of chromatin material and mixing of nuclear and cytoplasmic contents (particularly granular antimicrobial peptides [gAMPs]) prior to extrusion of this material from the cell as a meshwork of chromatin and gAMPs [216]. These structures can then surround the pathogenic material, be it a protozoa, bacteria, virus or fungi, and mediate either its destruction, via cytotoxic gAMPs (myeloperoxidase [MPO], LL-37, neutrophil elastase [NE]), or mark the pathogen for phagocytosis [215-219]. A number of intact pathogens and pathogen associated molecular patterns (lipopolysaccharide [LPS], zymosan) can induce NETosis [215, 216, 220, 221]. Sterile stimuli include activators of calcium signaling (phorbol myristate acetate [PMA] and calcium ionophore [A23187]), certain cytokines (IL-1β, -8, TNF), integrin stimulation (Mac-1) and auto-antibodies or immune complexes [177, 178, 215, 216, 222-225]. Though the precise mechanisms of NET formation have yet to be fully elucidated and may be stimuli-specific, a few pathways do appear to be necessary for NETosis. Stimuli activate the granulocyte's oxidative machinery and ROS production. Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) and superoxide dismutase (SOD) produce hydrogen peroxide (H_2O_2) to release NE from an azurophilic gAMP complex, then MPO activates NE's proteolytic activity and migration to the nucleus (Figure 1-1) [226]. In the nucleus, NE cleaves histone H4, promoting the decondensation of DNA [227]. Another enzyme that appears important for DNA decondensation in NETosis is peptidylarginine deiminase (PAD)-4 [228, 229]. Nuclear PAD-4 modifies histone arginine residues into citrulline residues, resulting in a change from a positive charge to a more neutral charge thereby decreasing the DNA:histone electrostatic interaction [228,

[230]. Once NETs form and serve their function, they should be rapidly degraded as they contain many cytotoxic gAMPs and expose otherwise cryptic self-epitopes to the periphery. In healthy individuals, this is performed by phagocytic macrophages and an abundance of constitutively present serum nucleases. However, each of these mechanisms, from NET activation to clearance, appears to be dysregulated in lupus patients.

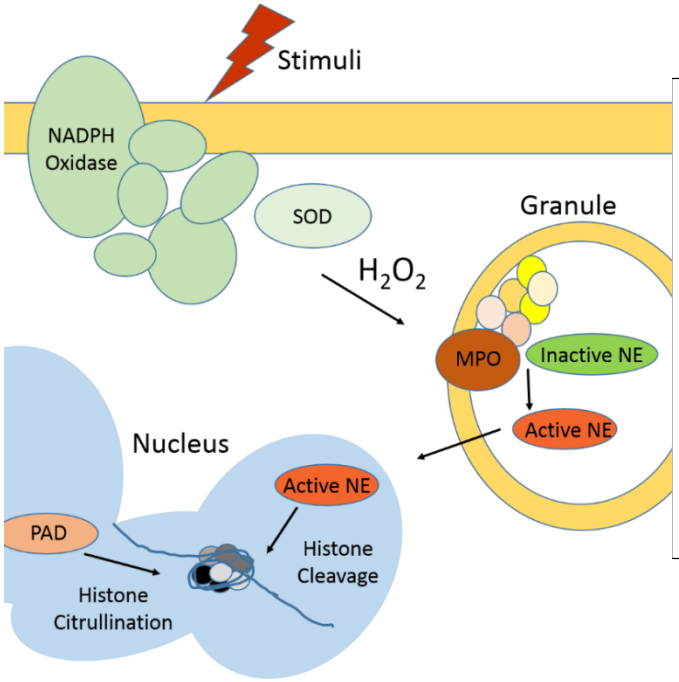


Figure 1-1. Mechanisms leading to nuclear decondensation prior to Neutrophil Extracellular Trap (NET) formation. Hydrogen peroxide (H_2O_2) from NADPH oxidase and superoxide dismutase (SOD) promotes myeloperoxidase (MPO), within a granule complex, to activate neutrophil elastase (NE). Activated NE travels to the nucleus and, in addition to peptidylarginine deiminase (PAD), modifies histones to promote nuclear decondensation and NET formation. Modified from Metzler et al. (2014).

A significant amount of work has been performed over the last five years linking aberrant NET formation and lupus. Granulocytes from SLE patients show an increased capacity to form NETs [178, 214]. In particular, lupus patients possess a unique granulocyte population of low-density granulocytes (LDGs), so named as they are identified and isolated from the peripheral blood mononuclear cell (PBMC) layer rather than the denser red blood cell layer after density gradient centrifugation [214, 231, 232]. Compared to healthy and lupus normal density granulocytes (NDGs), lupus LDGs show an enhanced capacity to synthesize inflammatory cytokines, including IFN-I, an increased, spontaneous formation of NETs, decreased phagocytic activity and an enhanced

expression of primary gAMPs [214, 231]. Importantly, NETs are potential sources of nuclear, modified auto-antigens and oxidative species [177, 233-236]. Both factors are significant to SLE pathogenesis and possibly SLE-related CVD [59, 76, 77, 166, 201, 237-246]. As NET degradation and anti-oxidant activity are impaired in SLE, this may promote extensive, chronic inflammation [98, 130, 247-250]. Therapies for blocking NET formation, namely through PAD inhibition, result in decreased IFN-I signatures and tissue inflammation in lupus-prone mouse models [33, 40].

Given the association of atherosclerosis with chronic inflammation, oxidative stress, and aberrant NETosis, pDC, monocyte and macrophage immune responses (described later in this thesis), many of the pathways discussed here appear to contribute to SLE-related CVD.

IV. Appearance of Clinical Features

Work from various groups indicates that the appearance of pathogenic auto-antibodies can appear in individuals predisposed to lupus many years before the onset of clinical disease [251]. The evolution from pre-clinical autoimmunity to clinically apparent disease is unclear, but it is likely that stochastic events and both genetic and environmental factors play crucial roles [251-254]. In addition to auto-antibodies, the lupus milieu is characterized by increased levels of cell death, innate immune cell activation, inflammatory cytokine production (IL-6, TNF, IFN-I), and adaptive immune cell expansion [17-19, 211, 251, 255, 256]. Acute manifestations of lupus are sometimes initiated after infection, ultra violet light exposure, or other types of environmental stresses [252-254, 257, 258].

With the initiation of more effective therapies, patients are managing to live for decades with this disease. Yet, the smoldering clinical presentation as well as the periods of flare and remission exposes patients to prolonged oxidative stress that promotes the development of end-stage chronic complications. Among them, atherosclerotic CVD is of particular concern given the clinical impact. It is believed that vascular damage starts to develop early on during the natural

history of lupus and is not identified in the majority of patients given the lack of established biomarkers [25, 251, 259].

D. Treatment:

Therapies for mild SLE typically include anti-malarials (e.g. hydroxychloroquine), non-steroidal anti-inflammatory drugs, or low dose corticosteroids. With increased severity, higher doses of corticosteroids are needed [260]. Yet, steroids have extensive metabolic effects that may contribute to CVD-risk [261-263]. Additionally, broad immunosuppressants including mycophenolate mofetil, methotrexate, cyclophosphamide, and azathioprine are often prescribed. Unfortunately, these can cause SLE patients to become severely immunocompromised, leading to death by infections [22-24, 264]. Targeted biologics are relatively new to SLE, with belimumab (anti-BAFF) as the only currently FDA-approved monoclonal antibody for the treatment of SLE [265]. The efficacy of anti-T cell, anti-B cell and anti-IFN therapies are under current clinical trial investigations [266-268]. Statins are also frequently prescribed to SLE patients, but it is unclear that they significantly decrease CVD risk in this patient population [269]. Indeed, while many of these therapies have improved the quality of life of SLE patients, to date no drug has been shown to decrease CVD risk in SLE. Furthermore, it remains unclear if the mechanisms implicated in premature CVD in lupus are similar to the ones observed in atherosclerosis within the general population (described below).

Atherosclerosis:

A. Definition, Demographics and Clinical Features:

Atherosclerosis is the build-up of plaque, composed of cholesterol and cellular infiltrates, in the sub-endothelial space of various major arterial trees. Atherosclerosis is a main contributor to the development of heart attacks and strokes. Specifically, coronary artery disease (CAD) is now considered the primary cause of mortality in men and women worldwide [270, 271]. The Framingham heart study, initiated in 1948, was instrumental in identifying significant risk factors for heart disease [272, 273]. "Classical" risk factors include sex (higher risk for males), age (higher risk after the age of 45), diabetes, smoking, raised body mass index (BMI), blood pressure, and an abnormal lipid profile (dyslipidemia): elevated total cholesterol, low-density lipoprotein cholesterol (LDL), triglycerides (TG), and low levels of high-density lipoprotein cholesterol (HDL) [274-276]. However, as discussed elsewhere in the text, these factors are largely not applicable to lupus patients as cardiovascular events in this disease typically occur in women, relatively early in life (35-44 years), and as such cannot be predicted by the Framingham risk equation [21, 25, 277].

Over the years, newer markers of altered lipoprotein composition or function have been linked to "typical" CVD: high apolipoprotein (apo) B:apoA-I ratios, modifications in the protein cargo of both HDL and LDL, and impairments in cholesterol efflux capacity (CEC). Additionally, atherosclerosis is known as a chronic inflammatory condition as inflammatory indicators are significant biomarkers for CVD in the general population: enhanced levels of high-sensitivity C-reactive protein, pro-inflammatory monocytes, IL-6 levels, and low endothelial progenitor cell (EPC) numbers [278-285]. As discussed later in the text, lupus patients also show an inflammatory signature, but mechanisms and markers of lupus-CVD still need to be established.

B. Mouse Models of Cardiovascular Disease

CVD is difficult to replicate in mouse models. One possible explanation for their protection from atherosclerosis is the high prevalence of HDL in mice compared to humans [286]. Many other mechanisms attributed to atherogenesis in humans are not observed in mice. For example, unlike humans, mice seem to have a bias towards more nitric oxide synthase (NOS) and less MPO activity, which affects the types of oxidative species generated and possibly macrophage polarization [287-289]. Additionally, most models require the mice to be on a special high fat, "Western" or "Paigen", diet to see full disease effects [290, 291]. Primary sites of atherogenesis also differ between humans and mice, with plaque development mainly in aortic regions in mice and in the coronary arteries in humans [286]. There are, however, the *LDLR-/-* and *apoE-/-* mice which are often used in CVD studies. These mutations and altered diet, however, create models of murine CVD which show extreme dysregulation of lipoprotein activity and composition that are not entirely physiologically relevant to human atherogenesis.

C. Pathogenesis

I. Genetic and Gender Links

In addition to environmental or behavioral risk factors (smoking, diet, exercise), there is a genetic component to CVD risk. Individuals with at least one parent who exhibits premature CVD are 2.6 (for males) to 2.3 (for females) times more likely to develop CVD [292, 293]. GWAS studies have identified SNPs in genes involved in cholesterol metabolism, vascular integrity, and inflammation as markers of CVD risk [293-318]. There is a significant increased risk for CVD in men and post-menopausal women [272, 273, 319]. This is different from SLE CVD where most cardiovascular events occur in younger, pre-menopausal, women [20, 21].

II. Lipoproteins

Several CVD risk factors relate to plasma lipoprotein composition and activity. The three general plasma lipoprotein classes are chylomicrons, LDL and HDL. Plasma lipoprotein particles are spherical or discoid phospholipid monolayer structures with the primary function of transporting insoluble cholesterols and TG through the body to where they are needed for energy, hormone, vitamin D, and bile salt synthesis [320, 321]. Structurally, the phospholipid surface of lipoproteins is embedded with proteins (apo, complement, lipases) and non-esterified (free) cholesterol that surrounds an internal core of TG and cholesteryl esters (CE).

Chylomicrons are the largest and least dense of the lipoproteins [320, 321]. Their major function is to transport their high TG content to adipose tissue for storage or active tissue for energy [320, 321]. In addition to LDL, two other subclasses of the "LDL family" exist and are differentiated based on their density and size: very low-, and intermediate-density lipoprotein (VLDL and IDL, respectively) [320, 321]. The "LDL family" of lipoproteins are smaller and denser than chylomicrons. LDL, in particular, is highly associated with CVD as it facilitates the removal of CE out of HDL and into cells [320, 321]. This supplies cells with energy, but can also induce inflammatory reactions and plaque formation, detailed below.

HDL is the smallest and densest lipoprotein, with the largest protein content. apoA-I, which makes up 70% of HDL's protein weight, mediates the vasoprotective pathway known as reverse cholesterol transport (RCT) [322]. In RCT, HDL's apoA-I binds ATP Binding Cassette (ABC)A1, ABCG1, and scavenger receptor (SR)B-I on cells to promote the removal of free cholesterol out of these cells. The cholesterol is eventually transformed into CE and delivered to the liver where it is excreted as bile acid. This mechanism is important as it prevents plaque and inflammatory macrophage foam cell development. The quantifiable ability of HDL to promote RCT is called the

CEC, and is inversely associated with CVD risk [323]. The interaction of apoA-I with ABCA1/G1 and SRB-I is also key to promoting anti-inflammatory signals in the vasculature [324-326]. Paraoxonase (PON) and sphingolipid sphingosine-1-phospate (S1P) carried within HDL stimulate additional anti-oxidative, vasoprotective mechanisms [320, 321, 327-331]. What is typically referred to as "HDL" can actually be divided into subclasses differentiated by their density and size: HDL_3 or HDL_2 (nomenclature based on density), or small, medium and large HDL (nomenclature based on size) [329]. While native, unmodified HDL possesses many vasoprotective functions, oxidative modifications to HDL can transform it into a pro-atherogenic lipoprotein, discussed below.

III. *Inflammation and Atherogenesis*

In a healthy cardiovascular system, the vascular endothelial cells prevent platelet adhesion by expressing prostacyclin and endothelial nitric oxide synthase (eNOS), which releases constitutively low amounts of nitric oxide (NO) to regulate vascular tone [285, 332-335]. In atherosclerosis, however, plaque formation begins with injury to the endothelium, leads to unresolved cell death and inflammation, and eventually thrombosis (Figure 1-2).

a. Initial Injury

Initial inflammatory vascular responses can increase adhesion molecule expression on endothelial cells [336, 337]. Adherent platelets will release inflammatory cytokines (IL-1β, TNF), chemokines, coagulants, and adhesion factors [338, 339]. Thinning of the endothelium exposes the intima layer's collagen and fibrinogen meshwork, which binds and traps platelets and lipids to promote further inflammation and leukocyte recruitment [340-343].

b. Attempt at Repair and Resolution

Endothelial progenitor cells (EPCs), found in the bone marrow and circulation, travel to sites of endothelium damage to differentiate and cover the exposed intima layer. EPCs also produce eNOS-derived NO, a process aided by HDL [285, 344-349]. Monocytes are one of the primary leukocytes recruited in an attempt to remove excess LDL and dead cells stuck in the intima layer [340-342]. Various scavenger receptors (e.g. SRB-I, LDL receptor) on recruited "classical", monocytes ($CD14^+$, $CD16^-$) will bind to and engulf LDL in an attempt to get rid of the excess lipoprotein [350, 351]. The monocytes then begin to transform into lipid-loaded foam cell macrophages.

Macrophages develop into an M2 phenotype if raised in an anti-inflammatory, Th2 cytokine environment (IL-4, -13, -10, transforming growth factor beta [TGF-β]) [352-355]. HDL also appears to promote M2 polarization [356]. The M2 macrophages then produce cytokines associated with vasorepair such as IL-10, TGF-β, platelet-derived growth factor (PDGF), vascular endothelium growth factor (VEGF) and arginase [357-359]. In lupus-prone MRL-lpr mice, for example, an inability to generate M2 macrophages is linked to defective repair and enhanced disease after ischemia/reperfusion injury [188]. Like monocytes, the macrophages attempt to remove the pathogenic LDL from the extracellular environment through scavenger receptors such as SRA, SRB-I, CD36, and lectin-like oxidized LDL receptor (LOX1R).

HDL is important for vasorepair mechanisms as it blocks foam cell formation through RCT, abrogates inflammatory and apoptosis signals via apoA-I, PON and S1P activity, inhibits reactive oxygen species (ROS), T cell and caspase activation, and induces eNOS- and activating transcription factor 3 (ATF3)-induced anti-inflammatory and re-endothelialization mechanisms [238, 324-326, 331, 348, 360-369]. Whether these vasoprotective functions are active in lupus patient is unknown, however.

c. Unresolved Inflammation and Cell Death

One non-traditional CVD risk factor is the high presence of "inflammatory"/intermediate monocytes ($CD14^+$, $CD16^+$), rather than "classical" monocytes [356, 370-376]. These non-classical monocytes produce higher levels of ROS, TNF, IL-12B, adhesion molecules, and antigen presentation factors [375, 377-381]. When monocytes differentiate in an inflammatory, Th1 cytokine environment (lipopolysaccharide, TNF, IFN-ɣ), it gives rise to the "classical", inflammatory M1 macrophages phenotype [352-355]. M1 macrophages produce high levels of ROS, reactive nitrogen species (RNS), IL-6, -1β, -12, and TNF [357-359]. Indeed, M1 macrophages persist in lupus-prone MRL-lpr mice after ischemia/reperfusion injury and have been linked by some groups to increased lupus organ damage [188]. This M1 macrophage persistence is also associated with unstable atherosclerotic plaques in CVD [353, 382].

Foam-cell macrophages are a potent source of oxidative enzymes that can modify apoB-100 in LDL [194, 383-391]. This modified LDL is very cytotoxic and inflammatory: it induces macrophage chemokine release, endothelial adhesion molecule expression and NFκB activation [392-394]. Similarly, certain modifications in HDL or apoA-I convert the lipoprotein into a pro-inflammatory form (oxHDL) [395-400]. Oxidized HDL is increased in CVD, promotes adhesion molecule expression, and impairs EPC function [401, 402]. Of the possible modifications, 3-chlorotyrosine (3-ClY) and 3-nitrotyrosine (3-NY) oxidized apoA-I is present in the plasma and atherosclerotic plaques of CVD patients [403-405]. MPO is the only enzyme capable of catalyzing the formation of 3-ClY, which leads to abrogation of RCT and increased inflammatory pathways [289, 391, 395, 403-410]. Part of this effect is due to oxHDL's decreased affinity for SRB-I and ABCA1 [403-405]. This also results in loss of ABCA1/G1-mediated anti-inflammatory and anti-apoptotic effects [326, 363, 411-413]. MPO has largely been associated with pro-atherogenic 3-NY oxidation, though NOS

may also play an important role in its formation [289, 384, 391, 395, 398, 404, 405, 407-410, 414-418]. While SLE patients exhibit many of these CVD-associated inflammatory pathways, it has been unclear if specific HDL post-translational modifications occur in SLE which modify the function of the lipoprotein and promote atherogenesis.

d. Role of Neutrophils in Atherosclerosis

CVD patients show elevated neutrophil counts that positively correlate with lipid core size in human carotid arteries, suggesting that these cells are important for atherogenesis [419-422]. Initially, neutrophils were rarely detected in plaques, but advancements in antibody specificity and two-photon excitation microscopy have increased our understanding of the role of these cells in CVD. Neutrophils have been detected in the shoulder, cap, and tunica adventitia layer of murine and human atheromas [421-424]. Recent evidence has also shown that luminal neutrophils can be activated by inflammatory platelets to undergo NETosis, visualized *in vivo*, and may play an important role in coagulation, pDC and macrophage inflammation, endothelial damage, and atheroma lesion size [178, 214, 425-432]. Additionally, mice deficient in *PAD-4*, an enzyme crucial in NET formation, or treated with PAD inhibitors are protected from thrombosis and show decreased atheroma lesion formation [425, 433, 434]. Neutrophils may be a source of HDL modification, as phagosomal-MPO can mediate 3-ClY and 3-NY oxidation [414, 435-437]. Whether NETosis is a significant contributor to HDL oxidation has yet to be explored as an important mechanism leading to SLE atherogenesis.

e. Late Stage Atherogenesis and Thrombosis

There is evidence of enhanced cell death occurring in the arterial wall intima layer due, in part, to their prolonged exposure to an inflammatory milieu. Specifically, cholesterol crystal deposited in endothelial cells, neutrophils, monocytes and macrophages can promote

inflammasome activation, cell death, and damage to the endothelium [196, 430, 438, 439]. This leads to the development of a necrotic lipid core in the intima layer (Figure 1-2). Other leukocytes eventually infiltrate into the atheroma, including T cells, dendritic cells, and mast cells. Dendritic cells exposed to oxidized LDL (oxLDL) become potent antigen presenting cells, leading to T and B cell auto-reactive responses against oxLDL and high anti-oxLDL auto-antibody levels in CAD patients [343, 440-443]. However, the significance of this remains to be better characterized. It should be noted that some auto-antibodies, particular IgM natural antibodies, may have vasoprotective capabilities [444-446].

With the build-up of inflammation and apoptotic debris in the atheroma, vascular smooth muscle cells (VSMCs) are recruited from the tunica media to the intima cap layer. These VSMCs produce a fibrous cap of collagen and elastin to "heal" the damaged endothelium and sequester the necrotic core from the vascular lumen [447, 448]. The plaque will bulge towards the lumen, increasing shear stress, promoting further endothelial cell damage and atherogenesis. Inflammatory macrophages, mast cell and particularly neutrophils (including NETs) will release proteases which digest the fibrotic cap, leading to plaque instability [407, 449-453]. Eventually the cap will rupture, leaking the necrotic core into the lumen [454]. This will activate the platelet coagulation cascade creating a thrombi or blot clot. Either a complete or partial blockage of blood circulation will occur and can lead to stroke or myocardial infarction.

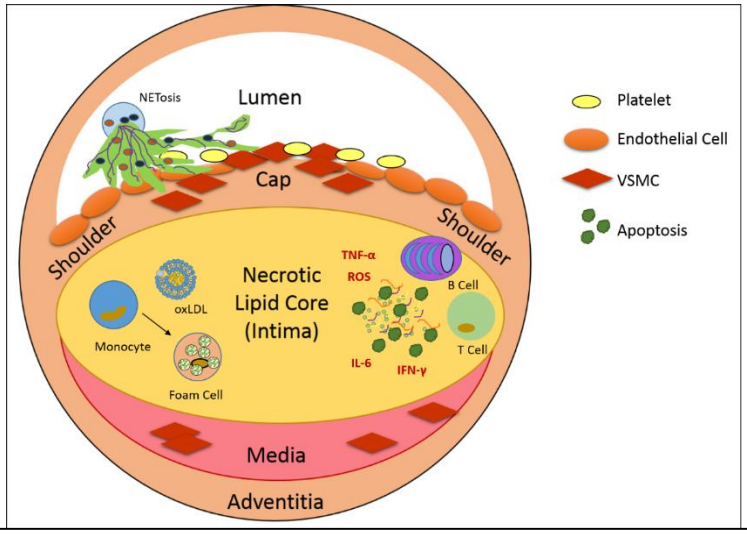


Figure 1-2. Plaque structure and cellular processes leading to atherothrombosis. Damage to vascular endothelial cells and platelet aggregation promotes monocyte and low-density lipoprotein (LDL) invasion into the intima layer. Monocytes engulf oxidized LDL (oxLDL) and differentiate into lipid-loaded foam cell macrophages. The necrotic core will build as cells die, leading to cryptic epitope exposure and B and T cell activation. Vascular smooth muscle cells (VSMC) from the medial layer will attempt to heal over the damaged endothelium. Platelet activation can enhance NET formation in the lumen of the vasculature. This prompts more endothelial cell death, digests the fibrous cap and eventually leads to the expulsion of the necrotic core material into the lumen (thrombus), leading to coagulation and blood vessel blockage.

IV. Lupus-related Cardiovascular Disease

The increase in cardiovascular risk in lupus is striking and cannot be predicted by the Framingham risk equation [20, 21, 25-27]. It has been proposed that SLE may represent the extreme phenotype of what is seen in "typical" atherogenesis, with an exaggerated inflammatory, immune reaction to endogenous substances (dead cell debris and lipoproteins), leading to enhanced cell death, followed by impaired clearance and repair mechanisms. Recently, the role of immune dysregulation in lupus premature CVD has been linked to a number of putative pathways.

a. Cytokine and Cellular Mechanisms of Lupus Atherosclerosis

Atherosclerosis is considered a chronic inflammatory disease, and both SLE and CVD in the general population share some common inflammatory pathways and abnormalities in innate and adaptive immune responses [6, 7, 15, 17-19, 214, 243, 455-460]. IFN-I, for example, may be significant to the pathogenesis of both diseases. An imbalance of vascular damage and repair is triggered by IFN-I. These cytokines promote enhanced apoptosis of EPCs and a dysregulated capacity for these cells to differentiate into mature endothelial cells [34, 231, 461-469]. Furthermore, IFN-I can promote the differentiation of macrophages into foam cells, as well as platelet activation leading to thrombosis enhancement [34, 470, 471]. Indeed, in the NZM lupus model, attenuation of IFN-I signaling improves vasculopathy and thrombosis risk, while administration of IFN-I (mimicking lupus flares) worsens endothelial function and promotes thrombosis, implicating these cytokines as key drivers of vascular damage in this disease [34, 468]. IFN-I signaling also enhances plaque formation in *apoE-/-* mice [34]. Additionally, in a human lupus cohort, IFN-I were identified as key associates with endothelial dysfunction and subclinical atherosclerosis in univariate and multivariate analyses [472]. Given the very high IFN-I levels in SLE, what may be potent pro-atherogenic mechanisms in CVD may be even more severe in SLE.

Another putative mechanism implicated in premature lupus and "typical" atherogenesis is NETosis. Lupus NETs are potent mediators of platelet activation, coagulation, endothelial cell damage and atherosclerosis [214, 231, 425-427, 430, 432, 451, 463, 464, 473-477]. NETs taken up by pDCs promote high IFN-I production, which may explain the critical role of pDCs in *apoE-/-* atherosclerosis [177, 180, 181, 464, 478, 479]. NETosis may also contribute to the high levels of oxidative markers in SLE, but how this enhanced externalized oxidative machinery may modify lipoproteins in atherogenesis remains to be determined [238, 239, 247-250, 480, 481].

b. Dysfunctional HDL Activity

As mentioned, there is a lack of research concerning lipoprotein signaling in SLE and its implications in atherosclerosis. Most literature to date involves clinical reports on the presence of auto-antibodies against lipoprotein components in SLE (anti-oxLDL, apoA-I, cardiolipin, β2-glycoprotein I, and HDL) but their role in mediating vascular disease remains to be determined [244, 245, 248, 482-489]. There is debate in the literature about whether IFN-I and the immune complexes which form with these auto-antibodies block LDL uptake, degradation and cell debris clearance [470, 473, 490-497]. What little work that has been done on the role of HDL in SLE inflammatory pathways is largely observational and does not identify specific pro-atherogenic signaling mechanisms [198, 498, 499].

The HDL of lupus patients is considered dysfunctional and "pro-inflammatory" [326, 367, 411, 500]. To date, lupus "pro-inflammatory" HDL has been quantified by the inability of HDL to block LDL oxidation [239, 501] This inability is higher in SLE than rheumatoid arthritis, in SLE patients with plaque, and correlates with inflammatory markers [239, 501]. Monocytes treated with SLE "pro-inflammatory" HDL show increased chemotaxis and TNF production [498]. In both lupus-prone mouse models and human patients, "pro-inflammatory" HDL activity correlates with low levels of the anti-oxidant enzyme PON [247, 502]. Decreased PON activity also correlates with high anti-apoA-I antibody titers, which increase with disease flares [248, 487, 502]. SLE HDL also demonstrates lower ABCA1/G1-mediated CEC than healthy controls or rheumatoid arthritis patients [503]. Hydroxychloroquine use has been associated with improved lipid profiles and decreased plaque thickness, but it may impair RCT [193, 262, 504, 505]. Interestingly, abrogation of IFN-I decreases levels of oxidized HDL in NZM lupus-prone mice [34].

D. Treatment:

Preventive measures are important in patients at risk for CVD or in patients that have experienced atherosclerotic events. These include smoking cessation, diet, exercise and control of blood pressure. In addition, many drugs are currently being used or explored as primary or secondary CVD prevention. Statins are frequently used in CVD [506-510]. Medications that increase HDL levels are being tested and it remains unclear whether they will be safe and useful in primary and secondary CVD prevention [511]. HDL mimetics are synthetic constructs made of apolipoproteins and lipids [423, 512]. As *in vitro* and *in vivo* animal models have shown an ability of mimetics to promote anti-atherosclerotic mechanisms, various HDL mimetics are in current clinical trials or were previously tested [339, 513, 514]. In an animal model of lupus atherosclerosis (*apoE-/-, Fas-/-* mice), treatment with an apoA-I mimetic showed lower auto-antibody production, indicating a potential therapeutic application in lupus CVD [499]. While many of these therapies have improved the lifespan of CVD patients, to date no drug has been shown to decrease CVD risk in SLE. There is a great need to further characterize the main pathways promoting atherogenesis in lupus as well as the most effective modulators of vascular risk in this disease.

Chapter 2

Mechanisms Leading to Enhanced HDL Oxidation in SLE

A. Introduction:

Lupus patients have an enhanced predisposition for early CVD, but neither clinical markers nor a well-defined innate immune pathway have truly been associated with this risk. As mentioned above, while others have indicated that HDL is dysfunctional in SLE and associated with subclinical atherosclerosis, it remains to be determined what specific modifications occur in lupus HDL to modify its function and what are the cellular sources of this modification.

We hypothesized that SLE patients possess increased levels of modified, dysfunctional HDL and that this modification would render the lipoprotein pro-atherogenic. To this effect, we quantified levels of 3-ClY and 3-NY in lupus HDL and plasma. We focused on these modifications as in previous studies they have been highly linked to the abrogation of HDL's vasoprotective capabilities such as CEC, anti-oxidative activity, and abrogation of inflammatory pathways [395, 397, 399, 401, 404]. Furthermore, we posited that NET formation, which is enhanced in SLE, exposes the active oxidative machinery (NOS, MPO, NOX) required for 3-ClY and 3-NY oxidation in humans and mice (Figure 2-1) [414, 416, 515-520].

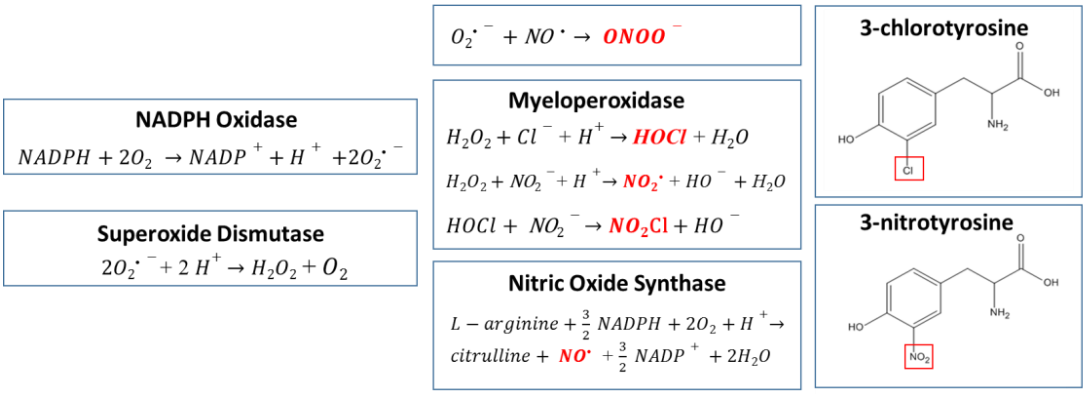


Figure 2-1. Enzymatic pathways leading to tyrosine oxidation. NADPH oxidase and superoxide dismutase generate the substrates used by myeloperoxidase and nitric oxide synthase. These enzymes then generate the reactive oxygen and nitrogen species (in red) capable of 3-chlorotyrosine and 3-nitrotyrosine modifications to apolipoprotein A-I, which are associated with cardiovascular disease.

B. Materials and methods

Subject Recruitment

Plasma samples were collected from SLE patients fulfilling the American College of Rheumatology diagnostic criteria for the disease [4]. Healthy controls (Ctrls) were recruited by advertisement. Study was approved by the University of Michigan and NIH IRBs. Lupus disease activity was quantified by the SLE Disease Activity Index (SLEDAI) [5]. Pregnant or lactating women and individuals with recent or current infections or liver dysfunction were excluded.

Mice

NZM2328 (NZM) breeding pairs were a gift from Dr. Chaim Jacob [34]. Balb/c mice were purchased from Jackson Laboratory (Bar Harbor, ME). Mice were bred and housed in a specific pathogen-free barrier facility at the University of Michigan. Female mice were euthanized at 26-weeks of age, before overt development of renal disease. Protocol was approved by University of Michigan's Committee on Use and Care of Animals.

Plasma HDL Isolation

HDL was isolated from human and murine plasma by buoyant-density sequential ultracentrifugation [521]. Protein concentration was estimated with Coomassie (Thermo Scientific, Rockford, IL). Samples were stored at -80 °C until analysis.

Quantification of Oxidized Amino Acids in Plasma, HDL, and apoA-I Peptides

Plasma proteins were precipitated and delipidated [521]; oxidized amino acids were quantified using isotopically labeled internal standards, $^{13}C_6$ tyrosine, $^{13}C_6$ 3-chlorotyrosine and $^{13}C_6$ 3-nitrotyrosine, by liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) with multiple reaction monitoring (MRM) MS/MS positive ion acquisition mode [522].

MRM Analysis of Oxidized Tyrosine-Containing apoA-I Peptides with LC/ESI/MS/MS

Plasma HDL samples were delipidated, and reduced with dithiothreitol (5 μM, Sigma-Aldrich, St. Louis, MO) before alkylation with iodoacetamide, (15 mM, Sigma-Aldrich) [521]. Samples were trypsin-digested and purified using solid-phase extraction C18 Sep-Pak columns (Waters Corporation, Milford, MA). Isotopically labeled oxidized (nitrated and chlorinated) peptides and native apoA-I peptides were spiked into samples following trypsin digestion. MRM analysis was performed with an Agilent 6490 Triple Quadrupole MS system equipped with an Agilent 1200 Infinity UPLC (Agilent Technologies, New Castle, DE) in positive ion mode [521].

Cholesterol efflux capacity (CEC) and MPO Quantification

CEC assay and MPO quantification were performed as described [521]. J774 cells were maintained in Dulbecco's modified Eagle's medium (DMEM)/10% fetal bovine serum (FBS, Life Technologies, Carlsbad, CA) [323]. To radiolabel cellular free cholesterol pool, cells were incubated with 1 μCi/ml [^{3}H] cholesterol (Perkin Elmer, Waltham, MA) in DMEM/ 1 mg/ml fatty acid-free

bovine serum albumin (FAFA, BSA) and 5 µg/ml ACAT inhibitor Sandoz 58-035 (Sigma-Aldrich) overnight. Cells were incubated with 0.5 mM 8-Br-cAMP to induce ABCA1 expression for 20 hours, then incubated with DMEM/FAFA with or without 2.8% apoB-depleted plasma for 4 hour at 37 ^{o}C and chilled on ice. Medium was collected and filtered, [^{3}H] cholesterol content of medium and cells was quantified, and fraction of total [^{3}H] cholesterol released into the medium was calculated [521].

HDL Proteomic Analysis

Blood was obtained in heparinized tubes and plasma was collected from 10 Ctrl and 10 SLE patients by Ficoll-Paque (GE Healthcare) density centrifugation. Plasma was separated by gel filtration chromatography using two Superose 6 (GE Healthcare) columns arranged in series on an AKTA fast protein liquid chromatography (FPLC) system (GE Healthcare) as described previously [523]. Fractions within the HDL peak were combined to generate pools representing large and small HDL. Pooled fractions were applied to a phospholipid binding resin to isolate HDL from abundant co-migrating plasma proteins and, after washing twice with 50 mM ammonium bicarbonate, lipoprotein bound proteins were digested with sequencing grade trypsin (1.5 µg/sample) overnight. Resulting peptides were reduced with dithiothreitol and carbamidomethylated with iodoacetamide prior to drying in a speedvac. Dried peptides were suspended in 0.1% trifluoroacetic acid and desalted using ZipTips (Millipore). Desalted peptides were dried and suspended in 20 µL of 0.1% formic acid. Samples were analyzed by ESI-MS/MS on an Orbitrap Elite mass spectrometer (Thermo Scientific) [523]. Peak lists were searched against the UniProtKB/Swiss-Prot database using the Mascot search engine and Scaffold (version Scaffold_2_04_00, Proteome Software, Portland, OR) was used to validate protein identifications.

Statistical significance of group comparisons, by spectral counting, was determined in GraphPad Prism (GraphPad, La Jolla, CA).

Induction and Purification of NETs and Co-culture Assays

Human Ctrl NDG and lupus LDGs were purified from peripheral blood, and murine neutrophils from bone marrow [33, 214]. Cells were plated at a density of 0.25 x 10^6 cells/cm^2 on tissue-culture plates in RPMI-1640 without phenol red (Life Technologies) for 3-ClY quantification, or in Krebs Ringer's Phosphate Glucose (KRPG) buffer (Sigma-Aldrich) for 3-NY quantification [214]. NDG were cultured in the presence or absence of 20 nM PMA (Sigma-Aldrich) for 3 hours (human), or 100 nM PMA for 5 hours (mouse) to induce NET formation [216]. As LDGs form NETs spontaneously, they were left unstimulated. To inhibit NET formation, 200 µM Cl-Amidine was added for the full incubation [33]. As NOX and MPO are required for NET formation but are also targets for the conditions where oxidation was inhibited, cells were allowed to form NETs for 1 hour before adding the following inhibitors: diphenylene iodonium (DPI, blocks NOX activity, 100 µM, Tocris, Bristol, UK), L-NG-monomethyl-L-arginine (L-NMMA, blocks NOS activity, 200 µM, Abcam, Cambridge, UK) and 3-amino-1,2,4-triazole (3-AT, blocks MPO activity, 10 mM, Sigma-Aldrich) [216, 225, 406, 524-526]. Inhibitors were replenished hourly during NET formation. NETs were isolated as described, using 100 U/mL DNase I (Roche, Branchburg, New Jersey) [189]. Supernatants resulting from the final spin contain NET-bound proteins and DNA. Ctrl, (negligibly oxidized) HDL (50 µg/mL) was incubated with these NETs in the presence or absence of L-NMMA, 3-AT and DPI for 30 minutes at 37° C. Relative abundance of HDL oxidation was calculated as fold change of each condition relative to HDL oxidation with NET formation.

Quantification of NOS and NOX in Human and Murine NETs

Immunoblot: To detect NOS and NOX externalization during NET formation, digested human and murine NETs and whole neutrophil pellets were harvested. NET proteins were precipitated with acetone; 50 µg of NET or whole cell pellet proteins were separated on a 10% SDS-PAGE gel. Proteins were transferred onto a nitrocellulose membrane, incubated in 5% bovine serum albumin (BSA) / phosphate buffered saline (PBS) 0.1% Tween-20 for 1 hour, and stained with goat anti-mouse p47 or p22 (1:200, Santa Cruz Biotechnology, Santa Cruz, CA), rabbit anti-eNOS or iNOS (1:100, Abcam) or anti-tubulin (negative control, 1:500, Sigma-Aldrich), followed by horseradish peroxidase-conjugated rabbit anti-goat (1:1000, Millipore, Temecula, CA) or goat anti-rabbit (1:5000, Jackson ImmunoResearch, West Grove, PA) secondary antibodies. Densitometry was performed with ImageJ (Bethesda, MD).

Fluorescence microscopy: Human NDG and lupus LDGs and mouse neutrophils were seeded onto poly-L-lysine (Sigma-Aldrich) coated coverslips, stimulated with PMA as above, or left unstimulated (for LDGs) before fixing in 4% paraformaldehyde and staining with goat anti-p47 or p22 (both 1:50 dilution, Santa Cruz), or rabbit anti-iNOS or eNOS (both 1:20 dilution, Abcam) for 1 hour at 4 °C, followed by secondary fluorochrome-conjugated antibodies (Jackson ImmunoResearch) and Hoechst 33342 (Life Technologies) [33, 214]. Coverslips were mounted with ProLong Gold Antifade (Life Technologies). Images were acquired on a Zeiss LSM510 META confocal laser-scanning microscope (Carl Zeiss Microimaging, Thornwood, NY) with a ×63 lens and quantified [33, 214].

Effect of Chloroquine on Neutrophils

Ctrl or lupus whole blood was incubated with 250 ng/mL chloroquine (Sigma-Aldrich, CQ) for two hours before neutrophils were purified as above [527]. Effect of CQ on NET formation was

quantified using SYTOX assay (Life Technologies), immunofluorescent staining and immunoblot [189]. To determine the effect of CQ on MPO, NOS and NOX activity, 1 x 10^6 neutrophils/mL, in either RPMI 1640 or KRPG with protease inhibitor cocktail (Roche), were homogenized at 35,000 rpm (Omni International, Kennesaw, GA) for three 10 second intervals on ice before treatment with or without 20 nM PMA for 30 minutes, then in the absence or presence of 250 ng/mL CQ for 30 minutes, and finally with or without 50 µg/mL HDL for 30 minutes. This solution was frozen and analyzed for 3-ClY and 3-NY oxidation.

In vivo Cl-Amidine Administration

PAD inhibitor Cl-amidine (Cl-Am) was synthesized [528]. Twelve-week old NZM female mice were administered daily subcutaneous injections of Cl-Am (10 mg/kg/day) or PBS (Life Technologies) for 14 weeks [33]. Isolated plasma and HDL were analyzed for 3-ClY and 3-NY oxidation [34].

Statistical Analysis

Pearson correlation coefficients were calculated between outcomes studied and patient characteristics. Multivariable linear models were used to explore significant predictors of the outcomes of interest. The method of best subsets with the R-squared selection criterion guided model selection process [521]. These models were also used to estimate and test differences between Ctrl and SLE groups. Skewed variables were *logarithm base 10* (log10) or natural log (ln) transformed to satisfy statistical assumptions. Normally distributed variables were not transformed. A p value <0.05 was considered significant. Analyses were conducted using SAS V.9.2 (SAS Institute Inc., Cary, North Carolina, USA) or GraphPad Prism (GraphPad). For Table 2-2, variables indicated with an "*" were transformed (natural log-scale) prior to correlation calculation. Values indicated with "**" are adjusted as follows: CEC (%): Adjusted for HDL

(n=50, R^2 =0.08); MPO (fmol/ml): Adjusted for Age, BMI, and LDL (n=49, r^2 =0.33); HDL-3-ClY: Adjusted for BMI (n=59, R^2 =0.10); HDL-3-NY: Adjusted for cholesterol (n=54, R^2 =0.34); Plasma-3-3-ClY: Adjusted for LDL (n=50, R^2 =0.21); Plasma-3-NY: Adjusted for LDL (n=50, R^2 =0.07).

	Healthy Control (Ctrl)	Lupus (SLE)
	n=20	n=40
Age (years)	47.5 ± 10.9	48.2 ± 13.5
Sex (males)	10.0% (2)	10.0% (4)
Body Mass Index (kg/m^2)	24.0 (6.6)	27.8 (12.2)
Cholesterol (mg/dL)	212.0 (58.0)	150.0 (87.0)
Triglycerides (mg/dL)	161.5 (89.0)	124.5 (99.0)
HDL (mg/dL)	57.5 (28.0)	52.0 (18.5)
LDL (mg/dL)*	128.1 ± 29.7	92.9 ± 39.6
ESR (mm/hr)	n/a	13.0 (13.0)
C-Reactive Protein (mg/L)	n/a	0.6 (0.7)
SLEDAI*	0	3.0 (6.0)
Steroid use*	0	42.5% (17)
Antimalarial use*	0	72.5% (29)
Statin Use*	0	22.5% (9)
Beta Blockers*	0	25.0% (10)
ACE inhibitor Use*	0	22.5% (9)
MPO (fmol/mL)*	326.7 (156.0)	425.5 (212.0)
Cholesterol Efflux Capacity (%)*	9.2 ± 1.6	7.8 ± 1.5

Table 2-1. Demographics of patients used for oxidation study. Statistically significant differences between groups noted with "*" ($p<0.05$). Normally distributed variables are displayed as mean ± standard deviation. Skewed variables are displayed as median (IQR). HDL= high-density lipoprotein; LDL= low-density lipoprotein; ESR= Erythrocyte Sedimentation Rate; SLEDAI= Systemic Lupus Erythematosus Disease Activity Index; ACE= Angiotensin-converting enzyme; MPO= myeloperoxidase. Duplicated from Smith et al. (2014).

C. Results

Patients Characteristics

Ctrl and lupus patients did not differ in most demographic characteristics (Table 2-1). SLE patients also exhibited total cholesterol, BMI, TG, LDL, and HDL levels within the normal range for CVD risk factors mentioned previously [274-276]. Levels of LDL were significantly lower in SLE compared to Ctrl, possibly associated with the prevalent use of statins (22.5%). SLE statin use correlated with elevated plasma 3-ClY levels, whereas antimalarial use was associated with higher plasma 3-NY content (Table 2-2). SLE patients displayed significantly higher levels of plasma MPO compared to Ctrls (Table 2-1). Elevated MPO levels significantly correlated with a higher ESR and low LDL levels (Table 2-2). Oxidation levels did not correlate with SLEDAI (Table 2-2).

CEC is Impaired in SLE

The ability of HDL to promote cholesterol efflux from macrophages (CEC) is a metric of HDL function and has a strong inverse association with CVD [408, 503, 527]. Plasma from patients with SLE displayed significantly diminished CEC when compared to Ctrl plasma (Table 2-1, Figure 2-2A). These results persisted after adjustment (listed in the Statistical Analysis section) for significant predictors of CEC (Table 2-2) and support previous indications that dysfunctional HDL present in SLE leads to impaired CEC and may promote pro-atherogenic responses [503].

Chlorinated and Nitrated HDL is Increased in SLE

Impaired CEC has been associated with HDL modifications such as 3-ClY and 3-NY [401, 408]. We quantified levels of 3-ClY, a highly specific product of MPO, and 3-NY, a product of MPO and other RNS-producing enzymes, in SLE and Ctrl isolated HDL and in total plasma [522, 529]. SLE HDL displayed 2-fold higher median levels of 3-NY (Figure 2-2B) and 121-fold higher

median levels of 3-ClY (Figure 2-2C), compared to Ctrl HDL even after adjustment (listed in the Statistical Analysis section). As previous studies have identified that chlorination of tyrosine (Y) residue 192 (Y-192) within the apoA-I protein most directly associates with impaired CEC, with six other tyrosine residues in apoA-I (Y-18, -29, -100, -115, -166, and -236) as other potential sites of oxidation, we determined if regiospecific nitration and chlorination patterns occur in SLE [408, 409, 529]. Levels of 3-NY HDL oxidation were highest at Y-192, 115, 100, 29 and 18 in SLE (Figure 2-2D). The highest levels of MPO-dependent 3-ClY HDL oxidation in SLE samples were observed at Y-192, -115 and -18 (Figure 2-2E), when compared to Ctrl samples. Taken together, this data suggests that regiospecific modifications to HDL by MPO and RNS-producing enzymes, specifically at Y-18, -115, and -192, may be of particular interest in the context of SLE-associated CVD.

When we examined correlations between levels of 3-NY and 3-ClY in SLE HDL, they significantly positively correlated with each other (Figure 2-2F), suggesting that these oxidative modifications are generated at similar sites in the body. There was no significant correlation between 3-NY and MPO levels (Figure 2-2G), but the levels of 3-ClY significantly correlated with plasma MPO levels (Figure 2-2H). This suggests that the RNS leading to HDL nitration are not primarily derived from MPO, but from another oxidative source. While Y-115 and -18 displayed the highest levels of oxidation in lupus, 3-ClY Y-192 showed the most significant positive correlation with plasma MPO levels (Figure 2-2I). Overall, these results support the notion that SLE patients possess high levels of oxidative species leading to HDL oxidation and impairment of lipoprotein function.

Pearson Correlations Among All Patients (n=60)						
	MPO*	**CE**	**HDL- Cl-Tyr***	**HDL- N-Tyr***	**Plasma- Cl-Tyr ***	**Plasma-N-Tyr**
Age (years)	r = -0.23, p=0.08	r = 0.11, p=0.39	r = 0.03, p=0.83	r = 0.07, p=0.61	r = -0.05, p=0.71	r = -0.02, p=0.89
Sex (males)	r = -0.21, p=0.11	r = -0.05, p=0.72	r = 0.21, p=0.12	r = 0.14, p=0.30	r = 0.08, p=0.54	r = 0.04, p=0.77
Body Mass Index* (kg/m2)	r = 0.20, p=0.13	r = -0.23, p=0.08	**r = 0.31, p=0.02**	r = 0.05, p=0.72	r = 0.18, p=0.18	r = 0.07, p=0.61
Cholesterol* (mg/dL)	r = -0.13, p=0.33	r = 0.14, p=0.31	r = -0.04, p=0.77	**r = 0.58, p<0.01**	r = -0.25, p=0.07	r = 0.23, p<0.10
Triglycerides* (mg/dL)	r = 0.11, p=0.44	r = -0.07, p=0.62	r = -0.14, p=0.32	r = 0.01, p=0.94	r = -0.02, p=0.90	r = -0.16, p=0.26
HDL* (mg/dL)	r = -0.25, p=0.08	**r = 0.28, p<0.05**	r = -0.15, p=0.28	r = 0.04, p=0.79	r = -0.17, p=0.22	r = 0.01, p=0.96
LDL (mg/dL)	**r = -0.44, p<0.01**	r = 0.18, p=0.22	**r = -0.29, p=0.04**	r = 0.12, p=0.41	**r = -0.46, p<0.01**	r = -0.27, p=0.06
Pearson Correlations Among Lupus Patients only (n=40)						
	MPO*	**CE**	**HDL- Cl-Tyr***	**HDL- N-Tyr***	**Plasma- Cl-Tyr ***	**Plasma-N-Tyr**
CRP* (mg/L)	r = 0.05, p=0.76	r = 0.18, p=0.27	r = 0.17, p=0.31	r = 0.11, p=0.52	r = 0.24, p=0.15	r = -0.16, p=0.34
ESR* (mm/hr)	**r = 0.36, p=0.02**	r = 0.06, p=0.71	r = -0.05, p=0.75	r = 0.19, p=0.26	r = 0.07, p=0.66	r = -0.14, p=0.40
SLEDAI	r = 0.14, p=0.39	r = 0.08, p=0.63	r = 0.15, p=0.34	r = 0.06, p=0.71	r = 0.15, p=0.36	r = 0.05, p=0.76
Steroid use	r= -0.09, p=0.59	r = 0.11, p=0.51	r = -0.20, p=0.22	r = -0.27, p=0.09	r = 0.11, p=0.49	r = 0.09, p=0.57
Antimalarial use	r = 0.03, p=0.83	r = 0.08, p=0.64	r = -0.08, p=0.62	r = 0.05, p=0.76	r = -0.20, p=0.23	**r = 0.32, p<0.05**
Statin use	r = -0.18, p=0.26	r = 0.13, p=0.42	r = -0.002, p=0.99	r = -0.01, p=0.95	**r = 0.32, p<0.05**	r = -0.12, p=0.48
Beta Blocker use	r = -0.07, p=0.66	r = 0.22, p=0.17	r = -0.15, p=0.36	r = -0.26, p=0.11	r = 0.16, p=0.32	r = -0.004, p=0.98
Ace inhibitor use	r = -0.06, p=0.70	r = 0.16, p=0.33	r = 0.09, p=0.59	r = 0.12, p=0.46	r = 0.01, p=0.96	r = -0.06, p=0.71

	Unadjusted Comparison				**Adjusted** Comparison**			
	Controls	**Lupus**	**p-value**	**Model R^2**	**Controls**	**Lupus**	**p-value**	**Model R^2**
CEC (%)	9.2 ± 0.3	7.8 ± 0.2	0.0022	0.15	9.2 ± 0.3	8.0 ± 0.3	0.0073	0.21
MPO (fmol/ml)*	5.7 ± 0.1	6.0 ± 0.1	0.0005	0.19	5.8 ± 0.1	6.0 ± 0.1	0.0198	0.4
HDL 3-chlorotyrosine*	1.4 ± 0.4	5.4 ± 0.3	<0.0001	0.51	1.5 ± 0.4	5.4 ± 0.3	<0.0001	0.56
HDL 3-nitrotyrosine*	2.5 ± 0.4	4.2 ± 0.3	0.0003	0.2	2.6 ± 0.3	4.1 ± 0.2	0.0002	0.49
Plasma 3-chlorotyrosine*	0.4 ± 0.3	3.3 ± 0.2	<0.0001	0.49	0.3 ± 0.3	3.3 ± 0.2	<0.0001	0.62
Plasma 3-nitrotyrosine*	3.4 ± 0.2	5.7 ± 0.1	<0.0001	0.64	3.3 ± 0.2	5.7 ± 0.2	<0.0001	0.63

Table 2-2. Correlations of lipoprotein characteristics and plasma oxidation with clinical features in all subjects and in patients with SLE only. Shaded boxes reach statistical significance ($p<0.05$). HDL = high-density lipoprotein; LDL= low-density lipoprotein; CRP= C-reactive protein; ESR = erythrocyte sedimentation rate; SLEDAI = SLE Disease Activity Index; ACE = angiotensin-converting enzyme; CEC= cholesterol efflux capacity; MPO = myeloperoxidase. Variables with "*" were transformed (natural log-scale) prior to correlation calculation. Values indicated with "**" are adjusted as stated in the Statistical Analysis section. Duplicated from Smith et al. (2014)

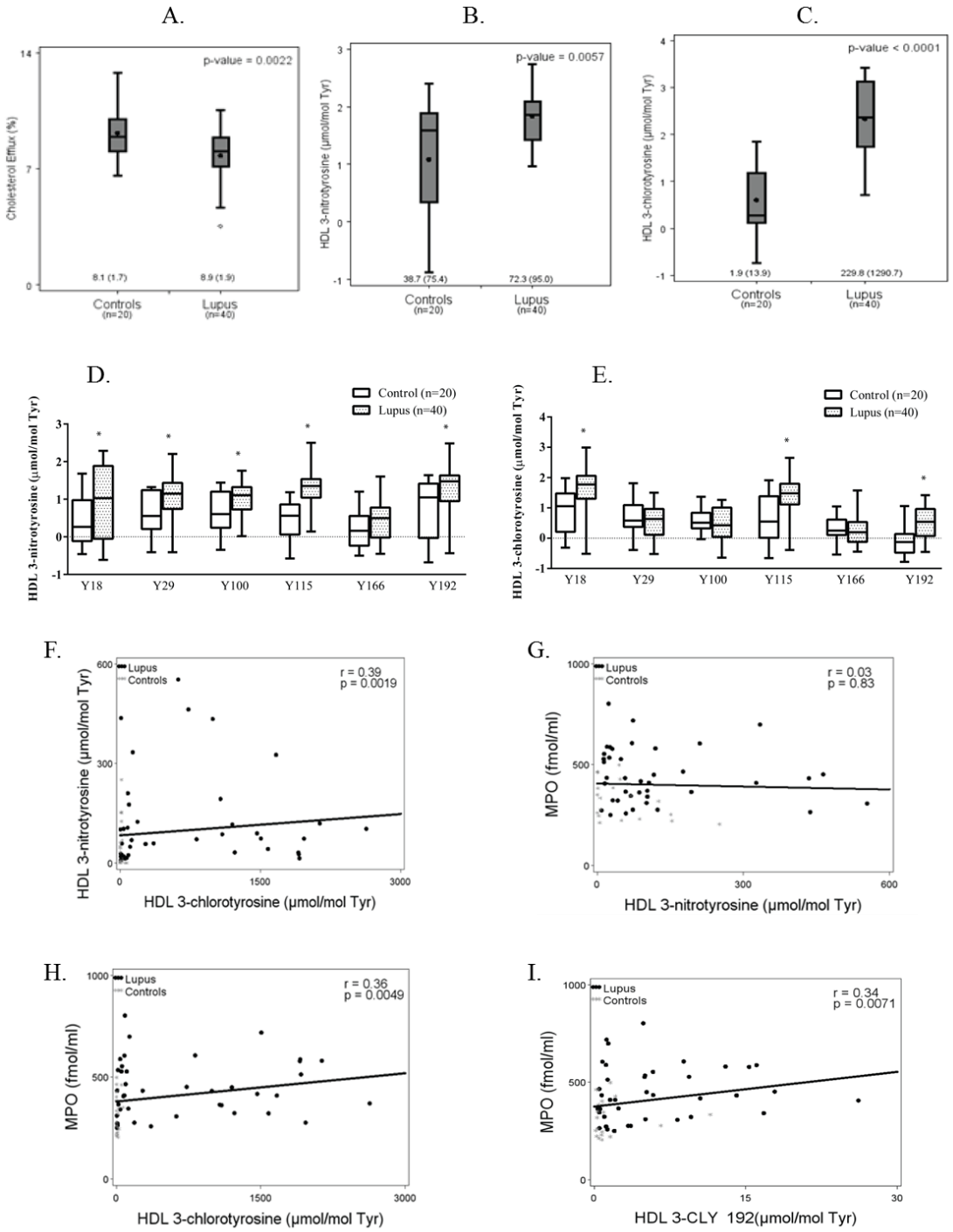


Figure 2-2. Plasma and HDL of SLE patients exhibit high levels of dysfunction and oxidation. (A) The cholesterol efflux capacity of plasma samples from Ctrl and lupus patients were examined in J774 cells. Levels of HDL (B) 3-NY and (C) 3-ClY were examined in control and lupus patients. The regiospecific tyrosine (Y) oxidation patterns of (D) 3-NY and (E) 3-ClY were determined for control and lupus HDL. Correlations between lupus and control (F) 3-NY and 3-ClY, (G) plasma MPO and 3-NY, (H) plasma MPO and 3-ClY, and (I) plasma MPO and Y192 3-ClY oxidation were determined. Modified from Smith et al. (2014). (N= 20 Ctrl and N=40 SLE donors, *p<0.05, **p<0.006, ***p<0.0001)

SLE HDL Exhibits an Altered Proteome

In addition to examining differences in oxidation levels, we quantified protein content variations by FPLC in HDL collected from the plasma of 10 Ctrl and 10 SLE donors. Compared to Ctrl HDL, SLE HDL showed significantly lower complement C4-B, apoA-II and higher levels of vasorin, plasma protease C1 inhibitor, protein Z-dependent protease inhibitor, immunoglobulin fragments, and complement factor I (Figure 2-3A). Vasorin is rarely reported on in the literature. It is largely expressed by VSMCs [530]. When cleaved by ADAM17 protease, it binds to TGF-β to prevent its signaling [531]. It appears to have a role in vascular remodeling, though whether it leads to repair or damaging fibrosis is not clear [530-532].

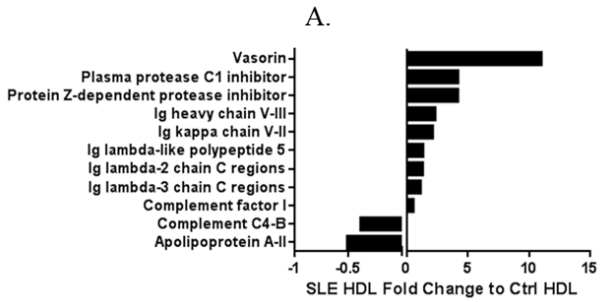


Figure 2-3. SLE HDL demonstrates a different protein content than Ctrl HDL. Plasma HDL proteomics was determined by FPLC. (N=10 Ctrl and SLE donor samples, $p<0.05$)

NET-derived MPO, NOX and NOS Promote HDL Oxidation In Vitro

As levels of RNS- and MPO-derived oxidative modifications are markedly elevated in lupus and correlate with functional impairment of CEC, we attempted to identify the putative sources of enhanced RNS and MPO activity in SLE. MPO HDL oxidation is classically attributed to plaque macrophages and phagosome activity [414, 415, 435-437, 515, 533]. Another possible source is peripheral blood MPO, reported enhanced in SLE and which we were able to confirm (Tables 2-1 and 2-2) [534]. As a subset of peripheral blood lupus granulocytes (LDGs) have a significantly enhanced capacity to form NETs, a source of externalized NOX and MPO, we examined if the

NETs could induce HDL oxidation [214, 216, 525]. Additionally, because we found that MPO levels did not correlate with 3-NY HDL levels (Figure 2-2G), we examined if an alternative producer of RNS (NOS and NOX formation of peroxynitrite, Figure 2-1) could be present in NETs and serve as a source of HDL nitration.

MPO (not shown), NOX (p22 and p47 subunits) and NOS (eNOS and iNOS) were all detected on both Ctrl NDG and LDG NETs by fluorescence microscopy and immunoblot (Figure 2-4A). Next, we exposed healthy HDL to NETs isolated from PMA-stimulated Ctrl NDG or spontaneously by lupus LDGs, in the absence or presence of MPO, NOX and NOS inhibitors (3-AT, DPI and L-NMMA, respectively), and examined HDL oxidation profile. As shown in Figures 2-4B and 2-4C, NETs from both lupus LDGs and Ctrl NDG significantly enhanced HDL oxidation. Hypochlorous acid (HOCl), which is synthesized by MPO downstream of NOX activity, is the major source of HDL chlorination in humans. When NETting Ctrl NDGs and lupus LDGs were treated with 3-AT and DPI to block MPO and NOX activity, respectively, HDL 3-ClY oxidation was abrogated (Figure 2-4B). These results support the hypothesis that MPO and NOX externalized on NETs can induce 3-ClY HDL oxidation in circulation.

Because both NOS and MPO are capable of producing the RNS required for HDL nitration, we used a KRPG solution with no added nitrite to examine the role of NOS alone in 3-NY oxidation. Under these conditions, only NOS could generate the nitric oxide radical ($NO^{\bullet}$) required for peroxynitrite formation that could lead to HDL nitration (Figure 2-1). MPO, under these conditions, could not be the source of RNS. Indeed, the MPO inhibitor 3-AT was ineffective at blocking HDL nitration, while the NOS inhibitor L-NMMA significantly abrogated HDL 3-NY oxidation for both lupus LDGs and Ctrl NDG (Figure 2-4C). These results suggest that NET-bound

NOS is a source of the RNS causing HDL nitration, and may explain why the levels of MPO did not correlate with the levels of 3-NY oxidized HDL in SLE.

Chloroquine (CQ) Blocks NET Formation In Vitro

As a majority (72.5%, Table 2-1) of the SLE patients studied were on anti-malarials, we determined the effect of physiologically relevant concentrations of CQ (250 ng/mL) on NET formation [527]. CQ significantly inhibited NET formation in Ctrl NDGs and lupus LDGs (Figure 2-5A-C). To assess if CQ modified MPO, NOS and NOX activity, neutrophils were homogenized, treated with CQ, and incubated with HDL. CQ did not significantly block 3-ClY or 3-NY oxidation in lupus LDGs or Ctrl NDGs (data not shown). This indicates that though CQ may block NET formation, this is not accomplished by inhibiting oxidative enzyme activity.

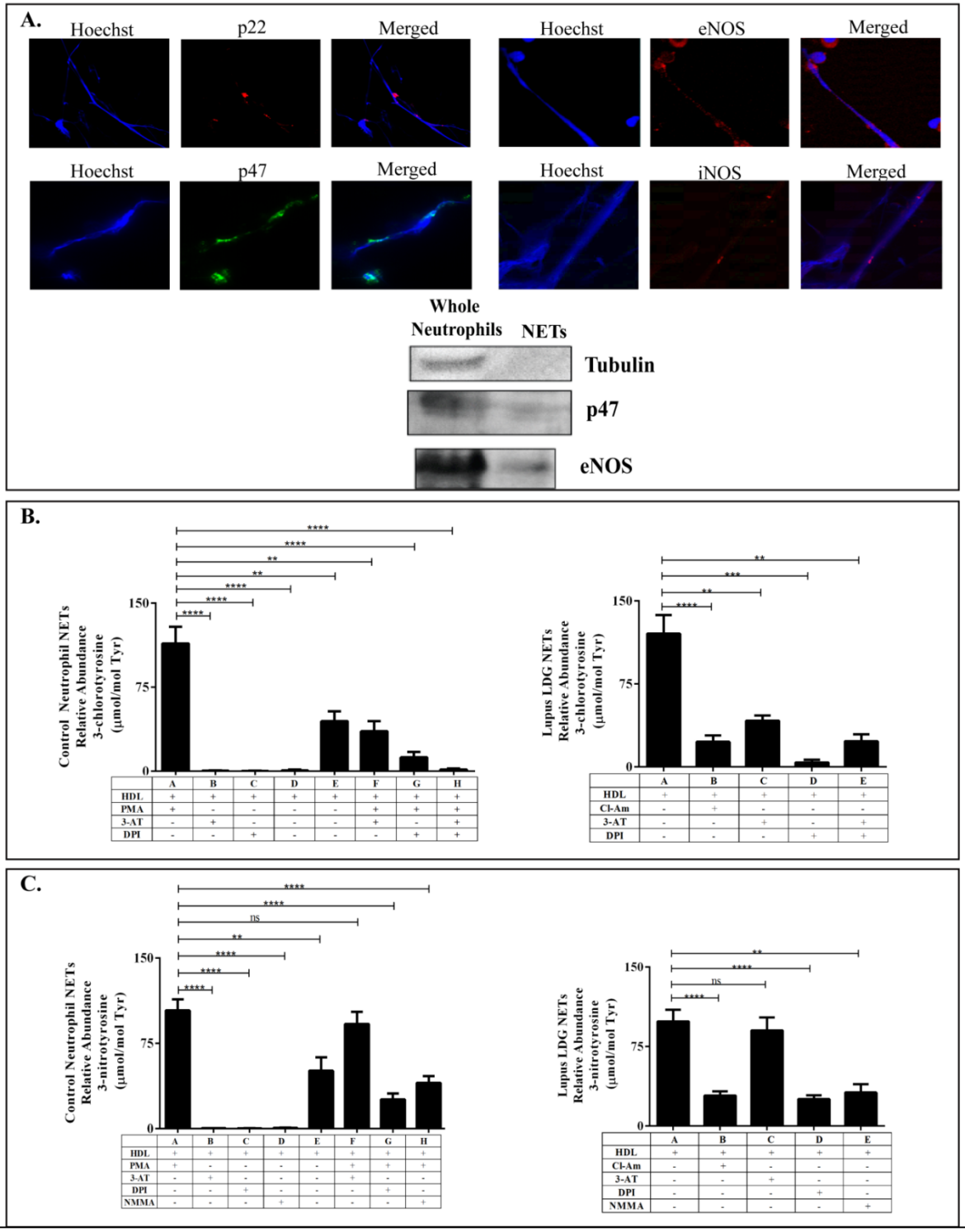


Figure 2-4. Human Ctrl NDGs and lupus LDGs contain the oxidative machinery and capacity to cause 3-ClY and 3-NY oxidation. (A) NOX (p22 and p47) and NOS were detected in NETs by immunofluorescence. The ability of NETs from NDGs and LDGs to induce (B) 3-ClY and (C) 3-NY HDL oxidation was examined. The activity of NET formation, MPO, NOS or NOX was inhibited by Cl-Am or lack of PMA, by 3-AT, by NMMA, or by DPI, respectively. Duplicated from Smith et al. (2014). (Data are displayed as mean ± SEM, N= 6/group, *p<0.03, ** p < 0.009, *** p < 0.0009)

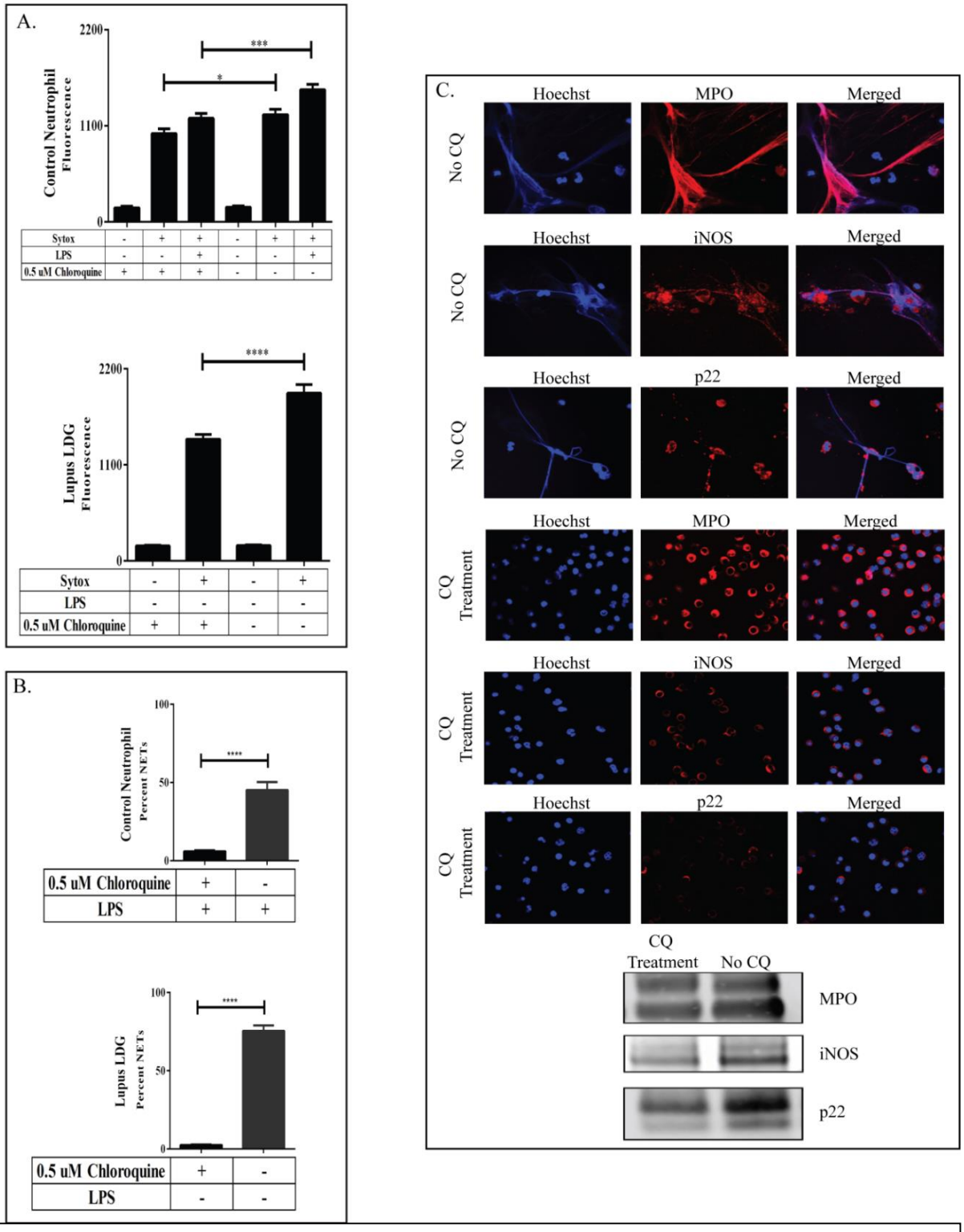


Figure 2-5. Chloroquine (CQ) impairs NET formation. Both blood NDGs and LDGs were incubated with CQ and examined for NET formation by (A) SYTOX fluorescent assay and (B) immunofluorescence. (C) Representative images of NET formation with CQ treatment, staining for MPO, iNOS and NOX (p22) components. Duplicated from Smith et al. (2014). (N=4/group, Data are displayed as mean ± SEM, *p<0.03, *** p < 0.0009, **** p < 0.0001)

NET-derived NOS and NOX are Sources of HDL Oxidation in Murine Systems

While present in murine NETs, MPO is not associated with murine atherosclerosis [533, 535]. Lupus-prone NZM mice display striking elevations of 3-NY HDL oxidation, but not 3-ClY, when compared to Ctrl mice, possibly because murine leukocytes contain less MPO, the sole known source of 3-ClY oxidation, than human leukocytes [34, 415, 533, 535]. We therefore examined if NOX and NOS were present in murine NETs and could produce RNS to form 3-NY oxHDL [524, 525]. We identified NOS and NOX (p22 and p47) machinery present in PMA-induced murine NETs (Figure 2-6A). To verify that these NET-bound enzymes could cause HDL oxidation, we exposed native HDL (purified from Ctrl Balb/c mice) to NZM or Balb/c NETs in the absence or presence of MPO, NOX and NOS inhibitors (3-AT, DPI and L-NMMA, respectively) and examined HDL oxidation profiles [216, 225, 406, 524-526]. Consistent with our previous report, we found no significant 3-ClY oxHDL patterns after HDL incubation with murine NETs (Figure 2-6B) [34]. However, a significant oxidation pattern for 3-NY oxHDL was observed (Figure 2-6C). While experiments were performed in nitrite containing media (RPMI) so that either NOS or MPO could potentially promote HDL nitration, only DPI and L-NMMA significantly blocked NET-induced 3-NY HDL oxidation. These results support the idea that MPO is less active in mice and that NOX and NOS are the primary sources of the oxidative species required for HDL nitration in murine systems.

Finally, to verify that NETs are an important source of HDL oxidation in the periphery, we inhibited NET formation in NZM mice *in vivo* and examined their oxidative profile. NZM mice received the PAD inhibitor Cl-Am daily for 14 weeks, as PAD activity is necessary for NET formation [33, 536]. Cl-Am-treated mice displayed significantly decreased 3-NY content in HDL, but not total plasma, when compared to vehicle treated mice (Figure 2-6D). These results indicate that *in vivo* inhibition of NET formation in lupus-prone mice decreases HDL oxidation.

D. Summary

We sought to determine if lupus HDL and plasma were oxidized and displayed impaired CEC, factors known to promote atherogenesis. We also pursued a putative innate immune mechanism that would explain the modified HDL in SLE. We found that compared to controls, SLE plasma demonstrated impaired CEC and SLE plasma proteins, including apoA-I, showed high 3-ClY and 3-NY oxidation levels. Because 3-ClY and 3-NY oxHDL levels correlated with each other, but 3-NY did not correlate with MPO levels, this suggested that both modifications occur at similar sites in the vasculature, though not through the same enzymes. As the plasma oxidation levels were so high in SLE, the results also suggested that the site of oxidation was largely in the circulation. Most of the literature to date has focused on macrophages in the atherosclerotic plaque as sources of MPO-driven HDL oxidation, yet circulating neutrophils contain higher levels of MPO than tissue-resident macrophages, and both neutrophils and NETs are present around thrombotic and atheroma plaques [415, 476, 533, 537-539]. Indeed, NOS, MPO and NOX externalized in human and murine NETs were capable of inducing a CVD risk pattern of HDL oxidation. Finally, we demonstrated that *in vivo* NET inhibition potently decreased levels of 3-NY oxHDL in lupus-prone mice. Therefore, NETs may play a crucial role in the transformation of HDL into its pro-atherosclerotic form.

These results indicate a novel mechanism of HDL oxidation, through NET activity which may occur primarily in the circulation rather than in sub-endothelial plaques. Given the high rate of spontaneous NET activity exhibited by LDGs, our results also contribute to the supposition that these granulocytes may be particularly pathogenic to lupus patients. Indeed, it would be interesting to see if LDGs can be detected in lupus atherosclerotic plaques or if levels of blood LDGs correlate with HDL oxidation levels. The increased presence of these cells in the blood of SLE patients

combined with their extensive NET activity may explain why the circulating plasma in SLE showed higher levels of oxidation and impaired CEC than seen even in "typical" CVD, found in the general population [409].

Given the high levels of circulating plasma oxidation, we focused more on HDL than LDL, which is a less mobile lipoprotein and is generally found stuck in plaques. However, as NET structures have also been detected in tissue, whether NETs in SLE also contribute to LDL oxidation should be explored in the future [33, 40, 214, 425, 540]. This may prove a potential biomarker for SLE cardiovascular health along with 3-ClY and 3-NY HDL oxidation. However, a larger cohort of patients than was used in this study would be needed to determine if these biomarkers are sensitive and specific to SLE CVD. Such markers are needed given the lack of association between Framingham risk factors and lupus atherosclerosis.

Pharmacological inhibition of NET formation, by Cl-Am e.g., has been proposed as a possible lupus therapy. Indeed, lupus-prone mice treated with such inhibitors show reduced tissue damage, levels of auto-reactive antibodies and inflammatory cytokines [33, 40, 425]. Our murine results indicate that such therapies may also be beneficial for SLE CVD. However, our study was limited by the lack of well-defined lupus-prone mouse models which also develop atherosclerosis. As mentioned previously, mice in general do not develop CVD. NZM mice do show endothelial cell dysfunction and high levels of HDL 3-NY oxidation, but they fail to develop atherosclerotic plaques [34]. As such, we did not measure the effect of Cl-Am on plaque formation in lupus mice. Additionally, as 3-NY oxidized HDL (predominant in mice) is not associated with impaired CEC, we did not measure the effect of Cl-Am treated on murine CEC [408]. However, as the HDL from lupus patients and mice does possess the hallmarks of a dysfunctional, pro-atherosclerotic lipoprotein, we asked whether these modifications would, in turn, amplify inflammatory responses.

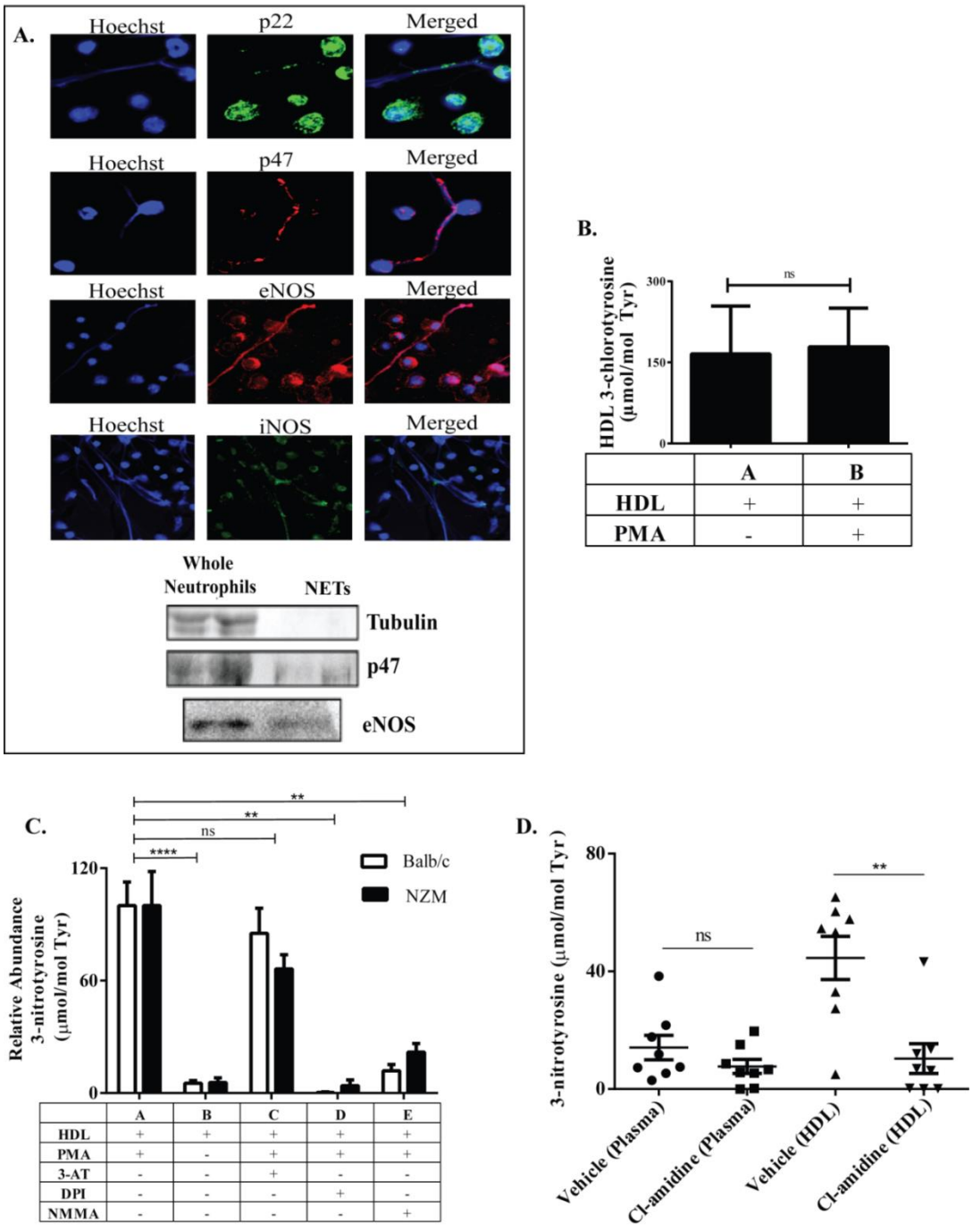


Figure 2-6. Murine granulocytes contain the oxidative machinery and capacity to cause 3-NY oxidation and abrogation of NET formation blocks 3-NY HDL levels. (A) NETs from mice were stained for NOX components (p22 and p47) and NOS. (B) The ability of NETs from Balb/c and NZM mice to induce 3-ClY HDL oxidation was examined (N=4/group). (C) The ability of NETs from Ctrl Balb/c and NZM mice to induce 3-NY HDL oxidation was quantified. The activity of NET formation, MPO, NOS or NOX was inhibited by lack of PMA, by DPI, or by NMMA, respectively (N=8/group). (D) Levels of plasma and HDL 3-NY in NZM mice that received daily s.c. injections of PBS (N= 10) or Cl-Am (N=10) for 14 weeks. Duplicated from Smith et al. (2014). (Data are displayed as mean ± SEM, ** $p < 0.01$, **** $p < 0.0001$)

Chapter 3

Lupus HDL Promotes Pro-inflammatory Responses in Macrophages by Binding LOX1R and Failing to Promote ATF3 Activity

A. Introduction:

"Healthy" HDL has a strong inverse correlation to CVD risk, and promotes numerous vasoprotective mechanisms: RCT, and inhibition of LDL oxidation, adhesion molecule expression, TLR-induced inflammatory responses and NFκB activation [319, 367, 368, 541, 542]. When HDL is oxidized, however, it loses many of these vasoprotective effects [239, 401, 408, 409, 501, 503, 543]. As both SLE and CVD appear to share high levels of HDL oxidation and altered monocyte and macrophage immune responses, we hypothesized that these pathways work together to induce enhanced atherosclerosis in SLE [197, 333].

We predicted that SLE HDL would have an impaired ability to block TLR-induced inflammation, and may even promote inflammatory cytokine production in macrophages. We further examined the role of SLE HDL on macrophage polarization and induction of anti-inflammatory activity through transcription factor regulation. As various scavenger receptors expressed on macrophages (LOX1R, CD36, SR-BI) interact with HDL to modify cholesterol and inflammation homeostasis, we analyzed the role of these receptors on SLE HDL-induced responses [197, 544]. Finally, we hypothesized that a recently described HDL mimetic (ETC-642) could abrogate inflammatory responses induced by oxidized HDL both *in vitro* human macrophages and *in vivo* lupus-prone NZM mice [512, 545]. These findings would enable us to

elucidate a novel pathway by which the highly oxidized HDL in SLE transforms healthy macrophages into pro-inflammatory, pro-atherogenic cells.

B. Materials and methods

Subject Recruitment

The same cohort of lupus patients were used as in chapter 2.

HDL and ETC-642 Preparation

HDL was purified as described above [529]. ETC-642 was prepared using ESP24218 apolipoprotein A-I mimetic peptide, sphingomyelin (SM) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) at 1:1:1 weight/weight ratios [512, 546]. All components were dissolved in organic solvent, lyophilized and hydrated with sterile PBS (Life Technologies) [546]. The purity and size distribution of the resulting ETC-642 particles were examined by gel permeation chromatography and dynamic light scattering, respectively. ESP24218 was prepared by custom synthesis (GenScript Corp, Piscataway, NJ) and phospholipids were purchased (Avanti Polar Lipids, Alabaster, AL). Experimental controls for ETC-642 components, suspension of DPPC-SM mixture and ESP2418 solution, were prepared by sonication of lipid powders in PBS.

Macrophage Culture

SLE and Ctrl PBMCs were isolated by Ficoll-Paque (GE Healthcare Life Sciences) density gradient. Cells were plated at a density of $0.5x10^6$ cell/cm^2 and allowed to adhere for 2 hours; non-adherent cells were removed and attached cells cultured in fresh X-Vivo-15 media (Lonza, Basel, Switzerland) with 10% FBS (Gemini, Sacramento, CA). Monocytes were differentiated into macrophages over the course of 1 week, with media changes every 3 days, and purity was determined by FACS analysis.

Culture Conditions, RNA Isolation and RT-qPCR

When determining the effect of HDL alone on inflammatory cytokine production, Ctrl macrophages were cultured in the presence or absence of Ctrl or SLE HDL (50 μg/mL) for 4 hours before adding TriPure Isolation Reagent (Roche, Indianapolis, IN). When determining the effect of HDL on responses to TLR agonists, the macrophages were incubated in the presence or absence of ETC-642, Ctrl or SLE HDL, or S1P1-3 (50 μg/mL) for 4 hours before media was removed, then incubated with Pam3CSK4 (300 ng/mL, InvivoGen, San Diego, CA), R848 (1 μg/mL, InvivoGen), or LPS (100 ng/mL, Sigma-Aldrich) plus 2% lipoprotein deficient serum (LPDS, Millipore) in DMEM (Life Technologies) for 4 hours. For macrophage polarization assessment, Ctrl and SLE macrophages were cultured in the presence or absence of Ctrl or SLE HDL (50 μg/mL) with 2% LPDS for 3 days. For human ETC-642 studies, various SLE HDL: ETC-642 ratios (1:1, 1:2, 1:4, 2:1, 4:1) were tested on Ctrl macrophages for 4 hours.

RNA was isolated using a Direct-zol RNA MiniPrep kit (Zymo Research, Irvine, CA) and purity determined by NanoDrop 1000 (Thermo Scientific). Total RNA (1 μg) was reverse transcribed using iScript Reverse Transcriptase (BioRad, Hercules, CA) and RT-qPCR was performed using SSoAdvanced Universal SYBR Green (BioRad). Primer sequences are included in Table 3-1 and 3-2. Samples were run in duplicate using a CFX96 C1000 Touch Real Touch Thermal Cycler (Bio-Rad). Data was analyzed using Bio-Rad CFX Manager software.

Target	Sequence
human β-Actin forward	CAT CAC GAT GCC AGT GGT ACG
human β-actin reverse	AAC CGC GAGAAG ATG ACC CAG
human GAPDH forward	GGT CGG AGT CAA CGG ATT TGG TCG
human GAPDH reverse	CCT CCG ACG CCT GCT TCA CCA C
human ATF3 forward	AAG AAC GAG AAG CAG CAT TTG AT
human ATF3 reverse	TTC TGA GCC CGG ACA ATA CAC
human CCL-22 forward	TGC CGT GAT TAC GTC CGT TAC
human CCL-22 reverse	AAG GCC ACG GTC ATC AGA GTA G
human CD206 forward	CGA GGA AGA GGT TCG GTT CAC C
human CD206 reverse	GCA ATC CCG GTT CTC ATG GC
human IL-6 forward	TCA GCC CTG AGA AAG GAG ACA T
human IL-6 reverse	TGG CAT TTG TGG TTG GGT CA
human IL-12B forward	GGA GTA CCC TGA CAC CTG GA
human IL-12B reverse	ACG CTA ATG CTG GCA TTT TT
human SOCS3 forward	TCC CCC CAG AAG AGC CTA TTA C
human SOCS3 reverse	TCC GAC AGA GAT GCT GAA GAG TG
human TNF forward	CCC AGG GAC CTC TCT CTA ATC
human TNF reverse	ATG GGC TAC AGG CTT GTC ACT
human IL-1β forward	GCA CGA TGC ACC TGT ACG AT
human IL-1β reverse	CAC CAA GCT TTT TTG CTG TGA GT

Table 3-1. Primers used for human RT-qPCR.

ELISA

Macrophages from 5 Ctrl donors were incubated with or without azide-free goat IgG isotype control (5 µg/mL, SouthernBiotech, Birmingham, AL) or goat IgG anti-LOX1R blocking antibody (5 µg/mL, R&D Systems, Minneapolis, MN) in DMEM for 30 minutes, before addition of HDL or Pam3CSK4 in DMEM with 2% LPDS for 18 hours. Commercially available ELISAs were performed to quantify human IL-6 and TNF in supernatants following manufacturer's instructions (ALPCO, Salem, NH). For murine *in vivo* studies, serum IL-6 was quantified by ELISA (eBioscience, San Diego, CA).

Immunofluorescence Microscopy

For TLR studies, Ctrl macrophages were cultured with LPS (100 ng/mL, Sigma-Aldrich), Pam3CSK4 (300 ng/mL, InvivoGen), no, Ctrl or SLE HDL (50 µg/mL) for 2 hours. For scavenger receptor studies, macrophages were cultured with isotype control, anti-LOX1R blocking antibody (see above), rabbit anti-CD36 blocking antibody (1:100, Abcam), or rat anti-SR-BI/II blocking antibody (1:100, Abcam) before incubation with HDL in DMEM/2% LPDS for 30 minutes (for ATF3 experiments), followed by fixation with 4% PFA (Santa Cruz). Fixed cells were permeabilized with 0.2% Triton X-100 (Sigma-Aldrich) for 10 minutes, washed, and then blocked with 0.2% gelatin for 30 minutes. Cells were incubated for 1.5 hours with rabbit anti-p65 (1:500, FIVEphoton Biochemicals, San Diego, CA), mouse anti-ATF3 (1:100, Abcam), mouse anti-TLR-4 (1:250, Abcam), or mouse anti-TLR-2 (1:100, Abcam) followed by incubation for 1 hour with donkey anti-rabbit, or donkey anti-mouse Alexa Fluor 555 (1:500, Life Technologies), then with Hoechst 33342 (1:1000, Life Technologies) for 5 minutes. Coverslips were mounted with ProLong Gold Antifade (Life Technologies). Images were acquired on a Zeiss LSM 510 META laser scanning confocal microscope (Carl Zeiss Microscopy) with a x63 lens and quantified using the Zeiss Zen 2012 SP1 software's weighted co-localization coefficient analysis.

Flow Cytometry

Ctrl or SLE PBMCs were isolated by Ficoll-Paque (GE Healthcare Life Sciences) density gradient. Cells were blocked in FACS solution (Biolegend) for 30 minutes. Surface markers were stained for 20 minutes at room temperature: Pacific Blue anti-human CD16, PE anti-human CD14, APC anti-human CD86 (Biolegend). Monocytes were classified as either non-classical ($CD14^{+}$, $CD16^{++}$), intermediate/"inflammatory" ($CD14^{++}$,$CD16^{+}$), and classical ($CD14^{++}$, $CD16^{-}$) as described [376]. For M1/M2 studies, cultured SLE or Ctrl macrophages were cultured in the presence

or absence of Ctrl or SLE HDL (50 µg/mL) with 2% LPDS in DMEM for 3 days. Cells were harvested using StemPro Accutase Reagent (Life Technologies), blocked in FACS solution (Biolegend, San Diego, CA) for 30 minutes, and incubated with anti-human CD206 APC and anti-human CD40 Pacific Blue for 20 minutes at room temperature. For ATF3 quantification, cells were incubated in permeabilization buffer (eBioscience) and stained according to manufacturer's instructions with rabbit FITC anti-ATF3 (1:100, Bioss, Woburn, MA). For human ETC-642 studies, monocytes were isolated by negative selection from Ctrl PBMCs using MACS Monocyte Isolation Kit II (Miltenyi, San Diego, CA). Monocytes were then incubated in DMEM (Life Technologies) with 2% LPDS for 18 hours with Ctrl or SLE HDL (50 µg/mL). Cells were stained for intracellular ATF3 as stated above.

Immunoblot

For studies on HDL-induced total ATF3 production, Ctrl macrophages were incubated in the presence or absence of Ctrl or SLE HDL for 18 hours. In additional experiments, Ctrl macrophages were incubated with isotype control or anti-LOX1R blocking antibody (see above) prior to adding HDL for 30 minutes (for ATF3) or 2 hours (for p65). Nuclear fractions were then isolated using a Nuclear Extraction Kit (Active Motif, Carlsbad, CA). Protein concentrations were determined by BCA analysis (Thermo Scientific). Total cell lysate (10 µg) or nuclear fractions (5 µg) were run on 4-12% NuPAGE Bis-Tris gels (Life Technologies). Blots were blocked (10% BSA) and incubated overnight with rabbit anti-p65 (1:500, FIVEphoton Biochemicals), mouse anti-ATF3 (1:250, Abcam) or rabbit anti-histone 3 (1:2000, Abcam), followed by 1:10,000 donkey anti-rabbit or anti-mouse IRDye 680RD or 800CW (Li-cor, Lincoln, NE) for 1 hour at room temperature. Blots were imaged on an Odyssey CLx (Li-cor).

In Vivo Administration of ETC-642

The protocol was approved by the University of Michigan's Committee on Use and Care of Animals. NZM breeding pairs were obtained from Dr. Chaim Jacob (University of Southern California) [543]. Balb/c mice were purchased from the Jackson Laboratory. Mice were bred and housed in a specific pathogen–free barrier facility at the University of Michigan. Starting at 10 weeks of age, NZM female mice were treated with ETC-642 (15 mg/kg, N=8) or equal volume PBS (N=8) for 13 weeks, three times/week via tail vein injection. Mice were euthanized at 23 weeks of age, before clinical development of renal disease became apparent. At euthanasia, spleens were homogenized with an Omni TH Motor and probes (Omni International, Kennesaw, GA) and resulting splenocyte RNA used for RT-qPCR (see above). Serum was collected from terminal bleeds.

Target	Sequence
murine ATF3 forward	GAG CTG AGA TTC GCC ATC CA
murine ATF3 reverse	CCG CCT CCT TTT CCT CTC AT
murine IL-6 forward	TGG CTA AGG ACC AAG ACC ATC CAA
murine IL-6 reverse	AAC GCA CTA GGT TTG CCG AGT AGA
murine IL-12B forward	AGA AAG GTG CGT TCC TCG TAG
murine IL-12B reverse	AGC CAA CCA AGC AGA AGA CAG
murine TNF forward	CCC TCA CAC TCA GAT CAT CTT CT
murine TNF reverse	GCT ACG ACG TGG GCT ACA G
murine IL-1β forward	CCC TGC AGC TGG AGA GTG TGG A
murine IL-1β reverse	CTG AGC GAC CTG TCT TGG CCG
murine MX1 forward	GAT CCG ACT TCA CTT CCA GAT GG
murine MX1 reverse	CAT CTC AGT GGT AGT CAA CCC
murine ISG15 forward	CAG AAG CAG ACT CCT TAA TTC
murine ISG15 reverse	AGA CCT CAT ATA TGT TGC TGT G
murine IRF-7 forward	TGC TGT TTG GAG ACT GGC TAT
murine IRF-7 reverse	TCC AAG CTC CCG GCT AAG T

Table 3-2. Primers used for murine RT-qPCR.

Statistical Analysis

Significance was determined as stated in chapter 2.

C. Results

SLE HDL Induces Pro-inflammatory Responses in Macrophages and Fails to Abrogate Inflammatory Cytokine Synthesis Triggered by TLR Agonists.

We showed that SLE HDL is enriched in 3-NY and 3-ClY HDL oxidation and displays impaired CEC when compared to Ctrl HDL, yet we wished to determine the inflammatory nature of this SLE HDL (Table 2-1, 2-2, and Figure 2-2) [259, 501, 503, 543]. Ctrl macrophages exposed to SLE HDL synthesized significantly higher levels of IL-6 and TNF than macrophages exposed to Ctrl HDL, both at the mRNA (Figure 3-1A) and protein levels (Figure 3-1B).

Ctrl HDL has recently been reported to block TLR-induced inflammatory cytokine production in macrophages [367, 368]. We confirmed that Ctrl HDL significantly decreased the synthesis of IL-6, TNF, IL-1β and IL-12B mRNA following TLR-4, -1/2, or -7/8 stimulation (Figure 3-1C). In contrast, SLE HDL was impaired in its ability to block TLR-induced inflammatory cytokine synthesis in macrophages. The differences in the inhibitory effects of Ctrl versus lupus HDL on TLR signaling could be due to TLR sequestration or activation, either resulting in TLR internalization. To address this, we treated Ctrl macrophages with either Ctrl or SLE HDL and examined TLR-2 and -4 surface expression. Neither Ctrl nor SLE HDL affected TLR-2 or -4 surface expression or internalization (Figure 3-1D). Because of the inflammatory nature of SLE HDL, we quantified the effect of each HDL cohort on NFκB activation, as measured by p65 nuclear translocation. SLE HDL promoted p65 nuclear translocation in Ctrl macrophages, compared to Ctrl HDL (Figure 3-1E). Overall, these results indicate that SLE HDL promotes

inflammatory responses in macrophages and has an impaired ability to block TLR-induced inflammatory signals, without modifying TLR cellular trafficking.

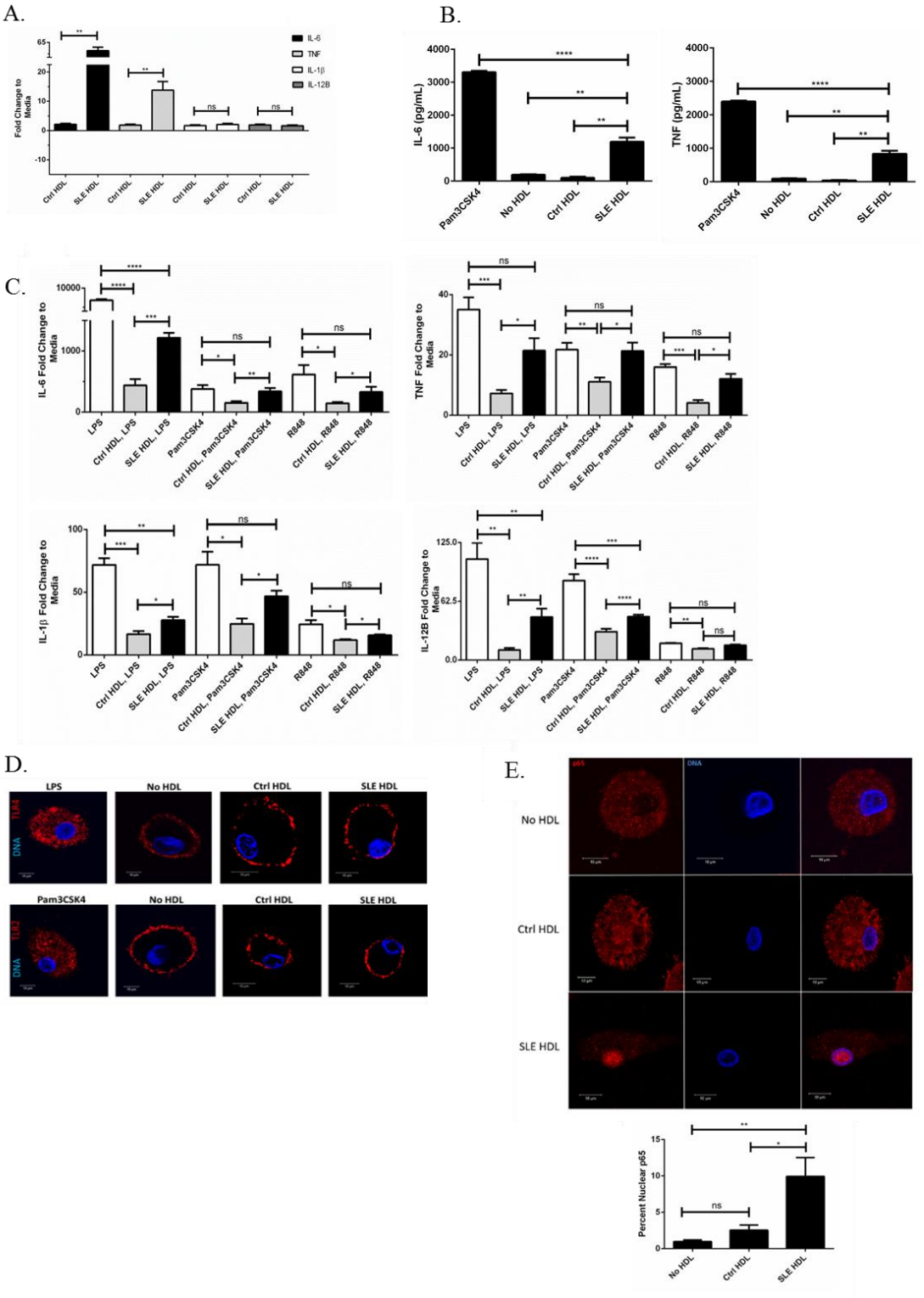


Figure 3-1. SLE HDL promotes inflammatory cytokine production, fails to block TLR-induced inflammation, and activates NFκB. (A) Ctrl macrophages incubated with Ctrl or SLE HDL were examined for IL-6, TNF, IL-1β and IL-12B mRNA levels by RT-qPCR (N=7). (B) Ctrl macrophages treated with Pam3CSK4, no, Ctrl, or SLE HDL and examined for IL-6 and TNF production by ELISA (N=5). (C) Ctrl macrophages were treated with no, Ctrl or SLE HDL before TLR agonist treatment (LPS, Pam3CSK4, R848, N=7). (D) Ctrl macrophages were treated with LPS, Pam3CSK4, no, Ctrl or SLE HDL and stained for TLR-2 or -4 (N=3). (E) Ctrl macrophages were treated with no, Ctrl or SLE HDL and stained for p65 to examine NFκB activation (N=7). (Data are displayed as mean ± SEM, *p<0.03, ** p < 0.009, *** p<0.0005, **** p < 0.0001)

SLE Patients Exhibit an Enhanced Inflammatory Monocyte and M1 Macrophage Bias, which is Increased by SLE HDL.

As inflammatory monocytes are linked to CVD risk, we wanted to quantify inflammatory monocyte presence in our cohort of SLE patients [372, 373, 375, 378, 379, 547]. Based on two different gating strategies (Figure 3-2A), SLE patients exhibit a higher level of non-classical and intermediate (inflammatory) monocytes than Ctrl donors (Figure 3-2 B and C) [376, 547]. It should be noted that the SLE LDGs (granulocyte population visible in the SLE SSC-A vs. FSC-A in Figure 3-2A) do not contribute to these monocyte percentages as LDGs are $CD86^-$.

M1 macrophages are associated with atherosclerosis and pro-inflammatory cytokine production, similar to what was induced *in vitro* by lupus HDL (Figure 3-1A). We therefore quantified M1 and M2 markers in monocyte-derived SLE and Ctrl macrophages. At the protein level, Ctrl macrophages had significantly higher CD206 (an M2 marker) expression compared to SLE macrophages when both were cultured in media alone (Figure 3-2D). The addition of Ctrl HDL to the SLE and Ctrl macrophages increased CD206 expression, but Ctrl HDL-treated SLE macrophages still showed significantly lower CD206 expression than Ctrl macrophages. Addition of SLE HDL to SLE or Ctrl macrophages did not increase CD206 expression. Additionally, Ctrl macrophages cultured in media alone showed lower CD40 (an M1 marker) expression compared to SLE macrophages cultured in media alone (Figure 3-2D) [195]. Ctrl macrophages did not

significantly modify CD40 expression with the addition of SLE or Ctrl HDL. SLE macrophages, however, showed decreased CD40 expression following exposure to Ctrl HDL (Figure 3-2D). At the mRNA level, Ctrl macrophages exposed to Ctrl HDL displayed enhancement of M2 markers (CCL22 and CD206), while SLE HDL up-regulated the M1 marker SOCS3 and down-regulated M2 markers (Figure 3-2E) [195, 548]. These results indicate that SLE monocytes and macrophages are biased towards an inflammatory signature, which is exacerbated by SLE HDL and dampened by exposure to Ctrl HDL.

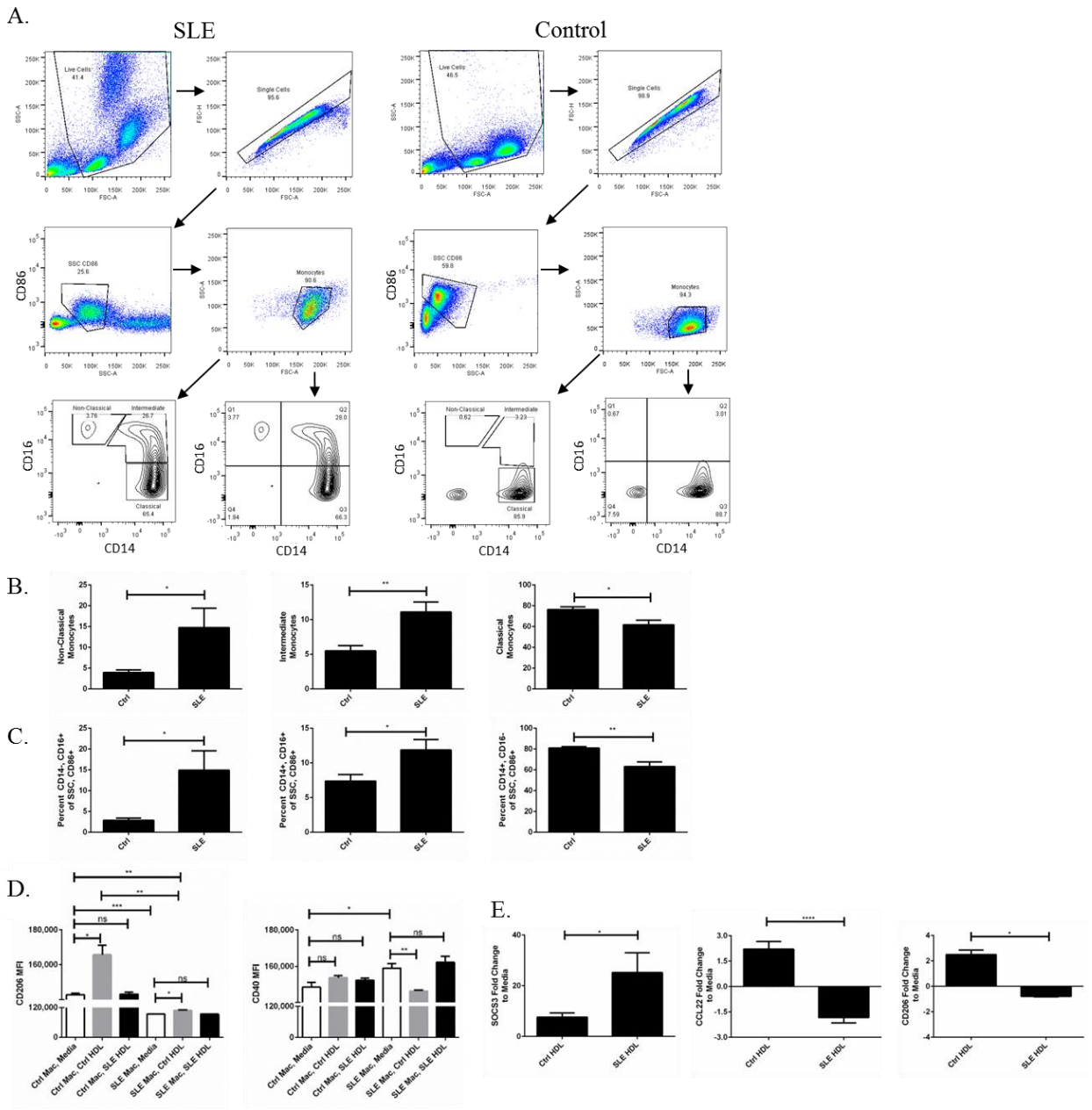


Figure 3-2. SLE patients show an enhanced inflammatory monocyte and M1 macrophage phenotype. (A) PBMCs from SLE and Ctrl donors were stained for CD86, CD14, and CD16 to quantify non-classical, intermediate (inflammatory), and classical monocyte populations, based on two gating strategies (B and C, N=14). (D) Ctrl and SLE macrophages were treated with SLE and Ctrl HDL and CD206 and CD40 surface expression quantified by flow cytometry (N=5). (E) Ctrl macrophages were treated with Ctrl or SLE HDL. M1 (SOCS3) and M2 (CCL22, CD206) genes were quantified by RT-qPCR (N=5). (Data are displayed as mean ± SEM, * $p<0.05$, ** $p<0.01$, *** $p<0.0005$, **** $p<0.0001$)

Lupus HDL is Impaired in its Capacity to Promote ATF3 Synthesis and Anti-inflammatory Activity.

ATF3 is ubiquitously expressed at low levels, and is up-regulated in response to inflammatory signals (IFN-ɣ, IL-1β, LPS), cell stress and calcium signaling [549, 550]. The 3' UTR of ATF3 mRNA contains many AUUUA sites, indicating that it is an immediate-early response gene [551]. Through an interaction with histone deacetylase 1 (HDAC1), ATF3 blocks IL-6, TNF, and IL-12B production downstream of inflammatory signals [365, 552]. Recent evidence indicates that HDL from healthy individuals modulates inflammatory responses in macrophages through induction of ATF3 [365, 367]. Given that lupus HDL promoted inflammatory cytokine production and showed an impaired capacity to down-regulate TLR-induced cytokines, we examined the effect of this lipoprotein on ATF3 nuclear translocation and synthesis.

When exposed to lupus HDL, Ctrl macrophages failed to up-regulate ATF3 mRNA by 2 hours when compared to Ctrl HDL treatment (Figure 3-3A). Furthermore, after 6 hours of exposure, Ctrl macrophages exposed to lupus HDL expressed significantly lower ATF3 mRNA levels compared to macrophages exposed to Ctrl HDL. At the protein level, Ctrl monocytes or monocyte-derived macrophages exposed to lupus HDL for 18 hours showed significantly lower ATF3 protein levels compared to Ctrl HDL, as demonstrated by immunoblot (Figure 3-3B) and flow cytometry (Figure 3-3C). Additionally, monocyte-derived SLE macrophages expressed lower levels of ATF3 than Ctrl macrophages (Figure 3-3D). These results suggest that lupus HDL fails to induce the expression of the inflammatory repressor ATF3 and this abnormality may play a role in the induction of macrophage pro-inflammatory responses observed in this disease.

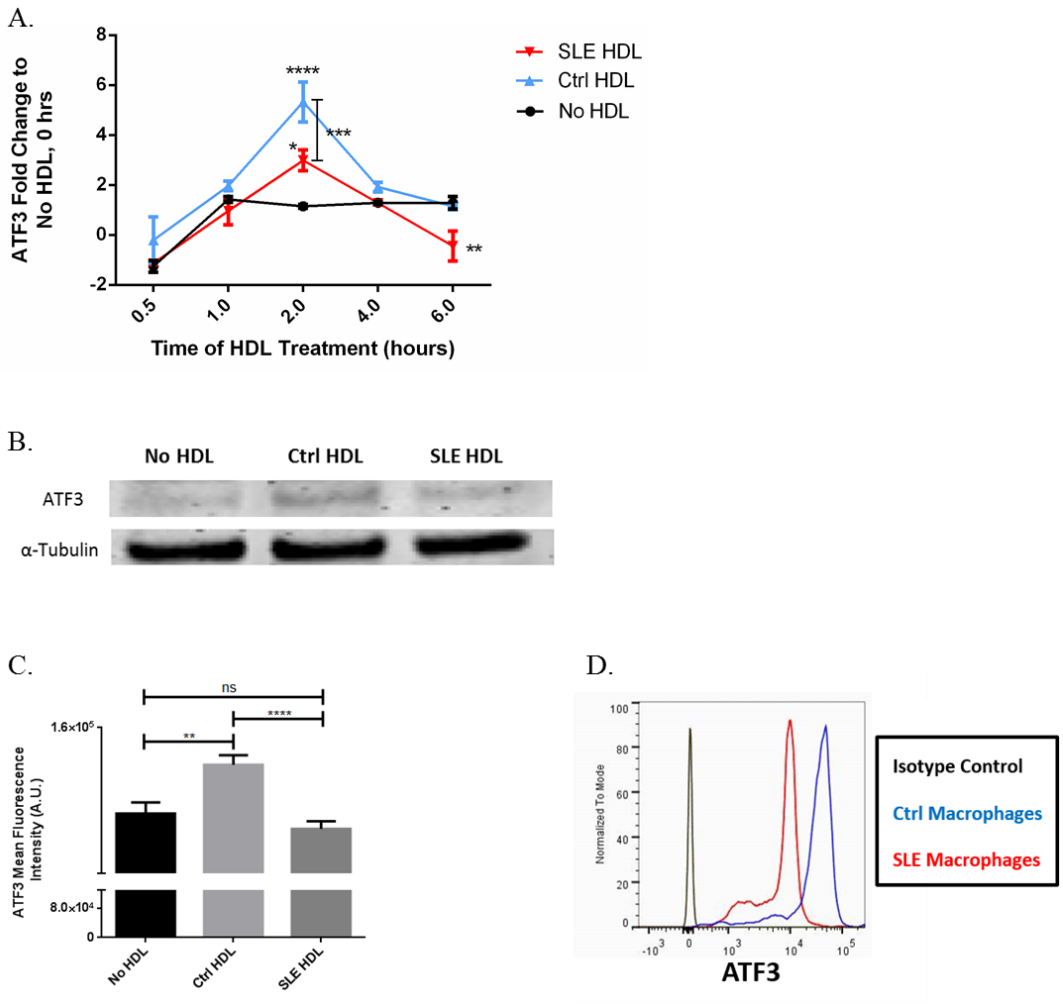


Figure 3-3. SLE HDL fails to induce ATF3 production. (A) Ctrl macrophages were treated with no, Ctrl or SLE HDL and examined for ATF3 mRNA levels by RT-qPCR. (B) Ctrl macrophages were treated with no, Ctrl or SLE HDL and lysates stained examined for ATF3 protein by immunoblot. Intracellular ATF3 was quantified by flow cytometry (C and D) in (C) Ctrl monocytes incubated with no, Ctrl or SLE HDL and (D) Ctrl and SLE macrophages. (N=7, Data are displayed as mean ± SEM, * p<0.05, ** p<0.01, *** p<0.0005, **** p<0.0001)

The Binding of Lupus HDL to LOX1R Prevents the Nuclear Translocation of ATF3 and Promotes Inflammatory Responses in Macrophages.

As ATF3 nuclear translocation is key to its activity, we examined the effect of lupus HDL on ATF3 intracellular mobilization. While Ctrl HDL-treated Ctrl macrophages demonstrated significant ATF3 nuclear translocation, SLE HDL treatment failed to promote ATF3 activation

(Figure 3-4A). One possible explanation for how SLE HDL blocks ATF3 nuclear translocation is the degradation of ATF3 protein and mRNA. However, as differences in ATF3 protein nuclear translocation can be detected as early as 30 minutes after challenging with HDL, this explanation is less likely.

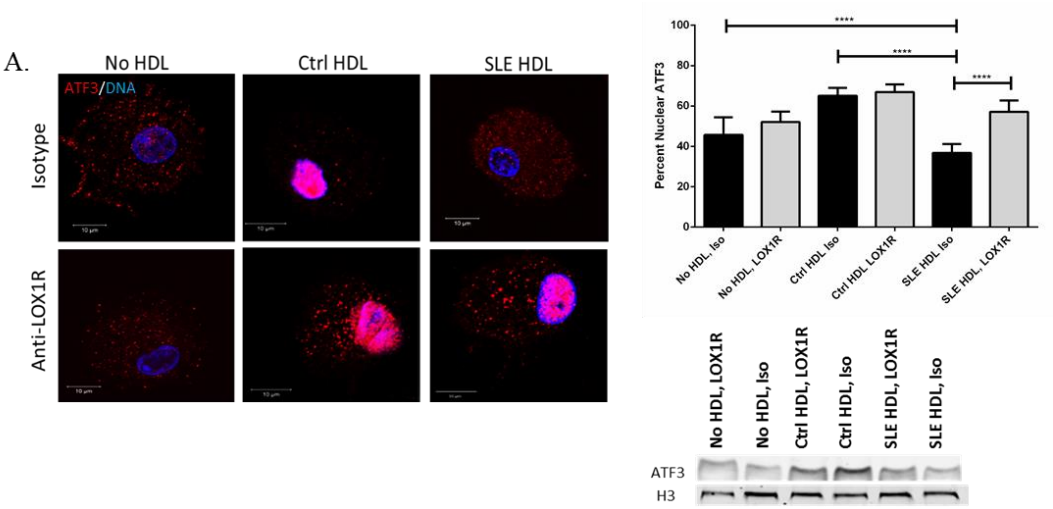
Previous work demonstrated that HDL oxidized by MPO or purified from patients with established CAD acquires the ability to bind LOX1R, and $CuSO_4$-oxidized HDL increases NFκB activation, and both TNF and LOX1R production [553-555]. While lectin-like oxidized LDL receptor 1 (LOX1R) expression has primarily been reported on endothelial cells, we and others find that it is also expressed on monocytes and its levels increase during differentiation into macrophages (data not shown) [556-558]. As lupus HDL contains enhanced levels of MPO-catalyzed 3-ClY and 3-NY, we assessed if the pro-inflammatory effects of lupus HDL required its binding to LOX1R [529, 543].

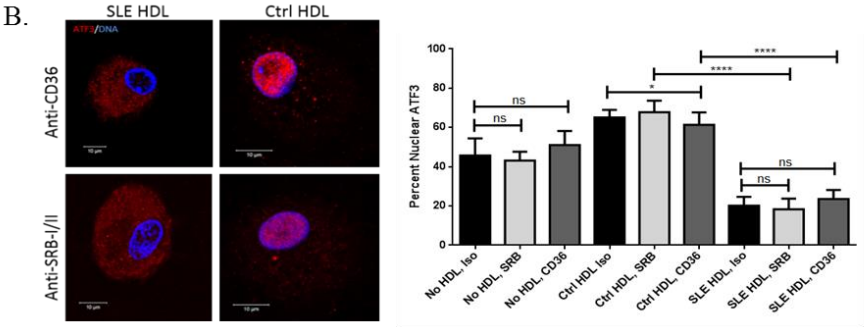
When we incubated healthy macrophages with blocking anti-LOX1R or isotype control antibodies prior to addition of SLE or Ctrl HDL and examined ATF3 nuclear translocation, we found that by preventing the binding of SLE oxHDL to LOX1R we restored ATF3 nuclear translocation (Figure 3-4A). Ctrl HDL-induced ATF3 activation was not affected by anti-LOX1R blocking antibody treatment. It is possible that SLE HDL binding to LOX1R activates an immediate signaling event which results in the inactivation of ATF3. Little is known about which post-translational modifications occur to ATF3, but future work should focus on how these modifications may affect the protein's activity.

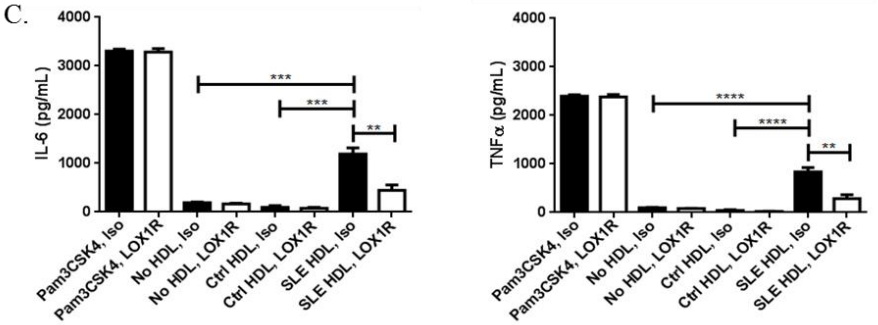
When we tried blocking other scavenger receptors previously implicated in binding oxidized HDL (CD36 and SRB-I) we saw no significant change in SLE HDL's effect on ATF3 activity (Figure 3-4B). It remains unclear which scavenger receptor binds healthy HDL to promote

ATF3 and block inflammation. Given that blocking CD36 decreased the induction of ATF3 by Ctrl HDL, however, it is possible that this scavenge receptor may play a role in the immunoregulatory roles of normal HDL function (Figure 3-4B). Overall, these results suggest that SLE HDL preferentially binds to LOX1R and initiates a pathway that leads to blockade of ATF3 activity.

Finally, Ctrl macrophages were incubated with blocking anti-LOX1R or isotype control antibody followed by SLE or Ctrl HDL for 18 hours, and supernatant IL-6 and TNF levels were measured by ELISA. LOX1R blockade significantly decreased the amount of IL-6 and TNF released due to lupus HDL treatment (Figure 3-4C). Blocking LOX1R prior to addition of SLE also decreased NFκB activation (p65 nuclear translocation, Figure 3-4D). These results indicate that lupus HDL engagement with LOX1R blocks the activity of ATF3, enhances NFκB activation, and promotes inflammatory cytokine synthesis by macrophages.


A.
No HDL
Ctrl HDL
SLE HDL
ATF3/DNA
Isotype
Anti-LOX1R
Percent Nuclear ATF3
No HDL, Iso
No HDL, LOX1R
Ctrl HDL Iso
Ctrl HDL LOX1R
SLE HDL Iso
SLE HDL, LOX1R
No HDL, LOX1R
No HDL, Iso
Ctrl HDL, LOX1R
Ctrl HDL, Iso
SLE HDL, LOX1R
SLE HDL, Iso
ATF3
H3


B.
SLE HDL
Ctrl HDL
Anti-CD36
Anti-SRB-I/II
Percent Nuclear ATF3
ns
No HDL, Iso
No HDL, SRB
No HDL, CD36
Ctrl HDL Iso
Ctrl HDL, SRB
Ctrl HDL, CD36
SLE HDL, Iso
SLE HDL, SRB
SLE HDL, CD36


C.
IL-6 (pg/mL)
TNFα (pg/mL)
Pam3CSK4, Iso
Pam3CSK4, LOX1R
No HDL, Iso
No HDL, LOX1R
Ctrl HDL, Iso
Ctrl HDL, LOX1R
SLE HDL, Iso
SLE HDL, LOX1R

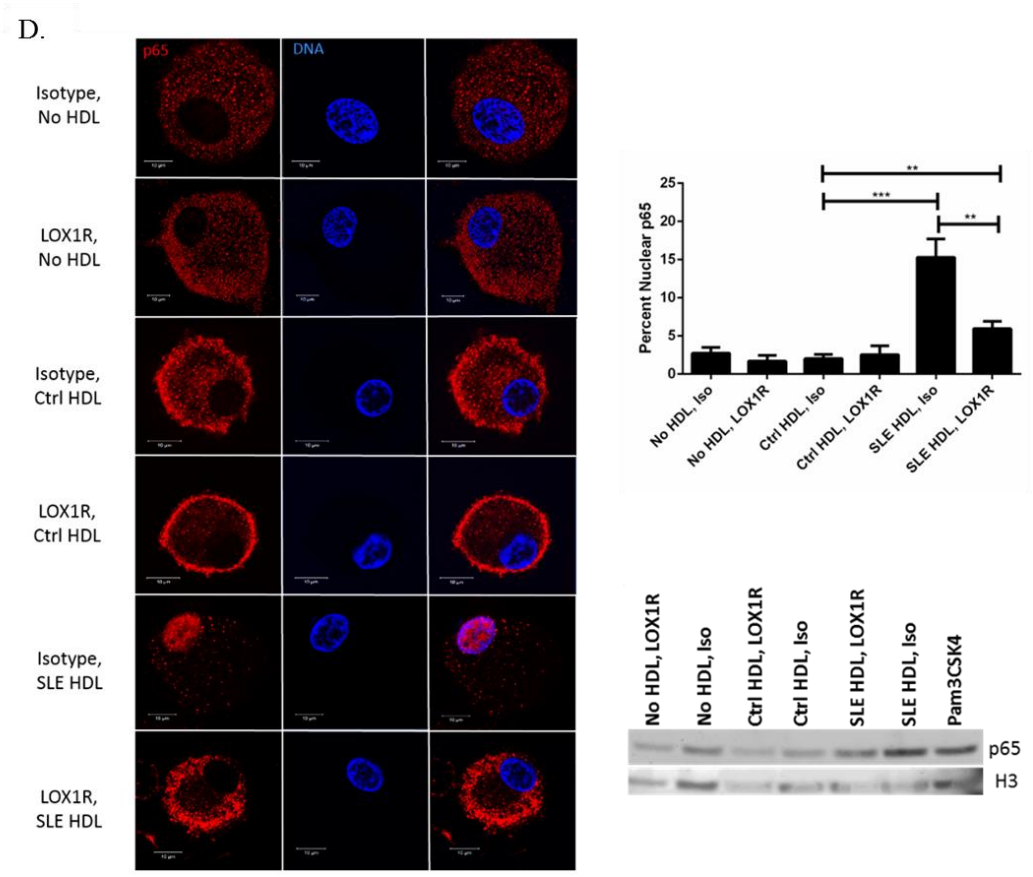


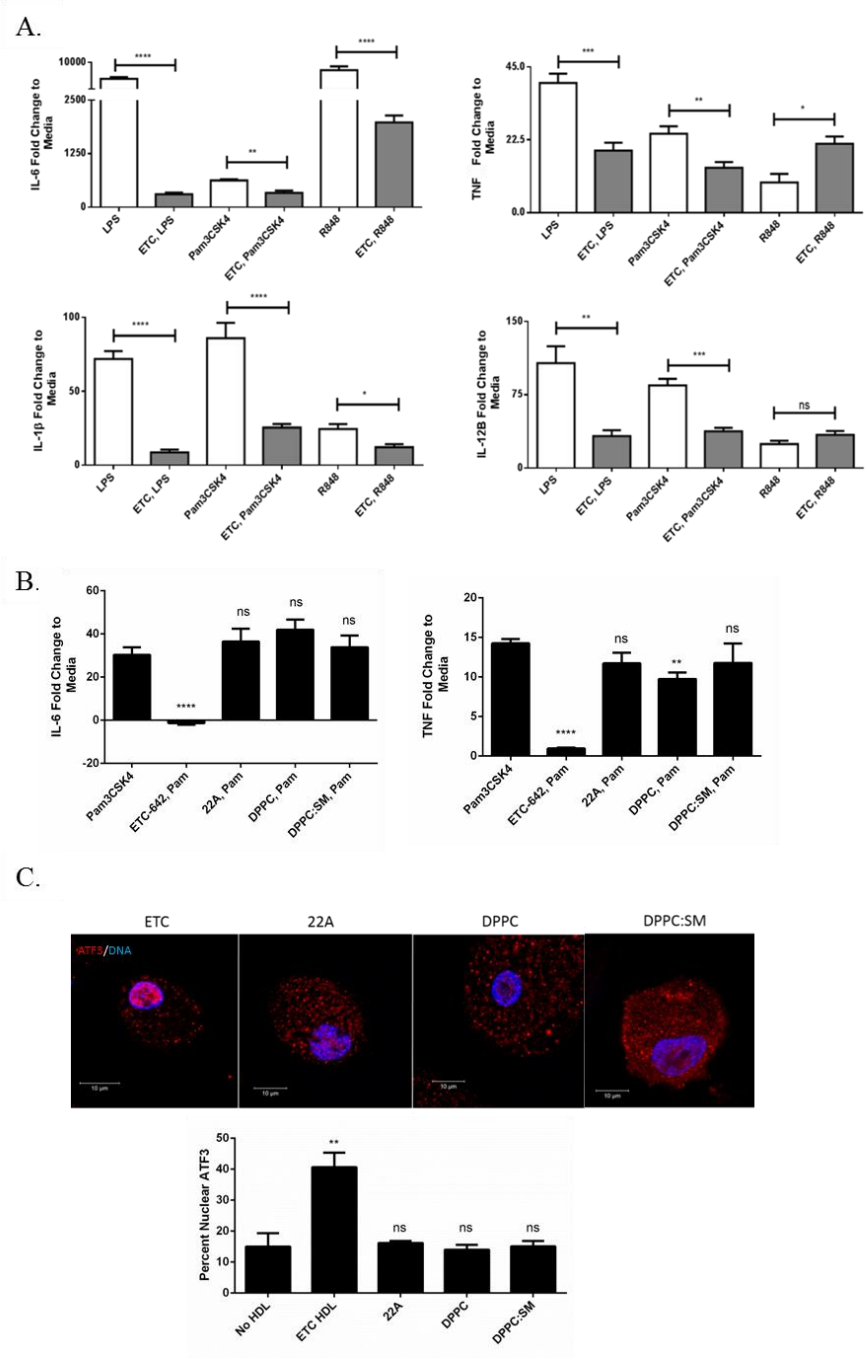
Figure 3-4. SLE HDL binding to LOX1R fails to promote ATF3 nuclear translocation and induces inflammatory cytokines and NFκB activation. (A) Ctrl macrophages were incubated in the absence or presence of isotype or anti-LOX1R blocking antibodies prior to no, Ctrl or SLE HDL incubation, and examined for ATF3 nuclear localization (N=7). (B) Ctrl macrophages were incubated with isotype or anti-CD36 or anti-SRB-I blocking antibodies prior to Ctrl or SLE HDL incubation, and examined for ATF3 nuclear localization (N=3). (C) Ctrl macrophages were incubated in the absence or presence of isotype or anti-LOX1R blocking antibodies prior to Pam3CSK4, no, Ctrl or SLE HDL incubation, and examined for IL-6 and TNF production by ELISA (N=5). (D) Ctrl macrophages were incubated with isotype or anti-LOX1R blocking antibodies prior to no, Ctrl or SLE HDL incubation, and examined for p65 nuclear localization (NFκB activation, N=7). (Data are displayed as mean ± SEM, * $p<0.05$, ** $p<0.01$, *** $p<0.0005$, **** $p<0.0001$)

The HDL Mimetic ETC-642 Abrogates the Inflammatory Effects Induced by Lupus HDL.

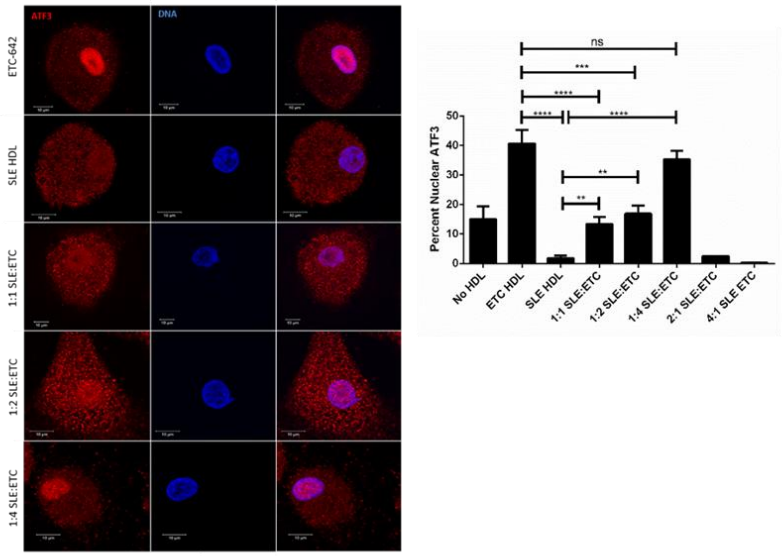
ETC-642 is a HDL mimetic compound composed of a 22 amino acid sequence (22A) derived from apoA-I, the most abundant protein in HDL, and the phospholipids 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), and sphingomyelin (SM). This compound improves cardiovascular parameters in rabbit models and has been shown to mobilize cholesterol in plasma

compartments following infusion in dyslipidemic patients.[512, 546, 559] Given that this compound mimics some of the anti-inflammatory effects of HDL, we assessed its role in down-regulating the deleterious effects of lupus HDL.

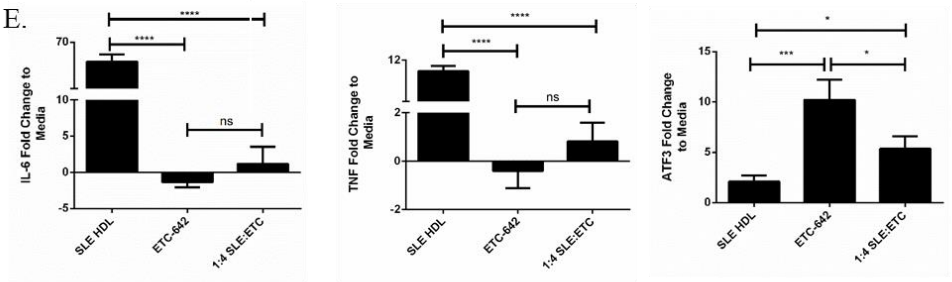
ETC-642 blocked TLR-induced inflammatory cytokine production in Ctrl macrophages (Figure 3-5A). In contrast, the individual protein or lipid components of ETC-642 did not block IL-6 or TNF mRNA induction by TLR agonists (Figure 3-5B). Similarly, ATF3 nuclear translocation was induced by ETC-642, but not by its individual components (Figure 3-5C).To address if ETC-642 could reverse the pro-inflammatory effects of SLE HDL, Ctrl macrophages were exposed to various ratios of ETC-642 and SLE HDL, then ATF3 nuclear translocation was assessed. The 1:4 SLE HDL:ETC-642 ratio significantly enhanced ATF3 nuclear translocation (Figure 3-5D) and blocked SLE HDL-induced IL-6 and TNF mRNA up-regulation (Figure 3-5E). Similarly, SLE HDL:ETC-642 1:4 increased ATF3 at the mRNA (Figure 3-5E) and protein levels (Figure 3-5F). ETC-642 and the SLE HDL:ETC-642 1:4 mixture also blocked NFκB activation (Figure 3-5G). Overall, these results indicate that ETC-642 can hamper macrophage inflammation induced by lupus HDL by mimicking the effects of healthy HDL on ATF3 and NFκB.


A.
IL-6 Fold Change to Media
10000
2500
1250
0
****
**
****
LPS
ETC, LPS
Pam3CSK4
ETC, Pam3CSK4
R848
ETC, R848
TNF Fold Change to Media
45.0
22.5
0.0
***
**
*
IL-1β Fold Change to Media
100
50
0
****
****
*
IL-12B Fold Change to Media
150
75
0
**
***
ns
B.
IL-6 Fold Change to Media
60
40
20
0
-20
ns
ns
ns
****
Pam3CSK4
ETC-642, Pam
22A, Pam
DPPC, Pam
DPPC:SM, Pam
TNF Fold Change to Media
20
15
10
5
0
ns
**
****
C.
ETC
22A
DPPC
DPPC:SM
ATF3/DNA
Percent Nuclear ATF3
50
40
30
20
10
0
**
ns
ns
ns
No HDL
ETC HDL
22A
DPPC
DPPC:SM

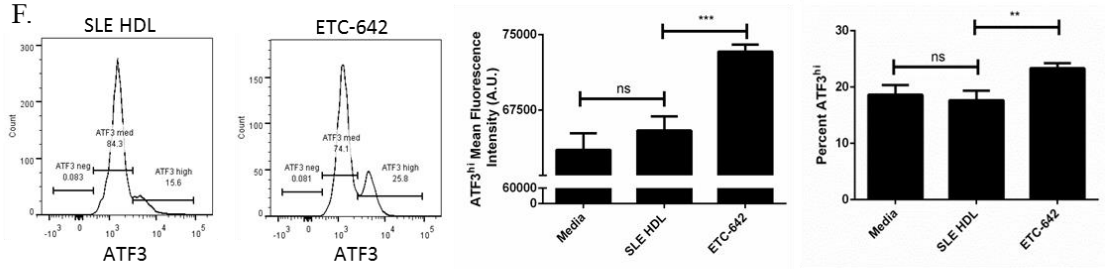
D.


ATF3
DNA
ETC-642
SLE HDL
1:1 SLE:ETC
1:2 SLE:ETC
1:4 SLE:ETC
Percent Nuclear ATF3
No HDL
ETC HDL
SLE HDL
1:1 SLE:ETC
1:2 SLE:ETC
1:4 SLE:ETC
2:1 SLE:ETC
4:1 SLE:ETC

E.


IL-6 Fold Change to Media
TNF Fold Change to Media
ATF3 Fold Change to Media
SLE HDL
ETC-642
1:4 SLE:ETC

F.


SLE HDL
ETC-642
ATF3 med
84.3
ATF3 neg
0.083
ATF3 high
15.6
ATF3 med
74.1
ATF3 neg
0.081
ATF3 high
25.8
ATF3
Count
ATF3hi Mean Fluorescence Intensity (A.U.)
Percent ATF3hi
Media
SLE HDL
ETC-642

G.

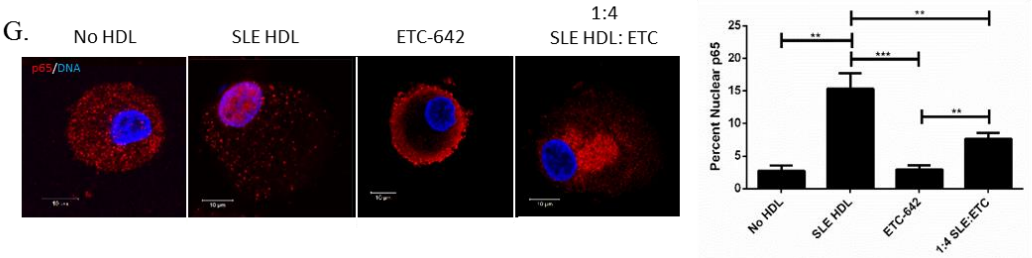

No HDL
SLE HDL
ETC-642
1:4
SLE HDL: ETC
p65/DNA
Percent Nuclear p65
No HDL
SLE HDL
ETC-642
1:4 SLE:ETC

Figure 3-5. ETC-642 blocks SLE HDL and TLR-induced inflammatory responses. Ctrl macrophages were treated with (A) ETC-642 prior to challenge with TLR agonists (LPS, Pam3CSK4 and R848) or (B) with the individual components of ETC-642 before Pam3CSK4 challenge and examined for inflammatory cytokine mRNA levels (N=5). Ctrl macrophages were treated with (C) individual components of ETC-642 or (D) various SLE HDL:ETC-642 ratios and examined for nuclear ATF3 (N=7). (E) Ctrl macrophages were treated with SLE HDL, ETC-642 or a 1:4 SLE:ETC ratio and examined for IL-6, TNF and ATF3 mRNA levels by RT-qPCR (N=5). (F) Ctrl monocytes were incubated with SLE HDL or ETC-642 and examined for intracellular ATF3 by flow cytometry (N=5). (G) Ctrl macrophages were treated with SLE HDL, ETC-642 or a 1:4 SLE:ETC and examined for nuclear p65 (NFκB activation, N=5). (Data are displayed as mean ± SEM, * $p<0.05$, ** $p<0.009$, *** $p<0.0008$, **** $p<0.0001$)

Sphingosine-1-phosphate Abrogates TLR-1/2 Induced Inflammation.

The HDL_3 subclass has been identified as one of the most vasoprotective versions of HDL [560, 561]. This may be attributed to its high concentration of the sphingolipid sphingosine-1-phosphate (S1P) [324, 328, 330, 331, 541]. This molecule has been linked to M2 macrophage polarization, endothelial NO production and its levels inversely correlated with ischemic heart disease risk and CAD [331, 562-566]. We therefore examined the effect of increasing concentrations of S1P (S1P-1 0.05 nmol/mg, S1P-2 0.5 nmol/mg, S1P-3 5 nmol/mg) mixed with DPPC and 22A on inflammatory cytokine production. Macrophages pre-incubated with the highest concentration of S1P (S1P-3) prior to Pam3CSK4 challenge showed significant abrogation of IL-6 (Figure 3-6A) and TNF (Figure 3-6B) mRNA levels. S1P-3 did not abrogate the inflammatory cytokine mRNA levels as extensively as ETC-642 however. This data indicates that S1P may be beneficial for regulating inflammatory cytokine production in SLE.

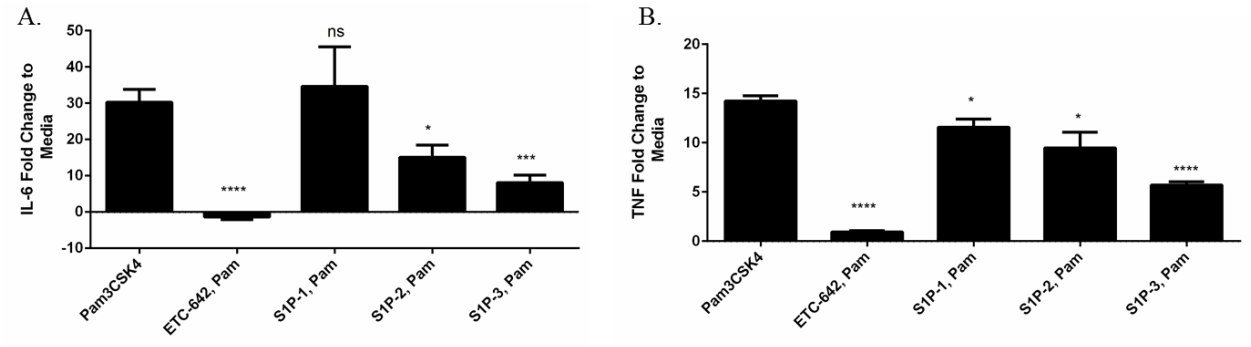


Figure 3-6. Sphingosine-1-phosphate (S1P) blocks inflammatory cytokine production. Ctrl macrophages were treated with ETC-642, its individual components, or ETC-642 with increasing concentrations of S1P (S1P-1 0.05 nmol/mg, S1P-2 0.5 nmol/mg, S1P-3 5 nmol/mg) before challenge with Pam3CSK4. Levels of (A) IL-6 and (B) TNF were quantified by RT-qPCR. Statistical comparisons made against Pam3CSK4 condition. (N=5, Data are displayed as mean ± SEM, * $p< 0.05$, ***$p<0.0003$, ****$p<0.0001$)

ETC-642 Dampens Inflammatory Cytokine Production in Lupus-prone NZM Mice.

Similar to human SLE, we previously showed that lupus-prone NZM mice display endothelial dysfunction and enhanced levels of oxidized HDL [34, 543]. We then assessed if *in vivo* systemic administration of ETC-642 could dampen inflammatory responses in female lupus-prone NZM mice. Compared to PBS-treated NZM mice, splenocytes from ETC-642-treated NZM mice displayed significant increases in ATF3 mRNA levels as well as significant decreases in mRNA levels of IL-6, TNF, IL-1β, IL-12B, and IFN stimulated genes (ISGs: MX1, ISG15, IRF-7) (Figure 3-7A). ETC-642-treated mice also showed lower IL-6 serum levels (Figure 3-7B). In contrast, ETC-642 treatment did not modify auto-antibodies or proteinuria (data not shown). Overall, these results indicate that *in vivo* administration of the HDL mimetic ETC-642 can dampen inflammatory responses in lupus.

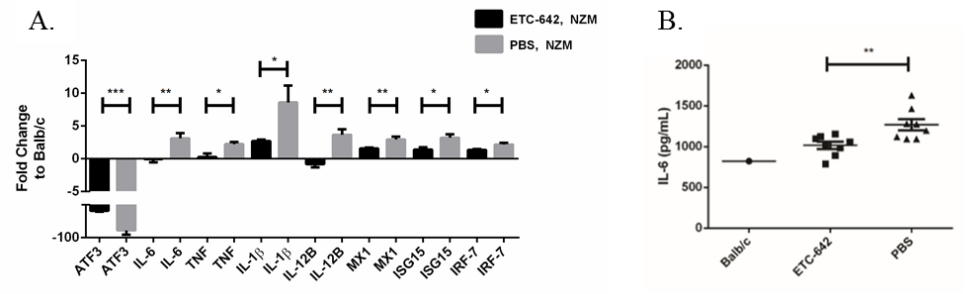


Figure 3-7. ETC-642 blocks *in vivo* inflammatory cytokine production in lupus-prone NZM2328 (NZM) mice. Lupus-prone NZM mice were treated with PBS or ETC-642 (15 mg/kg, 3x per week, i.v.) by tail vein injection for 13 weeks. (A) Splenocytes were examined for ATF3, inflammatory cytokines and interferon stimulated genes by RT-qPCR. (B) Serum IL-6 levels were quantified by ELISA. (N=8 mice, Data are displayed as mean ± SEM, * $p<0.05$, ** $p<0.009$, *** $p<0.0008$)

D. Summary

We sought to determine if the enhanced oxidation status of SLE HDL affects macrophage innate inflammatory responses related to CVD. We found that, in contrast to the anti-inflammatory effects of Ctrl HDL, Ctrl macrophage exposure to SLE HDL enhanced pro-inflammatory cytokine production (in the absence of TLR agonists) and failed to block TLR-induced cytokine production. As the SLE HDL proved to be pro-inflammatory in the absence of a TLR agonist, did not show any effect on TLR mobilization, but directly promoted NFκB activation, we explored TLR-independent signaling pathways involved in the pro-inflammatory effects of lupus HDL. We examined LOX1R's influence on SLE HDL-induced inflammation as it is one scavenger receptor linked to the pro-inflammatory properties of oxidized lipoproteins. Indeed, the SLE HDL-induced NFκB activation, cytokine production, and ATF3 repression was largely dependent on SLE HDL binding to LOX1R.

ETC-642 was able to overcome the inflammatory effects of SLE HDL in human macrophages. The individual components of ETC-642 (22A, DPPC and SM) had no significant effect on ATF3 or NFκB activation. *In vivo* treatment of lupus-prone mice with ETC-642 also

decreased systemic inflammatory markers. In conclusion, modified lupus HDL binds to LOX1R in macrophages, leading to induction of NFκB activity and failure to induce ATF3 synthesis and activation, thereby promoting pro-inflammatory responses.

In these studies, we focused on how SLE versus Ctrl HDL affects macrophage polarization and inflammatory responses in the context of CVD. However, aberrant macrophage function in lupus may also be linked to skin flares, kidney damage and pulmonary problems [188, 567-569]. Future studies could explore whether infusion of lupus-prone mice with ETC-642 or Ctrl murine HDL dampens inflammation in each of these organ systems through altered macrophage function or phenotype. Such studies may be hard to translate to human macrophage responses, however, given the differences in macrophage polarization markers and gene activity exhibited between humans and mice [195, 354, 544]. Nonetheless, these studies may identify added benefits of ETC-642 or other apoA-I mimetics as therapies for SLE.

In our hands, ETC-642 can prevent the pro-inflammatory effects of lupus HDL using *in vitro* models and general inflammation via an *in vivo* model. Whether ETC-642 treatment also improves vascular health in lupus-prone mice has yet to be determined. Again, the lack of well-defined lupus-prone mouse models which also develop atherosclerosis means that the effect of ETC-642 on atherogenesis could not be examined. It would be possible, however, to examine its effect on endothelial cell differentiation, angiogenesis, oxidative stress, or clotting following photochemical-induced thrombosis in lupus-prone mice [34, 468]. What affect lupus oxidized HDL has on LOX1R signaling in mice is also not known.

LOX1R-/- mice, especially when crossed with *apoE-/-* or *LDLR-/-* mice, do show improved vasorelaxation, and reduced inflammatory cytokine levels, intima thickening, and plaque macrophage accumulation [570-573]. Whether LOX1R has a higher affinity for 3-ClY or 3-NY

oxidized HDL has yet to be determined. This is worth establishing given that our lupus patients' HDL showed more 3-ClY than 3-NY, yet lupus-prone NZM mice show more 3-NY oxidized HDL. This difference in HDL oxidation patterns and how LOX1R responds to each may demonstrate another atherogenic mechanism which varies between mice and humans. Yet, it would be interesting to see if lupus-prone mice treated with LOX1R neutralizing antibody demonstrate improved vascular health. Such therapies, then, could be explored for SLE patients.

Chapter 4

Conclusions

A. Summary

The aims of our study were to characterize the oxidation status of SLE plasma and HDL, and examine aberrant lupus and CVD-related innate immune mechanisms up- and downstream of this lipoprotein. We found that lupus patients have dysfunctional and highly oxidized HDL and plasma, which fits patterns linked to CVD in the general population. These measurements, therefore, should be explored as biomarkers of CVD risk in SLE. Our data indicates that the high level of NETosis in SLE can contribute to these pro-atherosclerotic modifications, especially as abrogation of NET formation in lupus-prone mice led to decreased HDL oxidation. Finally, we showed that SLE oxHDL induces pro-inflammatory and defective anti-inflammatory characteristics in cultured control macrophages. Importantly, these inflammatory pathways can be blocked in mice by treatment with an HDL mimetic. These results highlight the significance of multiple aberrant innate immune mechanisms leading to lupus atherosclerosis, and could be applicable to other chronic inflammatory diseases.

To date, most research on SLE CVD has focused on its subclinical nature and association with IFN-I, TNF, NETosis, defective anti-oxidant activity, lupus-associated medications, anti-phospholipid antibodies, and endothelial cell death [574, 575]. The CVD literature includes reports demonstrating that NETs can exert direct damage to the endothelium and contribute to coagulation cascades. Other groups have shown how oxidized lipoproteins can contribute to atherogenesis [214, 391, 399, 401, 404, 408, 409, 538, 540]. By demonstrating how lupus NET activity contributes to specific pro-

inflammatory and pro-atherosclerotic modifications to HDL, our results not only fit in with these previously proposed atherosclerotic mechanisms but also combine heretofore separated pathways and build upon known mechanisms of SLE and CVD. Our results also have special clinical significance. By demonstrating the interplay between neutrophils, macrophages and lipoprotein innate signaling pathways, we confirmed that therapies which block NET activity, such as Cl-Am, or alter the activity of SLE HDL, such as ETC-642, may both be beneficial to lupus patients. The limitations that we faced in these studies, namely patient cohort size and available lupus-CVD mouse models, may be addressed by future studies and should elaborate on our findings.

B. Future Directions

I. SLE HDL's Effect on Neutrophil Activity

Given the potent effects of lipoproteins on macrophage function and the putative important contribution of NETs and neutrophils to SLE and CVD, it will be important to determine if lipoproteins also modify neutrophil biology. Indeed, healthy HDL can affect cell death pathways, ROS generation, and gAMP activity [324, 348, 500, 512, 545, 553, 559, 576, 577]. As these pathways are associated with NET formation, it is conceivable that HDL could alter the predisposition of a neutrophil to undergo NETosis. Whether these effects are lost upon oxidative modifications to HDL, such as seen in SLE, is also unclear. Additionally, the effect of ETC-642 or other mimetics on NETosis should be further characterized. The response of other neutrophil-related functions (degranulation, phagocytosis, ROS generation, etc.) to modified and unmodified lipoproteins could be further examined. As lipoproteins in general and HDL in particular are carriers not only of plasma proteins but also of miRNAs, these molecules may hold putative "messenger" capabilities.

II. SLE HDL Composition

The initial proteomics analysis of lupus HDL revealed an enrichment of vasorin, a protein that still requires significant characterization. Whether SLE HDL has a dampening effect on TGF-β signaling should be explored given that vasorin has been proposed to down-modulate this pathway [530-532]. This may be relevant to anti-inflammatory signaling, repair mechanisms, macrophage polarization and VSMC activity relating to SLE atherogenesis. However, validation of these findings in a larger cohort of SLE patients should be studied to verify our results and identify other potential immunomodulatory proteins in HDL. For example, previous groups have stated that the circulation is not the site of HDL oxidation because of the high presence of anti-oxidative compounds in the plasma [578]. Yet, it appears that SLE patients possess less anti-oxidant activity, including low PON levels in HDL [238, 239, 247-250]. Further proteomic analysis could demonstrate low anti-oxidative enzyme levels in SLE and, if performed in conjunction with NETosis activity assays, could strongly indicate the abnormally high circulating oxidative environment in SLE patients. In addition, lipidomic analysis of HDL should be explored in SLE to assess whether some of the differences in SLE versus control signaling pathways could be explained by altered lipoprotein lipid content.

For example, previous work indicates that S1P can block TLR-induced inflammation, and promote "classical", anti-inflammatory monocyte activity [579-581]. Whether S1P levels differ between SLE and Ctrl HDL should be validated. As lipids can also be oxidized, exploring differences in lipid oxidation levels between controls and SLE, which receptors they bind, and any downstream pro- or anti-inflammatory pathways may prove revealing. This could explain why, while blockade of LOX1R significantly abrogated the inflammatory cytokine production and NFκB activation induced by SLE HDL, it did not reduce levels down to that of Ctrl HDL

treatment (Figure 3-4C and D). This lipidomic analysis and comparisons could also reveal putative therapeutic targets. Additionally, as mentioned above, HDL's effect on target cells may be affected not only by receptor binding and signaling, but through the delivery of miRNAs to target cells.

HDL is a known carrier of miRNAs, with different miRNA "libraries" found in control versus CVD donors [582]. If these HDL-associated miRNAs are delivered into target cells, this could direct cholesterol homeostasis, macrophage polarization, and SLE pathogenesis mechanisms [583-587]. It is possible that some of the differential inflammatory and anti-inflammatory effects seen between control and lupus HDL are due to the transfer of miRNAs from the HDL to the macrophages.

Future studies in larger cohorts should therefore explore the association of the protein, lipid, and perhaps mRNA cargo of lipoproteins with anti-atherogenic effects and disease activity in lupus and other inflammatory diseases. Indeed, it remains to be determined whether our findings are applicable to most chronic inflammatory diseases, are distinct to SLE, or even distinct to humans.

III. Of Mice Versus Men

Previous groups have demonstrated the importance of IFN-I signaling to SLE and CVD pathogenesis. Suzuki et al. demonstrated the ability of HDL to block IFN-I responses [368]. Importantly, this study was performed in mouse models and with murine cell lines. In our hands, we did not see a consistent effect of healthy human HDL on the blockade of ISGs in human macrophages. As with De Nardo et al., we found that IL-6, TNF and IL-12B showed the largest effects with HDL treatment [367]. Nevertheless, lupus-prone mice exposed to ETC-642 displayed down-regulation of ISGs (Figure 3-7). As such, the effect of HDL on IFN-I responses may be

species specific. It will be important to better understand the differences in lipoprotein modulation between human and murine systems to identify the best experimental design to test novel therapies that modulate and signaling abnormal lipoprotein phenotype and function in chronic inflammatory conditions. For example, it is possible that this is due to differences in scavenger receptor expression in mice versus humans, which leads to altered immune responses to HDL treatment. This would not be entirely surprising given the already known significant differences between mouse and human mechanisms of atherogenesis and macrophage polarization [287-289].

C. Implications of Studies

The results of the studies presented above highlight the important role of innate immune responses, namely aberrant interactions between neutrophils, macrophages, the oxidative machinery, and lipoproteins in the promotion of accelerated atherogenesis and increased CVD risk in chronic inflammatory diseases such as lupus (Figure 3-8). These results may also be relevant to individuals with "typical" atherosclerosis, where innate immune responses may trigger and amplify vascular damage and abnormal lipoprotein biology.

Neutrophils, in particular, were largely ignored as mediators of atherogenic inflammation and oxidation until very recently. This was mostly due to an inability to detect neutrophils in plaques. With improved techniques, not only are neutrophil markers readily detectable in atheromas, but NETs too are visible in plaques, thrombi and in circulation [40, 215, 221, 425, 426, 429, 432, 477, 540]. NETs have already been linked to coagulation pathways and endothelial cell death, but our results demonstrate an additional mechanism by which they promote atherogenesis: oxidation of lipoproteins thereby affecting their CEC and anti-inflammatory function.

Lipoproteins too have been underappreciated as mediators of immune signaling. Previous groups have shown that unmodified HDL can block TLR-4 signaling and NFκB activation, while oxLDL and oxHDL increase NFκB signaling and adhesion molecule expression [392-395, 399, 401, 409]. Our findings on the anti-inflammatory nature of healthy HDL, especially with S1P, in addition to the pro-inflammatory effect of SLE HDL support the theory of lipoprotein-mediated immune cell signaling. Furthermore, we show that lipoproteins not only affect cytokine production but also alter macrophage polarization, an idea supported by other groups [356].

A number of other diseases have been recently linked to altered neutrophil and lipoprotein activity in the context of enhanced CVD risk: rheumatoid arthritis, psoriasis, vasculitis, liver disease and renal dysfunction [529, 540, 588-593]. The mechanisms elucidated here may be relevant to those disorders as well and should be explored in future experiments. Additionally, therapies which target NET activity and lipoprotein composition should be further examined with regards to their potential beneficial athero-protective and immunomodulatory role in SLE and CVD.

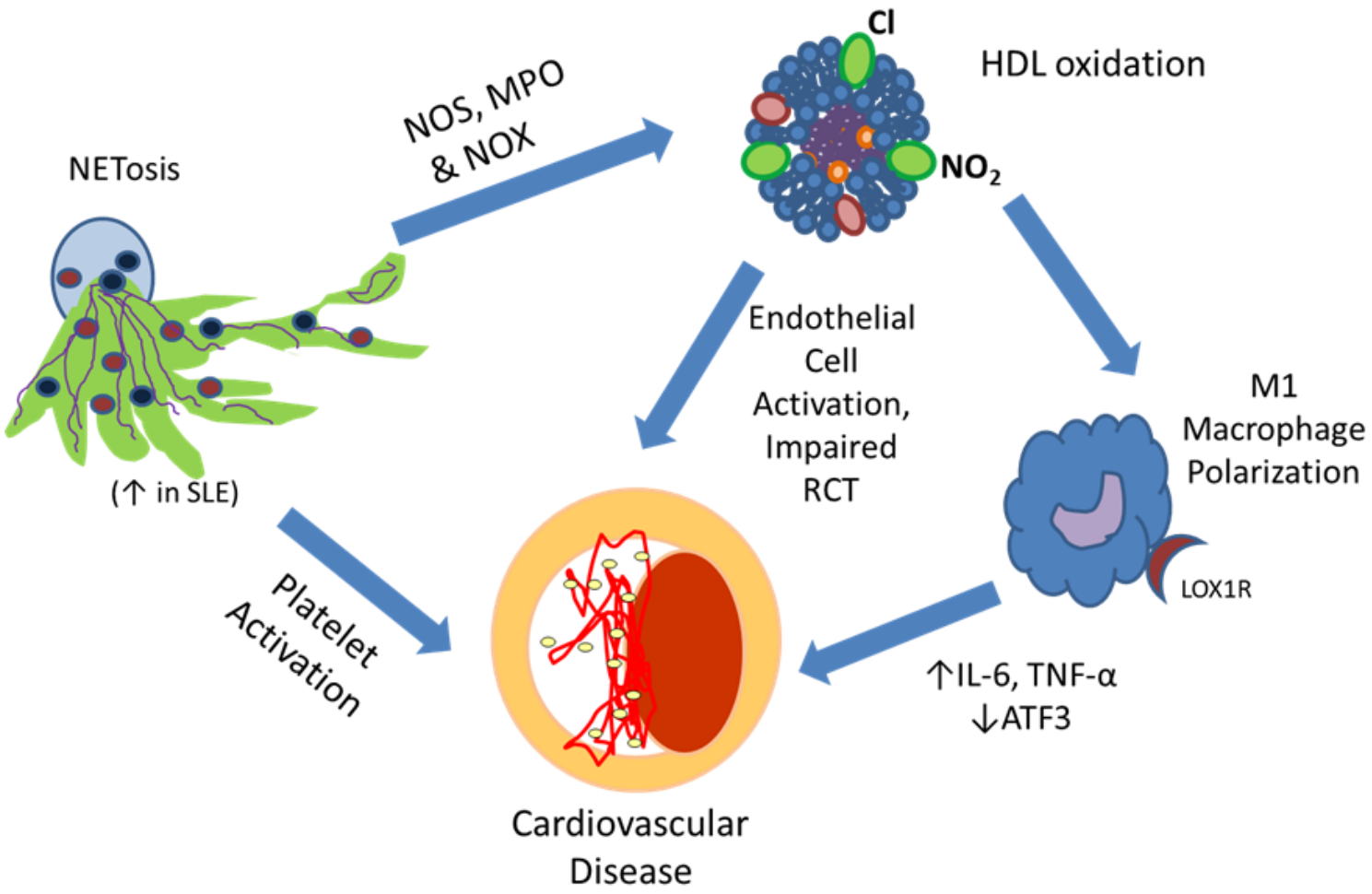


Figure 3-8. Proposed mechanism for NETosis-induced HDL oxidation and macrophage inflammation in SLE-related CVD. NET formation (which is enhanced in SLE) causes the exposure of the oxidative enzymes NOS, MPO and NOX to the periphery, where they cause 3-ClY and 3-NY HDL oxidation. This modified HDL then promotes an M1 macrophage phenotype with enhanced IL-6 and TNF production, matched with decreased ATF3 production and activation. This is largely dependent on oxidized HDL binding LOX1R. The toxic NETs, dysfunctional oxidized HDL and macrophage inflammatory cytokine production all contribute to SLE-related CVD.

References

1. Borchers AT, Naguwa SM, Shoenfeld Y, Gershwin ME. The geoepidemiology of systemic lupus erythematosus. *Autoimmunity reviews*. 2010;9:A277-287
2. McCarty DJ, Manzi S, Medsger TA, Jr., Ramsey-Goldman R, LaPorte RE, Kwoh CK. Incidence of systemic lupus erythematosus. Race and gender differences. *Arthritis and rheumatism*. 1995;38:1260-1270
3. Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, Schaller JG, Talal N, Winchester RJ. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis and rheumatism*. 1982;25:1271-1277
4. Hochberg MC. Updating the american college of rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis and rheumatism*. 1997;40:1725
5. Isenberg D, Bacon P, Bombardier C, Gladman D, Goldsmith CH, Kalunian K, Liang M, Maddison P, Nived O, Richter M, et al. Criteria for assessing disease activity in systemic lupus erythematosus. *The Journal of rheumatology*. 1989;16:1395-1396
6. von Muhlen CA, Tan EM. Autoantibodies in the diagnosis of systemic rheumatic diseases. *Seminars in arthritis and rheumatism*. 1995;24:323-358
7. Prabhakar BS, Saegusa J, Onodera T, Notkins AL. Lymphocytes capable of making monoclonal autoantibodies that react with multiple organs are a common feature of the normal b cell repertoire. *Journal of immunology (Baltimore, Md. : 1950)*. 1984;133:2815-2817

8. Thiagarajan P, Shapiro SS, De Marco L. Monoclonal immunoglobulin m lambda coagulation inhibitor with phospholipid specificity. Mechanism of a lupus anticoagulant. *The Journal of clinical investigation*. 1980;66:397-405

9. Conley CL HR. A hemorrhagic disorder caused by circulating anticoagulant in patients with disseminated lupus erythematosus. *J Lab Clin Invest*. 1952;31:621-622

10. Harris EN, Gharavi AE, Boey ML, Patel BM, Mackworth-Young CG, Loizou S, Hughes GR. Anticardiolipin antibodies: Detection by radioimmunoassay and association with thrombosis in systemic lupus erythematosus. *Lancet*. 1983;2:1211-1214

11. McNeil HP, Simpson RJ, Chesterman CN, Krilis SA. Anti-phospholipid antibodies are directed against a complex antigen that includes a lipid-binding inhibitor of coagulation: Beta 2-glycoprotein i (apolipoprotein h). *Proceedings of the National Academy of Sciences of the United States of America*. 1990;87:4120-4124

12. Gueirard P, Delpech A, Gilbert D, Godin M, Le Loet X, Tron F. Anti-myeloperoxidase antibodies: Immunological characteristics and clinical associations. *Journal of autoimmunity*. 1991;4:517-527

13. Firooz N, Albert DA, Wallace DJ, Ishimori M, Berel D, Weisman MH. High-sensitivity c-reactive protein and erythrocyte sedimentation rate in systemic lupus erythematosus. *Lupus*. 2011;20:588-597

14. Stojan G, Fang H, Magder L, Petri M. Erythrocyte sedimentation rate is a predictor of renal and overall sle disease activity. *Lupus*. 2013;22:827-834

15. Ytterberg SR, Schnitzer TJ. Serum interferon levels in patients with systemic lupus erythematosus. *Arthritis and rheumatism*. 1982;25:401-406

16. Palanichamy A, Bauer JW, Yalavarthi S, Meednu N, Barnard J, Owen T, Cistrone C, Bird A, Rabinovich A, Nevarez S, Knight JS, Dedrick R, Rosenberg A, Wei C, Rangel-Moreno J, Liesveld J, Sanz I, Baechler E, Kaplan MJ, Anolik JH. Neutrophil-mediated ifn activation in the bone marrow alters b cell development in human and murine systemic lupus erythematosus. *Journal of immunology (Baltimore, Md. : 1950)*. 2014;192:906-918

17. Robak E, Sysa-Jedrzejowska A, Robak T, Stepien H, Wozniacka A, Waszczykowsk E. Tumour necrosis factor alpha (tnf-alpha), interleukin-6 (il-6) and their soluble receptors (stnf-alpha-rp55 and sll-6r) serum levels in systemic lupus erythematodes. *Mediators of inflammation*. 1996;5:435-441

18. Meijer C, Huysen V, Smeenk RT, Swaak AJ. Profiles of cytokines (tnf alpha and il-6) and acute phase proteins (crp and alpha 1ag) related to the disease course in patients with systemic lupus erythematosus. *Lupus*. 1993;2:359-365

19. Linker-Israeli M, Deans RJ, Wallace DJ, Prehn J, Ozeri-Chen T, Klinenberg JR. Elevated levels of endogenous il-6 in systemic lupus erythematosus. A putative role in pathogenesis. *Journal of immunology (Baltimore, Md. : 1950)*. 1991;147:117-123

20. Hejtmancik MR, Wright JC, Quint R, Jennings FL. The cardiovascular manifestations of systemic lupus erythematosus. *American heart journal*. 1964;68:119-130

21. Manzi S, Meilahn EN, Rairie JE, Conte CG, Medsger TA, Jr., Jansen-McWilliams L, D'Agostino RB, Kuller LH. Age-specific incidence rates of myocardial infarction and angina in women with systemic lupus erythematosus: Comparison with the framingham study. *American journal of epidemiology*. 1997;145:408-415

22. Yap DY, Tang CS, Ma MK, Lam MF, Chan TM. Survival analysis and causes of mortality in patients with lupus nephritis. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association*. 2012;27:3248-3254

23. Lerang K, Gilboe IM, Steinar Thelle D, Gran JT. Mortality and years of potential life loss in systemic lupus erythematosus: A population-based cohort study. *Lupus*. 2014;23:1546-1552

24. Mak A, Cheung MW, Chiew HJ, Liu Y, Ho RC. Global trend of survival and damage of systemic lupus erythematosus: Meta-analysis and meta-regression of observational studies from the 1950s to 2000s. *Seminars in arthritis and rheumatism*. 2012;41:830-839

25. Esdaile JM, Abrahamowicz M, Grodzicky T, Li Y, Panaritis C, du Berger R, Cote R, Grover SA, Fortin PR, Clarke AE, Senecal JL. Traditional framingham risk factors fail to fully account for accelerated atherosclerosis in systemic lupus erythematosus. *Arthritis and rheumatism*. 2001;44:2331-2337

26. Kashef S, Ghaedian MM, Rajaee A, Ghaderi A. Dyslipoproteinemia during the active course of systemic lupus erythematosus in association with anti-double-stranded DNA (anti-dsdna) antibodies. *Rheumatology international*. 2007;27:235-241

27. Scalzi LV, Hollenbeak CS, Wang L. Racial disparities in age at time of cardiovascular events and cardiovascular-related death in patients with systemic lupus erythematosus. *Arthritis and rheumatism*. 2010;62:2767-2775

28. Doeing DC, Borowicz JL, Crockett ET. Gender dimorphism in differential peripheral blood leukocyte counts in mice using cardiac, tail, foot, and saphenous vein puncture methods. *BMC clinical pathology*. 2003;3:3

29. Mestas J, Hughes CC. Of mice and not men: Differences between mouse and human immunology. *Journal of immunology (Baltimore, Md. : 1950)*. 2004;172:2731-2738

30. Zhuang H, Szeto C, Han S, Yang L, Reeves WH. Animal models of interferon signature positive lupus. *Frontiers in immunology*. 2015;6:291

31. Perry D, Sang A, Yin Y, Zheng Y-Y, Morel L. Murine models of systemic lupus erythematosus. *Journal of Biomedicine and Biotechnology*. 2011;2011

32. Waters ST, Fu SM, Gaskin F, Deshmukh US, Sung SS, Kannapell CC, Tung KS, McEwen SB, McDuffie M. Nzm2328: A new mouse model of systemic lupus erythematosus with unique genetic susceptibility loci. *Clinical immunology (Orlando, Fla.)*. 2001;100:372-383

33. Knight JS, Zhao W, Luo W, Subramanian V, O'Dell AA, Yalavarthi S, Hodgin JB, Eitzman DT, Thompson PR, Kaplan MJ. Peptidylarginine deiminase inhibition is immunomodulatory and vasculoprotective in murine lupus. *The Journal of clinical investigation*. 2013;123:2981-2993

34. Thacker SG, Zhao W, Smith CK, Luo W, Wang H, Vivekanandan-Giri A, Rabquer BJ, Koch AE, Pennathur S, Davidson A, Eitzman DT, Kaplan MJ. Type i interferons modulate vascular function, repair, thrombosis, and plaque progression in murine models of lupus and atherosclerosis. *Arthritis and rheumatism*. 2012;64:2975-2985

35. Agrawal H, Jacob N, Carreras E, Bajana S, Putterman C, Turner S, Neas B, Mathian A, Koss MN, Stohl W, Kovats S, Jacob CO. Deficiency of type i ifn receptor in lupus-prone new zealand mixed 2328 mice decreases dendritic cell numbers and activation and protects from disease. *Journal of immunology (Baltimore, Md. : 1950)*. 2009;183:6021-6029

36. Murphy ED, Roths JB. A single gene model for massive lymphoproliferation with immune complex disease in new mouse strain mrl. *Proceedings of the 16th International Congress in Hematology. Excerpta Medica, Amsterdam*. 1977;69-72

37. Amoura Z, Chabre H, Koutouzov S, Lotton C, Cabrespines A, Bach JF, Jacob L. Nucleosome-restricted antibodies are detected before anti-dsdna and/or antihistone antibodies in serum of mrl-mp lpr/lpr and +/+ mice, and are present in kidney eluates of lupus mice with proteinuria. *Arthritis and rheumatism*. 1994;37:1684-1688

38. Roths JB, Murphy ED, Eicher EM. A new mutation, gld, that produces lymphoproliferation and autoimmunity in c3h/hej mice. *The Journal of experimental medicine*. 1984;159:1-20

39. Tarzi RM, Sharp PE, McDaid JP, Fossati-Jimack L, Herbert PE, Pusey CD, Cook HT, Warrens AN. Mice with defective fas ligand are protected from crescentic glomerulonephritis. *Kidney international*. 2012;81:170-178

40. Knight JS, Subramanian V, O'Dell AA, Yalavarthi S, Zhao W, Smith CK, Hodgin JB, Thompson PR, Kaplan MJ. Peptidylarginine deiminase inhibition disrupts net formation and protects against kidney, skin and vascular disease in lupus-prone mrl/lpr mice. *Annals of the rheumatic diseases*. 2014

41. Kuo CF, Grainge MJ, Valdes AM, See LC, Luo SF, Yu KH, Zhang W, Doherty M. Familial aggregation of systemic lupus erythematosus and coaggregation of autoimmune diseases in affected families. *JAMA internal medicine*. 2015

42. Shen N, Fu Q, Deng Y, Qian X, Zhao J, Kaufman KM, Wu YL, Yu CY, Tang Y, Chen JY, Yang W, Wong M, Kawasaki A, Tsuchiya N, Sumida T, Kawaguchi Y, Howe HS, Mok MY, Bang SY, Liu FL, Chang DM, Takasaki Y, Hashimoto H, Harley JB,

Guthridge JM, Grossman JM, Cantor RM, Song YW, Bae SC, Chen S, Hahn BH, Lau YL, Tsao BP. Sex-specific association of x-linked toll-like receptor 7 (tlr7) with male systemic lupus erythematosus. *Proceedings of the National Academy of Sciences of the United States of America*. 2010;107:15838-15843

43. Yamada M, Oritani K, Kaisho T, Ishikawa J, Yoshida H, Takahashi I, Kawamoto S, Ishida N, Ujiie H, Masaie H, Botto M, Tomiyama Y, Matsuzawa Y. Complement c1q regulates lps-induced cytokine production in bone marrow-derived dendritic cells. *European journal of immunology*. 2004;34:221-230

44. Jacob CO, Zhu J, Armstrong DL, Yan M, Han J, Zhou XJ, Thomas JA, Reiff A, Myones BL, Ojwang JO, Kaufman KM, Klein-Gitelman M, McCurdy D, Wagner-Weiner L, Silverman E, Ziegler J, Kelly JA, Merrill JT, Harley JB, Ramsey-Goldman R, Vila LM, Bae SC, Vyse TJ, Gilkeson GS, Gaffney PM, Moser KL, Langefeld CD, Zidovetzki R, Mohan C. Identification of irak1 as a risk gene with critical role in the pathogenesis of systemic lupus erythematosus. *Proceedings of the National Academy of Sciences of the United States of America*. 2009;106:6256-6261

45. Graham RR, Kyogoku C, Sigurdsson S, Vlasova IA, Davies LR, Baechler EC, Plenge RM, Koeuth T, Ortmann WA, Hom G, Bauer JW, Gillett C, Burtt N, Cunninghame Graham DS, Onofrio R, Petri M, Gunnarsson I, Svenungsson E, Ronnblom L, Nordmark G, Gregersen PK, Moser K, Gaffney PM, Criswell LA, Vyse TJ, Syvanen AC, Bohjanen PR, Daly MJ, Behrens TW, Altshuler D. Three functional variants of ifn regulatory factor 5 (irf5) define risk and protective haplotypes for human lupus. *Proceedings of the National Academy of Sciences of the United States of America*. 2007;104:6758-6763

46. Pontillo A, Girardelli M, Kamada AJ, Pancotto JA, Donadi EA, Crovella S, Sandrin-Garcia P. Polimorphisms in inflammasome genes are involved in the predisposition to systemic lupus erythematosus. *Autoimmunity*. 2012;45:271-278

47. Zhang J, Zhu Q, Meng F, Lei H, Zhao Y. Association study of tlr-9 polymorphisms and systemic lupus erythematosus in northern chinese han population. *Gene*. 2014;533:385-388

48. Hawn TR, Wu H, Grossman JM, Hahn BH, Tsao BP, Aderem A. A stop codon polymorphism of toll-like receptor 5 is associated with resistance to systemic lupus erythematosus. *Proceedings of the National Academy of Sciences of the United States of America*. 2005;102:10593-10597

49. Demirci FY, Manzi S, Ramsey-Goldman R, Kenney M, Shaw PS, Dunlop-Thomas CM, Kao AH, Rhew EY, Bontempo F, Kammerer C, Kamboh MI. Association study of toll-like receptor 5 (tlr5) and toll-like receptor 9 (tlr9) polymorphisms in systemic lupus erythematosus. *The Journal of rheumatology*. 2007;34:1708-1711

50. Sanchez E, Callejas-Rubio JL, Sabio JM, Gonzalez-Gay MA, Jimenez-Alonso J, Mico L, de Ramon E, Camps M, Suarez A, Gutierrez C, Garcia-Portales R, Tolosa C, Ortego-Centeno N, Sanchez-Roman J, Garcia-Hernandez FJ, Gonzalez-Escribano MF, Martin J. Investigation of tlr5 and tlr7 as candidate genes for susceptibility to systemic lupus erythematosus. *Clinical and experimental rheumatology*. 2009;27:267-271

51. Laska MJ, Troldborg A, Hansen B, Stengaard-Pedersen K, Junker P, Nexo BA, Voss A. Polymorphisms within toll-like receptors are associated with systemic lupus erythematosus in a cohort of danish females. *Rheumatology (Oxford, England)*. 2014;53:48-55

52. Cunninghame Graham DS, Morris DL, Bhangale TR, Criswell LA, Syvanen AC, Ronnblom L, Behrens TW, Graham RR, Vyse TJ. Association of ncf2, ikzf1, irf8, ifih1, and tyk2 with systemic lupus erythematosus. *PLoS genetics*. 2011;7:e1002341

53. Sigurdsson S, Nordmark G, Goring HH, Lindroos K, Wiman AC, Sturfelt G, Jonsen A, Rantapaa-Dahlqvist S, Moller B, Kere J, Koskenmies S, Widen E, Eloranta ML, Julkunen H, Kristjansdottir H, Steinsson K, Alm G, Ronnblom L, Syvanen AC. Polymorphisms in the tyrosine kinase 2 and interferon regulatory factor 5 genes are associated with systemic lupus erythematosus. *American journal of human genetics*. 2005;76:528-537

54. Li SW, He Y, Zheng ZH, Liu DW, Liu ZS. Single-nucleotide polymorphisms of irf8 gene are associated with systemic lupus erythematosus in chinese han population. *International journal of immunogenetics*. 2014;41:112-118

55. Chai HC, Chua KH, Lim SK, Phipps ME. Insight into gene polymorphisms involved in toll-like receptor/interferon signalling pathways for systemic lupus erythematosus in south east asia. *Journal of immunology research*. 2014;2014:529167

56. Kawasaki A, Furukawa H, Nishida N, Warabi E, Kondo Y, Ito S, Matsumoto I, Kusaoi M, Amano H, Suda A, Nagaoka S, Setoguchi K, Nagai T, Hirohata S, Shimada K, Sugii S, Okamoto A, Chiba N, Suematsu E, Ohno S, Katayama M, Okamoto A, Kono H, Tokunaga K, Takasaki Y, Hashimoto H, Sumida T, Tohma S, Tsuchiya N. Association of functional polymorphisms in interferon regulatory factor 2 (irf2) with susceptibility to systemic lupus erythematosus: A case-control association study. *PloS one*. 2014;9:e109764

57. Skarsvag S, Hansen KE, Holst A, Moen T. Distribution of hla class ii alleles among scandinavian patients with systemic lupus erythematosus (sle): An increased risk of sle

among non[drb1*03,dqa1*0501,dqb1*0201] class ii homozygotes? *Tissue antigens*. 1992;40:128-133

58. Dong RP, Kimura A, Hashimoto H, Akizuki M, Nishimura Y, Sasazuki T. Difference in hla-linked genetic background between mixed connective tissue disease and systemic lupus erythematosus. *Tissue antigens*. 1993;41:20-25

59. Fredi M, Bianchi M, Andreoli L, Greco G, Olivieri I, Orcesi S, Fazzi E, Cereda C, Tincani A. Typing trex1 gene in patients with systemic lupus erythematosus. *Reumatismo*. 2015;67:1-7

60. Yao Z, Seelig HP, Ehrfeld H, Renz M, Hartung K, Deicher H, Keller E, Nevinny-Stickel C, Albert ED. Hla class ii genes and antibodies against recombinant u1-nrnp proteins in patients with systemic lupus erythematosus. Sle study group. *Rheumatology international*. 1994;14:63-69

61. Yao Z, Kimura A, Hartung K, Haas PJ, Volgger A, Brunnler G, Bonisch J, Albert ED. Polymorphism of the dqa1 promoter region (qap) and drb1, qap, dqa1, dqb1 haplotypes in systemic lupus erythematosus. Sle study group members. *Immunogenetics*. 1993;38:421-429

62. Miyagawa S, Shinohara K, Nakajima M, Kidoguchi K, Fujita T, Fukumoto T, Yoshioka A, Dohi K, Shirai T. Polymorphisms of hla class ii genes and autoimmune responses to ro/ss-a-la/ss-b among japanese subjects. *Arthritis and rheumatism*. 1998;41:927-934

63. Rudwaleit M, Tikly M, Gibson K, Pile K, Wordsworth P. Hla class ii antigens associated with systemic lupus erythematosus in black south africans. *Annals of the rheumatic diseases*. 1995;54:678-680

64. Hong GH, Kim HY, Takeuchi F, Nakano K, Yamada H, Matsuta K, Han H, Tokunaga K, Ito K, Park KS. Association of complement c4 and hla-dr alleles with systemic lupus erythematosus in koreans. *The Journal of rheumatology*. 1994;21:442-447

65. Harley JB, Alarcon-Riquelme ME, Criswell LA, Jacob CO, Kimberly RP, Moser KL, Tsao BP, Vyse TJ, Langefeld CD, Nath SK, Guthridge JM, Cobb BL, Mirel DB, Marion MC, Williams AH, Divers J, Wang W, Frank SG, Namjou B, Gabriel SB, Lee AT, Gregersen PK, Behrens TW, Taylor KE, Fernando M, Zidovetzki R, Gaffney PM, Edberg JC, Rioux JD, Ojwang JO, James JA, Merrill JT, Gilkeson GS, Seldin MF, Yin H, Baechler EC, Li QZ, Wakeland EK, Bruner GR, Kaufman KM, Kelly JA. Genome-wide association scan in women with systemic lupus erythematosus identifies susceptibility variants in itgam, pxk, kiaa1542 and other loci. *Nature genetics*. 2008;40:204-210

66. Hom G, Graham RR, Modrek B, Taylor KE, Ortmann W, Garnier S, Lee AT, Chung SA, Ferreira RC, Pant PV, Ballinger DG, Kosoy R, Demirci FY, Kamboh MI, Kao AH, Tian C, Gunnarsson I, Bengtsson AA, Rantapaa-Dahlqvist S, Petri M, Manzi S, Seldin MF, Ronnblom L, Syvanen AC, Criswell LA, Gregersen PK, Behrens TW. Association of systemic lupus erythematosus with c8orf13-blk and itgam-itgax. *The New England journal of medicine*. 2008;358:900-909

67. Kyogoku C, Dijstelbloem HM, Tsuchiya N, Hatta Y, Kato H, Yamaguchi A, Fukazawa T, Jansen MD, Hashimoto H, van de Winkel JG, Kallenberg CG, Tokunaga K. Fcgamma receptor gene polymorphisms in japanese patients with systemic lupus erythematosus: Contribution of fcgr2b to genetic susceptibility. *Arthritis and rheumatism*. 2002;46:1242-1254

68. Kyogoku C, Langefeld CD, Ortmann WA, Lee A, Selby S, Carlton VE, Chang M, Ramos P, Baechler EC, Batliwalla FM, Novitzke J, Williams AH, Gillett C, Rodine P, Graham RR, Ardlie KG, Gaffney PM, Moser KL, Petri M, Begovich AB, Gregersen PK, Behrens TW. Genetic association of the r620w polymorphism of protein tyrosine phosphatase ptpn22 with human sle. *American journal of human genetics*. 2004;75:504-507

69. Kozyrev SV, Abelson AK, Wojcik J, Zaghlool A, Linga Reddy MV, Sanchez E, Gunnarsson I, Svenungsson E, Sturfelt G, Jonsen A, Truedsson L, Pons-Estel BA, Witte T, D'Alfonso S, Barizzone N, Danieli MG, Gutierrez C, Suarez A, Junker P, Laustrup H, Gonzalez-Escribano MF, Martin J, Abderrahim H, Alarcon-Riquelme ME. Functional variants in the b-cell gene bank1 are associated with systemic lupus erythematosus. *Nature genetics*. 2008;40:211-216

70. Koene HR, Kleijer M, Swaak AJ, Sullivan KE, Bijl M, Petri MA, Kallenberg CG, Roos D, von dem Borne AE, de Haas M. The fc gammariiia-158f allele is a risk factor for systemic lupus erythematosus. *Arthritis and rheumatism*. 1998;41:1813-1818

71. Racila DM, Sontheimer CJ, Sheffield A, Wisnieski JJ, Racila E, Sontheimer RD. Homozygous single nucleotide polymorphism of the complement c1qa gene is associated with decreased levels of c1q in patients with subacute cutaneous lupus erythematosus. *Lupus*. 2003;12:124-132

72. Hashimoto H, Nishimura Y, Dong RP, Kimura A, Sasazuki T, Yamanaka K, Tokano Y, Murashima A, Kabasawa K, Hirose S. Hla antigens in japanese patients with systemic lupus erythematosus. *Scandinavian journal of rheumatology*. 1994;23:191-196

73. Prokunina L, Castillejo-Lopez C, Oberg F, Gunnarsson I, Berg L, Magnusson V, Brookes AJ, Tentler D, Kristjansdottir H, Grondal G, Bolstad AI, Svenungsson E, Lundberg I,

Sturfelt G, Jonssen A, Truedsson L, Lima G, Alcocer-Varela J, Jonsson R, Gyllensten UB, Harley JB, Alarcon-Segovia D, Steinsson K, Alarcon-Riquelme ME. A regulatory polymorphism in pdcd1 is associated with susceptibility to systemic lupus erythematosus in humans. *Nature genetics*. 2002;32:666-669

74. Surolia I, Pirnie SP, Chellappa V, Taylor KN, Cariappa A, Moya J, Liu H, Bell DW, Driscoll DR, Diederichs S, Haider K, Netravali I, Le S, Elia R, Dow E, Lee A, Freudenberg J, De Jager PL, Chretien Y, Varki A, MacDonald ME, Gillis T, Behrens TW, Bloch D, Collier D, Korzenik J, Podolsky DK, Hafler D, Murali M, Sands B, Stone JH, Gregersen PK, Pillai S. Functionally defective germline variants of sialic acid acetylesterase in autoimmunity. *Nature*. 2010;466:243-247

75. Lee-Kirsch MA, Gong M, Chowdhury D, Senenko L, Engel K, Lee YA, de Silva U, Bailey SL, Witte T, Vyse TJ, Kere J, Pfeiffer C, Harvey S, Wong A, Koskenmies S, Hummel O, Rohde K, Schmidt RE, Dominiczak AF, Gahr M, Hollis T, Perrino FW, Lieberman J, Hubner N. Mutations in the gene encoding the 3'-5' DNA exonuclease trex1 are associated with systemic lupus erythematosus. *Nature genetics*. 2007;39:1065-1067

76. Yasutomo K, Horiuchi T, Kagami S, Tsukamoto H, Hashimura C, Urushihara M, Kuroda Y. Mutation of dnase1 in people with systemic lupus erythematosus. *Nature genetics*. 2001;28:313-314

77. Al-Mayouf SM, Sunker A, Abdwani R, Abrawi SA, Almurshedi F, Alhashmi N, Al Sonbul A, Sewairi W, Qari A, Abdallah E, Al-Owain M, Al Motywee S, Al-Rayes H, Hashem M, Khalak H, Al-Jebali L, Alkuraya FS. Loss-of-function variant in dnase1l3 causes a familial form of systemic lupus erythematosus. *Nature genetics*. 2011;43:1186-1188

78. Duits AJ, Bootsma H, Derksen RH, Spronk PE, Kater L, Kallenberg CG, Capel PJ, Westerdaal NA, Spierenburg GT, Gmelig-Meyling FH, et al. Skewed distribution of igg fc receptor iia (cd32) polymorphism is associated with renal disease in systemic lupus erythematosus patients. *Arthritis and rheumatism*. 1995;38:1832-1836

79. Andrade RM, Alarcon GS, Fernandez M, Apte M, Vila LM, Reveille JD. Accelerated damage accrual among men with systemic lupus erythematosus: Xliv. Results from a multiethnic us cohort. *Arthritis and rheumatism*. 2007;56:622-630

80. Jacobson DL, Gange SJ, Rose NR, Graham NM. Epidemiology and estimated population burden of selected autoimmune diseases in the united states. *Clinical immunology and immunopathology*. 1997;84:223-243

81. Phiel KL, Henderson RA, Adelman SJ, Elloso MM. Differential estrogen receptor gene expression in human peripheral blood mononuclear cell populations. *Immunology letters*. 2005;97:107-113

82. Inui A, Ogasawara H, Naito T, Sekigawa I, Takasaki Y, Hayashida Y, Takamori K, Ogawa H. Estrogen receptor expression by peripheral blood mononuclear cells of patients with systemic lupus erythematosus. *Clinical rheumatology*. 2007;26:1675-1678

83. Lin HL, Yen JH, Chiou SS, Tsai WC, Ou TT, Wu CC, Liu HW. Estradiol upregulates calcineurin expression via overexpression of estrogen receptor alpha gene in systemic lupus erythematosus. *The Kaohsiung journal of medical sciences*. 2011;27:125-131

84. Bynoe MS, Grimaldi CM, Diamond B. Estrogen up-regulates bcl-2 and blocks tolerance induction of naive b cells. *Proceedings of the National Academy of Sciences of the United States of America*. 2000;97:2703-2708

85. Walters E, Rider V, Abdou NI, Greenwell C, Svojanovsky S, Smith P, Kimler BF. Estradiol targets t cell signaling pathways in human systemic lupus. *Clinical immunology (Orlando, Fla.)*. 2009;133:428-436

86. Rider V, Jones S, Evans M, Bassiri H, Afsar Z, Abdou NI. Estrogen increases cd40 ligand expression in t cells from women with systemic lupus erythematosus. *The Journal of rheumatology*. 2001;28:2644-2649

87. Kanda N, Tsuchida T, Tamaki K. Testosterone inhibits immunoglobulin production by human peripheral blood mononuclear cells. *Clinical and experimental immunology*. 1996;106:410-415

88. Peeva E, Venkatesh J, Diamond B. Tamoxifen blocks estrogen-induced b cell maturation but not survival. *Journal of immunology (Baltimore, Md. : 1950)*. 2005;175:1415-1423

89. Lu Q, Wu A, Tesmer L, Ray D, Yousif N, Richardson B. Demethylation of cd40lg on the inactive x in t cells from women with lupus. *Journal of immunology (Baltimore, Md. : 1950)*. 2007;179:6352-6358

90. Masi AT, Kaslow RA. Sex effects in systemic lupus erythematosus: A clue to pathogenesis. *Arthritis and rheumatism*. 1978;21:480-484

91. Schweighoffer E, Vanes L, Nys J, Cantrell D, McCleary S, Smithers N, Tybulewicz Victor LJ. The baff receptor transduces survival signals by co-opting the b cell receptor signaling pathway. *Immunity*. 2013;38:475-488

92. Schneider P. The role of april and baff in lymphocyte activation. *Current opinion in immunology*. 2005;17:282-289

93. Braun D, Caramalho I, Demengeot J. Ifn-α/β enhances bcr-dependent b cell responses. *International immunology*. 2002;14:411-419

94. Badr G, Saad H, Waly H, Hassan K, Abdel-Tawab H, Alhazza IM, Ahmed EA. Type i interferon (ifn-alpha/beta) rescues b-lymphocytes from apoptosis via pi3kdelta/akt, rho-a, nfkappab and bcl-2/bcl(xl). *Cellular immunology*. 2010;263:31-40

95. Park JW, Moon SY, Lee JH, Park JK, Lee DS, Jung KC, Song YW, Lee EB. Bone marrow analysis of immune cells and apoptosis in patients with systemic lupus erythematosus. *Lupus*. 2014;23:975-985

96. Chang NH, Li TT, Kim JJ, Landolt-Marticorena C, Fortin PR, Gladman DD, Urowitz MB, Wither JE. Interferon-alpha induces altered transitional b cell signaling and function in systemic lupus erythematosus. *Journal of autoimmunity*. 2015;58:100-110

97. Brandt L, Hedberg H. Impaired phagocytosis by peripheral blood granulocytes in systemic lupus erythematosus. *Scand J Haematol*. 1969;6:348-353

98. Hakkim A, Furnrohr BG, Amann K, Laube B, Abed UA, Brinkmann V, Herrmann M, Voll RE, Zychlinsky A. Impairment of neutrophil extracellular trap degradation is associated with lupus nephritis. *Proceedings of the National Academy of Sciences of the United States of America*. 2010;107:9813-9818

99. Herrmann M, Voll RE, Zoller OM, Hagenhofer M, Ponner BB, Kalden JR. Impaired phagocytosis of apoptotic cell material by monocyte-derived macrophages from patients with systemic lupus erythematosus. *Arthritis Rheum*. 1998;41:1241-1250

100. Tas SW, Quartier P, Botto M, Fossati-Jimack L. Macrophages from patients with sle and rheumatoid arthritis have defective adhesion in vitro, while only sle macrophages have impaired uptake of apoptotic cells. *Ann Rheum Dis*. 2006;65:216-221

101. Ren Y, Tang J, Mok MY, Chan AW, Wu A, Lau CS. Increased apoptotic neutrophils and macrophages and impaired macrophage phagocytic clearance of apoptotic neutrophils in systemic lupus erythematosus. *Arthritis and rheumatism*. 2003;48:2888-2897

102. Le Coz C, Joublin A, Pasquali JL, Korganow AS, Dumortier H, Monneaux F. Circulating tfh subset distribution is strongly affected in lupus patients with an active disease. *PloS one*. 2013;8:e75319

103. Simpson N, Gatenby PA, Wilson A, Malik S, Fulcher DA, Tangye SG, Manku H, Vyse TJ, Roncador G, Huttley GA, Goodnow CC, Vinuesa CG, Cook MC. Expansion of circulating t cells resembling follicular helper t cells is a fixed phenotype that identifies a subset of severe systemic lupus erythematosus. *Arthritis and rheumatism*. 2010;62:234-244

104. Mackay F, Leung H. The role of the baff/april system on t cell function. *Seminars in immunology*. 2006;18:284-289

105. Ye Q, Wang L, Wells AD, Tao R, Han R, Davidson A, Scott ML, Hancock WW. Baff binding to t cell-expressed baff-r costimulates t cell proliferation and alloresponses. *European journal of immunology*. 2004;34:2750-2759

106. Liossis SN, Ding XZ, Dennis GJ, Tsokos GC. Altered pattern of tcr/cd3-mediated protein-tyrosyl phosphorylation in t cells from patients with systemic lupus erythematosus. Deficient expression of the t cell receptor zeta chain. *The Journal of clinical investigation*. 1998;101:1448-1457

107. Nambiar MP, Fisher CU, Warke VG, Krishnan S, Mitchell JP, Delaney N, Tsokos GC. Reconstitution of deficient t cell receptor zeta chain restores t cell signaling and augments

t cell receptor/cd3-induced interleukin-2 production in patients with systemic lupus erythematosus. *Arthritis and rheumatism*. 2003;48:1948-1955

108. Jury EC, Kabouridis PS, Flores-Borja F, Mageed RA, Isenberg DA. Altered lipid raft-associated signaling and ganglioside expression in t lymphocytes from patients with systemic lupus erythematosus. *The Journal of clinical investigation*. 2004;113:1176-1187

109. Kidani Y, Bensinger SJ. Lipids rule: Resetting lipid metabolism restores t cell function in systemic lupus erythematosus. *The Journal of clinical investigation*. 2014;124:482-485

110. Her M, Kim D, Oh M, Jeong H, Choi I. Increased expression of soluble inducible costimulator ligand (icosl) in patients with systemic lupus erythematosus. *Lupus*. 2009;18:501-507

111. Rauen T, Hedrich CM, Juang YT, Tenbrock K, Tsokos GC. Camp-responsive element modulator (crem)alpha protein induces interleukin 17a expression and mediates epigenetic alterations at the interleukin-17a gene locus in patients with systemic lupus erythematosus. *The Journal of biological chemistry*. 2011;286:43437-43446

112. Yang J, Chu Y, Yang X, Gao D, Zhu L, Yang X, Wan L, Li M. Th17 and natural treg cell population dynamics in systemic lupus erythematosus. *Arthritis and rheumatism*. 2009;60:1472-1483

113. Wan S, Xia C, Morel L. Il-6 produced by dendritic cells from lupus-prone mice inhibits cd4+cd25+ t cell regulatory functions. *Journal of immunology (Baltimore, Md. : 1950)*. 2007;178:271-279

114. Valencia X, Yarboro C, Illei G, Lipsky PE. Deficient cd4+cd25high t regulatory cell function in patients with active systemic lupus erythematosus. *Journal of immunology (Baltimore, Md. : 1950)*. 2007;178:2579-2588

115. Mizui M, Koga T, Lieberman LA, Beltran J, Yoshida N, Johnson MC, Tisch R, Tsokos GC. Il-2 protects lupus-prone mice from multiple end-organ damage by limiting cd4-cd8- il-17-producing t cells. *Journal of immunology (Baltimore, Md. : 1950)*. 2014;193:2168-2177

116. Ohl K, Wiener A, Schippers A, Wagner N, Tenbrock K. Interleukin-2 treatment reverses effects of camp-responsive element modulator alpha-over-expressing t cells in autoimmune-prone mice. *Clinical and experimental immunology*. 2015;181:76-86

117. Bruley-Rosset M, Dardenne M, Schuurs A. Functional and quantitative changes of immune cells of ageing nzb mice treated with nandrolone decanoate. I. Effect on survival and autoantibody development. *Clinical and experimental immunology*. 1985;62:630-638

118. von Spee-Mayer C, Siegert E, Abdirama D, Rose A, Klaus A, Alexander T, Enghard P, Sawitzki B, Hiepe F, Radbruch A, Burmester GR, Riemekasten G, Humrich JY. Low-dose interleukin-2 selectively corrects regulatory t cell defects in patients with systemic lupus erythematosus. *Annals of the rheumatic diseases*. 2015

119. Wong CK, Wong PT, Tam LS, Li EK, Chen DP, Lam CW. Activation profile of toll-like receptors of peripheral blood lymphocytes in patients with systemic lupus erythematosus. *Clinical and experimental immunology*. 2010;159:11-22

120. Chauhan SK, Singh VV, Rai R, Rai M, Rai G. Distinct autoantibody profiles in systemic lupus erythematosus patients are selectively associated with tlr7 and tlr9 upregulation. *Journal of clinical immunology*. 2013;33:954-964

121. Fadok VA, Bratton DL, Konowal A, Freed PW, Westcott JY, Henson PM. Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production

through autocrine/paracrine mechanisms involving tgf-beta, pge2, and paf. *The Journal of clinical investigation*. 1998;101:890-898

122. Fraser DA, Laust AK, Nelson EL, Tenner AJ. C1q differentially modulates phagocytosis and cytokine responses during ingestion of apoptotic cells by human monocytes, macrophages, and dendritic cells. *Journal of immunology (Baltimore, Md. : 1950)*. 2009;183:6175-6185

123. Huynh ML, Fadok VA, Henson PM. Phosphatidylserine-dependent ingestion of apoptotic cells promotes tgf-beta1 secretion and the resolution of inflammation. *The Journal of clinical investigation*. 2002;109:41-50

124. Uderhardt S, Herrmann M, Oskolkova OV, Aschermann S, Bicker W, Ipseiz N, Sarter K, Frey B, Rothe T, Voll R, Nimmerjahn F, Bochkov VN, Schett G, Kronke G. 12/15-lipoxygenase orchestrates the clearance of apoptotic cells and maintains immunologic tolerance. *Immunity*. 2012;36:834-846

125. Wickman G, Julian L, Olson MF. How apoptotic cells aid in the removal of their own cold dead bodies. *Cell death and differentiation*. 2012;19:735-742

126. Duvall E, Wyllie AH, Morris RG. Macrophage recognition of cells undergoing programmed cell death (apoptosis). *Immunology*. 1985;56:351-358

127. Manfredi AA, Rovere P, Galati G, Heltai S, Bozzolo E, Soldini L, Davoust J, Balestrieri G, Tincani A, Sabbadini MG. Apoptotic cell clearance in systemic lupus erythematosus. I. Opsonization by antiphospholipid antibodies. *Arthritis and rheumatism*. 1998;41:205-214

128. Abeler-Dorner L, Rieger CC, Berger B, Weyd H, Graf D, Pfrang S, Tarner IH, Schwarting A, Lorenz HM, Muller-Ladner U, Krammer PH, Kuhn A. Interferon-alpha

abrogates the suppressive effect of apoptotic cells on dendritic cells in an in vitro model of systemic lupus erythematosus pathogenesis. *The Journal of rheumatology*. 2013;40:1683-1696

129. Leffler J, Martin M, Gullstrand B, Tyden H, Lood C, Truedsson L, Bengtsson AA, Blom AM. Neutrophil extracellular traps that are not degraded in systemic lupus erythematosus activate complement exacerbating the disease. *Journal of immunology (Baltimore, Md. : 1950)*. 2012;188:3522-3531

130. Leffler J, Gullstrand B, Jonsen A, Nilsson JA, Martin M, Blom AM, Bengtsson AA. Degradation of neutrophil extracellular traps co-varies with disease activity in patients with systemic lupus erythematosus. *Arthritis research & therapy*. 2013;15:R84

131. Lu M, Yu S, Xu W, Gao B, Xiong S. Hmgb1 promotes systemic lupus erythematosus by enhancing macrophage inflammatory response. *Journal of immunology research*. 2015;2015:946748

132. Zhang S, Lu X, Shu X, Tian X, Yang H, Yang W, Zhang Y, Wang G. Elevated plasma cfdna may be associated with active lupus nephritis and partially attributed to abnormal regulation of neutrophil extracellular traps (nets) in patients with systemic lupus erythematosus. *Internal medicine (Tokyo, Japan)*. 2014;53:2763-2771

133. Bruns A, Blass S, Hausdorf G, Burmester GR, Hiepe F. Nucleosomes are major t and b cell autoantigens in systemic lupus erythematosus. *Arthritis and rheumatism*. 2000;43:2307-2315

134. Lu L, Kaliyaperumal A, Boumpas DT, Datta SK. Major peptide autoepitopes for nucleosome-specific t cells of human lupus. *The Journal of clinical investigation*. 1999;104:345-355

135. Pancer LB, Milazzo MF, Morris VL, Singhal SK, Bell DA. Immunogenicity and characterization of supernatant DNA released by murine spleen cells. *Journal of immunology (Baltimore, Md. : 1950)*. 1981;127:98-104

136. Bell DA, Morrison B, VandenBygaart P. Immunogenic DNA-related factors. Nucleosomes spontaneously released from normal murine lymphoid cells stimulate proliferation and immunoglobulin synthesis of normal mouse lymphocytes. *The Journal of clinical investigation*. 1990;85:1487-1496

137. Cairns AP, Crockard AD, McConnell JR, Courtney PA, Bell AL. Reduced expression of cd44 on monocytes and neutrophils in systemic lupus erythematosus: Relations with apoptotic neutrophils and disease activity. *Annals of the rheumatic diseases*. 2001;60:950-955

138. Li Y, Lee PY, Sobel ES, Narain S, Satoh M, Segal MS, Reeves WH, Richards HB. Increased expression of fcgammari/cd64 on circulating monocytes parallels ongoing inflammation and nephritis in lupus. *Arthritis research & therapy*. 2009;11:R6

139. Guilliams M, Bruhns P, Saeys Y, Hammad H, Lambrecht BN. The function of fcgamma receptors in dendritic cells and macrophages. *Nature reviews. Immunology*. 2014;14:94-108

140. Lau CM, Broughton C, Tabor AS, Akira S, Flavell RA, Mamula MJ, Christensen SR, Shlomchik MJ, Viglianti GA, Rifkin IR, Marshak-Rothstein A. Rna-associated autoantigens activate b cells by combined b cell antigen receptor/toll-like receptor 7 engagement. *The Journal of experimental medicine*. 2005;202:1171-1177

141. Zhuang H, Narain S, Sobel E, Lee PY, Nacionales DC, Kelly KM, Richards HB, Segal M, Stewart C, Satoh M, Reeves WH. Association of anti-nucleoprotein autoantibodies

with upregulation of type i interferon-inducible gene transcripts and dendritic cell maturation in systemic lupus erythematosus. *Clinical immunology (Orlando, Fla.)*. 2005;117:238-250

142. Pawar RD, Ramanjaneyulu A, Kulkarni OP, Lech M, Segerer S, Anders HJ. Inhibition of toll-like receptor-7 (tlr-7) or tlr-7 plus tlr-9 attenuates glomerulonephritis and lung injury in experimental lupus. *Journal of the American Society of Nephrology : JASN*. 2007;18:1721-1731

143. Pawar RD, Patole PS, Zecher D, Segerer S, Kretzler M, Schlondorff D, Anders HJ. Toll-like receptor-7 modulates immune complex glomerulonephritis. *Journal of the American Society of Nephrology : JASN*. 2006;17:141-149

144. Migita K, Miyashita T, Maeda Y, Nakamura M, Yatsuhashi H, Kimura H, Ishibashi H, Eguchi K. Toll-like receptor expression in lupus peripheral blood mononuclear cells. *The Journal of rheumatology*. 2007;34:493-500

145. Petri M, Singh S, Tesfasyone H, Dedrick R, Fry K, Lal P, Williams G, Bauer J, Gregersen P, Behrens T, Baechler E. Longitudinal expression of type i interferon responsive genes in systemic lupus erythematosus. *Lupus*. 2009;18:980-989

146. Bengtsson AA, Sturfelt G, Truedsson L, Blomberg J, Alm G, Vallin H, Ronnblom L. Activation of type i interferon system in systemic lupus erythematosus correlates with disease activity but not with antiretroviral antibodies. *Lupus*. 2000;9:664-671

147. Bennett L, Palucka AK, Arce E, Cantrell V, Borvak J, Banchereau J, Pascual V. Interferon and granulopoiesis signatures in systemic lupus erythematosus blood. *The Journal of experimental medicine*. 2003;197:711-723

148. Baechler EC, Batliwalla FM, Karypis G, Gaffney PM, Ortmann WA, Espe KJ, Shark KB, Grande WJ, Hughes KM, Kapur V, Gregersen PK, Behrens TW. Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. *Proceedings of the National Academy of Sciences of the United States of America*. 2003;100:2610-2615

149. Fairhurst AM, Xie C, Fu Y, Wang A, Boudreaux C, Zhou XJ, Cibotti R, Coyle A, Connolly JE, Wakeland EK, Mohan C. Type i interferons produced by resident renal cells may promote end-organ disease in autoantibody-mediated glomerulonephritis. *Journal of immunology (Baltimore, Md. : 1950)*. 2009;183:6831-6838

150. Feng X, Wu H, Grossman JM, Hanvivadhanakul P, FitzGerald JD, Park GS, Dong X, Chen W, Kim MH, Weng HH, Furst DE, Gorn A, McMahon M, Taylor M, Brahn E, Hahn BH, Tsao BP. Association of increased interferon-inducible gene expression with disease activity and lupus nephritis in patients with systemic lupus erythematosus. *Arthritis and rheumatism*. 2006;54:2951-2962

151. Ronnblom LE, Alm GV, Oberg KE. Possible induction of systemic lupus erythematosus by interferon-alpha treatment in a patient with a malignant carcinoid tumour. *Journal of internal medicine*. 1990;227:207-210

152. Wandl UB, Nagel-Hiemke M, May D, Kreuzfelder E, Kloke O, Kranzhoff M, Seeber S, Niederle N. Lupus-like autoimmune disease induced by interferon therapy for myeloproliferative disorders. *Clinical immunology and immunopathology*. 1992;65:70-74

153. Fleischmann M, Celerier P, Bernard P, Litoux P, Dreno B. Long-term interferon-alpha therapy induces autoantibodies against epidermis. *Dermatology (Basel, Switzerland)*. 1996;192:50-55

154. Wilson LE, Widman D, Dikman SH, Gorevic PD. Autoimmune disease complicating antiviral therapy for hepatitis c virus infection. *Seminars in arthritis and rheumatism*. 2002;32:163-173

155. Sladkova V, Mares J, Lubenova B, Hlustik P, Kanovsky P. Drug-induced systemic lupus erythematosus in interferon beta-1b therapy. *Neuro endocrinology letters*. 2011;32:4-6

156. Cuda CM, Agrawal H, Misharin AV, Haines GK, 3rd, Hutcheson J, Weber E, Schoenfeldt JA, Mohan C, Pope RM, Perlman H. Requirement of myeloid cell-specific fas expression for prevention of systemic autoimmunity in mice. *Arthritis and rheumatism*. 2012;64:808-820

157. Yuan W, DiMartino SJ, Redecha PB, Ivashkiv LB, Salmon JE. Systemic lupus erythematosus monocytes are less responsive to interleukin-10 in the presence of immune complexes. *Arthritis and rheumatism*. 2011;63:212-218

158. Santer DM, Wiedeman AE, Teal TH, Ghosh P, Elkon KB. Plasmacytoid dendritic cells and c1q differentially regulate inflammatory gene induction by lupus immune complexes. *Journal of immunology (Baltimore, Md. : 1950)*. 2012;188:902-915

159. Henriques A, Ines L, Carvalheiro T, Couto M, Andrade A, Pedreiro S, Laranjeira P, Morgado JM, Pais ML, da Silva JA, Paiva A. Functional characterization of peripheral blood dendritic cells and monocytes in systemic lupus erythematosus. *Rheumatology international*. 2012;32:863-869

160. Lee PY, Weinstein JS, Nacionales DC, Scumpia PO, Li Y, Butfiloski E, van Rooijen N, Moldawer L, Satoh M, Reeves WH. A novel type i ifn-producing cell subset in murine lupus. *Journal of immunology (Baltimore, Md. : 1950)*. 2008;180:5101-5108

161. Yoshimoto S, Nakatani K, Iwano M, Asai O, Samejima K, Sakan H, Terada M, Harada K, Akai Y, Shiiki H, Nose M, Saito Y. Elevated levels of fractalkine expression and accumulation of cd16+ monocytes in glomeruli of active lupus nephritis. *American journal of kidney diseases : the official journal of the National Kidney Foundation*. 2007;50:47-58

162. Sullivan KE, Suriano A, Dietzmann K, Lin J, Goldman D, Petri MA. The tnfalpha locus is altered in monocytes from patients with systemic lupus erythematosus. *Clinical immunology (Orlando, Fla.)*. 2007;123:74-81

163. Steinbach F, Henke F, Krause B, Thiele B, Burmester GR, Hiepe F. Monocytes from systemic lupus erythematous patients are severely altered in phenotype and lineage flexibility. *Annals of the rheumatic diseases*. 2000;59:283-288

164. Mikolajczyk TP, Osmenda G, Batko B, Wilk G, Krezelok M, Skiba D, Sliwa T, Pryjma JR, Guzik TJ. Heterogeneity of peripheral blood monocytes, endothelial dysfunction and subclinical atherosclerosis in patients with systemic lupus erythematosus. *Lupus*. 2015

165. Ruiz-Limon P, Barbarroja N, Perez-Sanchez C, Aguirre MA, Bertolaccini ML, Khamashta MA, Rodriguez-Ariza A, Almaden Y, Segui P, Khraiwesh H, Gonzalez-Reyes JA, Villalba JM, Collantes-Estevez E, Cuadrado MJ, Lopez-Pedrera C. Atherosclerosis and cardiovascular disease in systemic lupus erythematosus: Effects of in vivo statin treatment. *Annals of the rheumatic diseases*. 2015;74:1450-1458

166. Nagy G, Barcza M, Gonchoroff N, Phillips PE, Perl A. Nitric oxide-dependent mitochondrial biogenesis generates ca2+ signaling profile of lupus t cells. *Journal of immunology (Baltimore, Md. : 1950)*. 2004;173:3676-3683

167. Zhang Z, Song L, Maurer K, Petri MA, Sullivan KE. Global h4 acetylation analysis by chip-chip in systemic lupus erythematosus monocytes. *Genes and immunity*. 2010;11:124-133

168. Lopez P, Scheel-Toellner D, Rodriguez-Carrio J, Caminal-Montero L, Gordon C, Suarez A. Interferon-alpha-induced b-lymphocyte stimulator expression and mobilization in healthy and systemic lupus erthymatosus monocytes. *Rheumatology (Oxford, England)*. 2014;53:2249-2258

169. Kuroiwa T, Schlimgen R, Illei GG, Boumpas DT. Monocyte response to th1 stimulation and effector function toward human mesangial cells are not impaired in patients with lupus nephritis. *Clinical immunology (Orlando, Fla.)*. 2003;106:65-72

170. Blanco P, Palucka AK, Gill M, Pascual V, Banchereau J. Induction of dendritic cell differentiation by ifn-alpha in systemic lupus erythematosus. *Science (New York, N.Y.)*. 2001;294:1540-1543

171. Joo H, Coquery C, Xue Y, Gayet I, Dillon SR, Punaro M, Zurawski G, Banchereau J, Pascual V, Oh S. Serum from patients with sle instructs monocytes to promote igg and iga plasmablast differentiation. *The Journal of experimental medicine*. 2012;209:1335-1348

172. Huang X, Shen N, Bao C, Gu Y, Wu L, Chen S. Interferon-induced protein ifit4 is associated with systemic lupus erythematosus and promotes differentiation of monocytes into dendritic cell-like cells. *Arthritis research & therapy*. 2008;10:R91

173. Ding D, Mehta H, McCune WJ, Kaplan MJ. Aberrant phenotype and function of myeloid dendritic cells in systemic lupus erythematosus. *Journal of immunology (Baltimore, Md. : 1950)*. 2006;177:5878-5889

174. Decker P, Kotter I, Klein R, Berner B, Rammensee HG. Monocyte-derived dendritic cells over-express cd86 in patients with systemic lupus erythematosus. *Rheumatology (Oxford, England)*. 2006;45:1087-1095

175. Lovgren T, Eloranta ML, Kastner B, Wahren-Herlenius M, Alm GV, Ronnblom L. Induction of interferon-alpha by immune complexes or liposomes containing systemic lupus erythematosus autoantigen- and sjogren's syndrome autoantigen-associated rna. *Arthritis and rheumatism*. 2006;54:1917-1927

176. Sacre K, Criswell LA, McCune JM. Hydroxychloroquine is associated with impaired interferon-alpha and tumor necrosis factor-alpha production by plasmacytoid dendritic cells in systemic lupus erythematosus. *Arthritis research & therapy*. 2012;14:R155

177. Lande R, Ganguly D, Facchinetti V, Frasca L, Conrad C, Gregorio J, Meller S, Chamilos G, Sebasigari R, Riccieri V, Bassett R, Amuro H, Fukuhara S, Ito T, Liu YJ, Gilliet M. Neutrophils activate plasmacytoid dendritic cells by releasing self-DNA-peptide complexes in systemic lupus erythematosus. *Science translational medicine*. 2011;3:73ra19

178. Garcia-Romo GS, Caielli S, Vega B, Connolly J, Allantaz F, Xu Z, Punaro M, Baisch J, Guiducci C, Coffman RL, Barrat FJ, Banchereau J, Pascual V. Netting neutrophils are major inducers of type i ifn production in pediatric systemic lupus erythematosus. *Science translational medicine*. 2011;3:73ra20

179. Farkas L, Beiske K, Lund-Johansen F, Brandtzaeg P, Jahnsen FL. Plasmacytoid dendritic cells (natural interferon- alpha/beta-producing cells) accumulate in cutaneous lupus erythematosus lesions. *The American journal of pathology*. 2001;159:237-243

180. Rowland SL, Riggs JM, Gilfillan S, Bugatti M, Vermi W, Kolbeck R, Unanue ER, Sanjuan MA, Colonna M. Early, transient depletion of plasmacytoid dendritic cells ameliorates autoimmunity in a lupus model. *The Journal of experimental medicine*. 2014;211:1977-1991

181. Guiducci C, Tripodo C, Gong M, Sangaletti S, Colombo MP, Coffman RL, Barrat FJ. Autoimmune skin inflammation is dependent on plasmacytoid dendritic cell activation by nucleic acids via tlr7 and tlr9. *The Journal of experimental medicine*. 2010;207:2931-2942

182. Crispin JC, Vargas-Rojas MI, Monsivais-Urenda A, Alcocer-Varela J. Phenotype and function of dendritic cells of patients with systemic lupus erythematosus. *Clinical immunology (Orlando, Fla.)*. 2012;143:45-50

183. Teichmann LL, Ols ML, Kashgarian M, Reizis B, Kaplan DH, Shlomchik MJ. Dendritic cells in lupus are not required for activation of t and b cells but promote their expansion, resulting in tissue damage. *Immunity*. 2010;33:967-978

184. Pogue SL, Preston BT, Stalder J, Bebbington CR, Cardarelli PM. The receptor for type i ifns is highly expressed on peripheral blood b cells and monocytes and mediates a distinct profile of differentiation and activation of these cells. *Journal of interferon & cytokine research : the official journal of the International Society for Interferon and Cytokine Research*. 2004;24:131-139

185. Jin O, Kavikondala S, Sun L, Fu R, Mok MY, Chan A, Yeung J, Lau CS. Systemic lupus erythematosus patients have increased number of circulating plasmacytoid dendritic cells, but decreased myeloid dendritic cells with deficient cd83 expression. *Lupus*. 2008;17:654-662

186. Schiffer L, Bethunaickan R, Ramanujam M, Huang W, Schiffer M, Tao H, Madaio MP, Bottinger EP, Davidson A. Activated renal macrophages are markers of disease onset and disease remission in lupus nephritis. *Journal of immunology (Baltimore, Md. : 1950)*. 2008;180:1938-1947

187. Chalmers SA, Chitu V, Herlitz LC, Sahu R, Stanley ER, Putterman C. Macrophage depletion ameliorates nephritis induced by pathogenic antibodies. *Journal of autoimmunity*. 2015;57:42-52

188. Iwata Y, Bostrom EA, Menke J, Rabacal WA, Morel L, Wada T, Kelley VR. Aberrant macrophages mediate defective kidney repair that triggers nephritis in lupus-susceptible mice. *Journal of immunology (Baltimore, Md. : 1950)*. 2012;188:4568-4580

189. Kahlenberg JM, Carmona-Rivera C, Smith CK, Kaplan MJ. Neutrophil extracellular trap-associated protein activation of the nlrp3 inflammasome is enhanced in lupus macrophages. *Journal of immunology (Baltimore, Md. : 1950)*. 2013;190:1217-1226

190. Yang CA, Huang ST, Chiang BL. Sex-dependent differential activation of nlrp3 and aim2 inflammasomes in sle macrophages. *Rheumatology (Oxford, England)*. 2015;54:324-331

191. Pereira-Lopes S, Celhar T, Sans-Fons G, Serra M, Fairhurst AM, Lloberas J, Celada A. The exonuclease trex1 restrains macrophage proinflammatory activation. *Journal of immunology (Baltimore, Md. : 1950)*. 2013;191:6128-6135

192. Namjou B, Kothari PH, Kelly JA, Glenn SB, Ojwang JO, Adler A, Alarcon-Riquelme ME, Gallant CJ, Boackle SA, Criswell LA, Kimberly RP, Brown E, Edberg J, Stevens AM, Jacob CO, Tsao BP, Gilkeson GS, Kamen DL, Merrill JT, Petri M, Goldman RR, Vila LM, Anaya JM, Niewold TB, Martin J, Pons-Estel BA, Sabio JM, Callejas JL, Vyse

TJ, Bae SC, Perrino FW, Freedman BI, Scofield RH, Moser KL, Gaffney PM, James JA, Langefeld CD, Kaufman KM, Harley JB, Atkinson JP. Evaluation of the trex1 gene in a large multi-ancestral lupus cohort. *Genes and immunity*. 2011;12:270-279

193. Ammirati E, Bozzolo EP, Contri R, Baragetti A, Palini AG, Cianflone D, Banfi M, Uboldi P, Bottoni G, Scotti I, Pirillo A, Grigore L, Garlaschelli K, Monaco C, Catapano AL, Sabbadini MG, Manfredi AA, Norata GD. Cardiometabolic and immune factors associated with increased common carotid artery intima-media thickness and cardiovascular disease in patients with systemic lupus erythematosus. *Nutrition, metabolism, and cardiovascular diseases : NMCD*. 2014;24:751-759

194. Bae YS, Lee JH, Choi SH, Kim S, Almazan F, Witztum JL, Miller YI. Macrophages generate reactive oxygen species in response to minimally oxidized low-density lipoprotein: Toll-like receptor 4- and spleen tyrosine kinase-dependent activation of nadph oxidase 2. *Circulation research*. 2009;104:210-218, 221p following 218

195. Chistiakov DA, Bobryshev YV, Nikiforov NG, Elizova NV, Sobenin IA, Orekhov AN. Macrophage phenotypic plasticity in atherosclerosis: The associated features and the peculiarities of the expression of inflammatory genes. *International journal of cardiology*. 2015;184:436-445

196. Duewell P, Kono H, Rayner KJ, Sirois CM, Vladimer G, Bauernfeind FG, Abela GS, Franchi L, Nunez G, Schnurr M, Espevik T, Lien E, Fitzgerald KA, Rock KL, Moore KJ, Wright SD, Hornung V, Latz E. Nlrp3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. *Nature*. 2010;464:1357-1361

197. Moore KJ, Sheedy FJ, Fisher EA. Macrophages in atherosclerosis: A dynamic balance. *Nature reviews. Immunology*. 2013;13:709-721

198. Al Gadban MM, Alwan MM, Smith KJ, Hammad SM. Accelerated vascular disease in systemic lupus erythematosus: Role of macrophage. *Clinical immunology (Orlando, Fla.)*. 2015;157:133-144

199. Carvalheiro T, Gomes D, Pinto LA, Ines L, Lopes A, Henriques A, Pedreiro S, Martinho A, Trindade H, Young HA, da Silva JA, Paiva A. Sera from patients with active systemic lupus erythematosus patients enhance the toll-like receptor 4 response in monocyte subsets. *Journal of inflammation (London, England)*. 2015;12:38

200. Borghi MO, Raschi E, Grossi C, Chighizola CB, Meroni PL. Toll-like receptor 4 and beta2 glycoprotein i interaction on endothelial cells. *Lupus*. 2014;23:1302-1304

201. Lee TP, Huang JC, Liu CJ, Chen HJ, Chen YH, Tsai YT, Yang W, Sun KH. Interactions of surface-expressed tlr-4 and endosomal tlr-9 accelerate lupus progression in anti-dsdna antibody transgenic mice. *Experimental biology and medicine (Maywood, N.J.)*. 2014;239:715-723

202. Tsao JT, Hsieh SC, Chiang BL, Yu CL, Lin SC. Altered il-10 and tnf-alpha production in peripheral blood mononuclear cells of systemic lupus erythematosus patients after toll-like receptor 2, 4, or 9 activation. *Clinical and experimental medicine*. 2012;12:153-158

203. Urbonaviciute V, Starke C, Pirschel W, Pohle S, Frey S, Daniel C, Amann K, Schett G, Herrmann M, Voll RE. Toll-like receptor 2 is required for autoantibody production and development of renal disease in pristane-induced lupus. *Arthritis and rheumatism*. 2013;65:1612-1623

204. Tall AR, Yvan-Charvet L. Cholesterol, inflammation and innate immunity. *Nature reviews. Immunology*. 2015;15:104-116

205. Rock G, Chauhan K, Jamieson GA, Tandon NN. Anti-cd36 antibodies in patients with lupus anticoagulant and thrombotic complications. *British journal of haematology*. 1994;88:878-880

206. Rivas L, Zabaleta M, Toro F, Bianco NE, De Sanctis JB. Decreased transcription, expression and function of low-density lipoprotein receptor in leukocytes from patients with systemic lupus erythematosus. *Autoimmunity*. 2009;42:266-268

207. Wermeling F, Chen Y, Pikkarainen T, Scheynius A, Winqvist O, Izui S, Ravetch JV, Tryggvason K, Karlsson MC. Class a scavenger receptors regulate tolerance against apoptotic cells, and autoantibodies against these receptors are predictive of systemic lupus. *The Journal of experimental medicine*. 2007;204:2259-2265

208. Chen XW, Shen Y, Sun CY, Wu FX, Chen Y, Yang CD. Anti-class a scavenger receptor autoantibodies from systemic lupus erythematosus patients impair phagocytic clearance of apoptotic cells by macrophages in vitro. *Arthritis research & therapy*. 2011;13:R9

209. Mukherjee R, Kanti Barman P, Kumar Thatoi P, Tripathy R, Kumar Das B, Ravindran B. Non-classical monocytes display inflammatory features: Validation in sepsis and systemic lupus erythematous. *Scientific reports*. 2015;5:13886

210. Magna M, Pisetsky DS. The role of cell death in the pathogenesis of sle: Is pyroptosis the missing link? *Scandinavian journal of immunology*. 2015

211. Emlen W, Niebur J, Kadera R. Accelerated in vitro apoptosis of lymphocytes from patients with systemic lupus erythematosus. *Journal of immunology (Baltimore, Md. : 1950)*. 1994;152:3685-3692

212. Courtney PA, Crockard AD, Williamson K, McConnell J, Kennedy RJ, Bell AL. Lymphocyte apoptosis in systemic lupus erythematosus: Relationships with fas expression, serum soluble fas and disease activity. *Lupus*. 1999;8:508-513

213. Nakamura N, Shidara Y, Kawaguchi N, Azuma C, Mitsuda N, Onishi S, Yamaji K, Wada Y. Lupus anticoagulant autoantibody induces apoptosis in umbilical vein endothelial cells: Involvement of annexin v. *Biochemical and biophysical research communications*. 1994;205:1488-1493

214. Villanueva E, Yalavarthi S, Berthier CC, Hodgin JB, Khandpur R, Lin AM, Rubin CJ, Zhao W, Olsen SH, Klinker M, Shealy D, Denny MF, Plumas J, Chaperot L, Kretzler M, Bruce AT, Kaplan MJ. Netting neutrophils induce endothelial damage, infiltrate tissues, and expose immunostimulatory molecules in systemic lupus erythematosus. *Journal of immunology (Baltimore, Md. : 1950)*. 2011;187:538-552

215. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, Weinrauch Y, Zychlinsky A. Neutrophil extracellular traps kill bacteria. *Science (New York, N.Y.)*. 2004;303:1532-1535

216. Fuchs TA, Abed U, Goosmann C, Hurwitz R, Schulze I, Wahn V, Weinrauch Y, Brinkmann V, Zychlinsky A. Novel cell death program leads to neutrophil extracellular traps. *The Journal of cell biology*. 2007;176:231-241

217. Saitoh T, Komano J, Saitoh Y, Misawa T, Takahama M, Kozaki T, Uehata T, Iwasaki H, Omori H, Yamaoka S, Yamamoto N, Akira S. Neutrophil extracellular traps mediate a host defense response to human immunodeficiency virus-1. *Cell host & microbe*. 2012;12:109-116

218. Bruns S, Kniemeyer O, Hasenberg M, Aimanianda V, Nietzsche S, Thywissen A, Jeron A, Latge JP, Brakhage AA, Gunzer M. Production of extracellular traps against aspergillus fumigatus in vitro and in infected lung tissue is dependent on invading neutrophils and influenced by hydrophobin roda. *PLoS pathogens*. 2010;6:e1000873

219. Abi Abdallah DS, Lin C, Ball CJ, King MR, Duhamel GE, Denkers EY. Toxoplasma gondii triggers release of human and mouse neutrophil extracellular traps. *Infection and immunity*. 2012;80:768-777

220. Urban CF, Ermert D, Schmid M, Abu-Abed U, Goosmann C, Nacken W, Brinkmann V, Jungblut PR, Zychlinsky A. Neutrophil extracellular traps contain calprotectin, a cytosolic protein complex involved in host defense against candida albicans. *PLoS pathogens*. 2009;5:e1000639

221. Ermert D, Urban CF, Laube B, Goosmann C, Zychlinsky A, Brinkmann V. Mouse neutrophil extracellular traps in microbial infections. *Journal of innate immunity*. 2009;1:181-193

222. Gupta AK, Giaglis S, Hasler P, Hahn S. Efficient neutrophil extracellular trap induction requires mobilization of both intracellular and extracellular calcium pools and is modulated by cyclosporine a. *PloS one*. 2014;9:e97088

223. Neeli I, Dwivedi N, Khan S, Radic M. Regulation of extracellular chromatin release from neutrophils. *Journal of innate immunity*. 2009;1:194-201

224. Khandpur R, Carmona-Rivera C, Vivekanandan-Giri A, Gizinski A, Yalavarthi S, Knight JS, Friday S, Li S, Patel RM, Subramanian V, Thompson P, Chen P, Fox DA, Pennathur S, Kaplan MJ. Nets are a source of citrullinated autoantigens and stimulate inflammatory responses in rheumatoid arthritis. *Science translational medicine*. 2013;5:178ra140

225. Metzler KD, Fuchs TA, Nauseef WM, Reumaux D, Roesler J, Schulze I, Wahn V, Papayannopoulos V, Zychlinsky A. Myeloperoxidase is required for neutrophil extracellular trap formation: Implications for innate immunity. *Blood*. 2011;117:953-959

226. Metzler KD, Goosmann C, Lubojemska A, Zychlinsky A, Papayannopoulos V. A myeloperoxidase-containing complex regulates neutrophil elastase release and actin dynamics during netosis. *Cell reports*. 2014;8:883-896

227. Papayannopoulos V, Metzler KD, Hakkim A, Zychlinsky A. Neutrophil elastase and myeloperoxidase regulate the formation of neutrophil extracellular traps. *The Journal of cell biology*. 2010;191:677-691

228. Wang Y, Li M, Stadler S, Correll S, Li P, Wang D, Hayama R, Leonelli L, Han H, Grigoryev SA, Allis CD, Coonrod SA. Histone hypercitrullination mediates chromatin decondensation and neutrophil extracellular trap formation. *The Journal of cell biology*. 2009;184:205-213

229. Li P, Li M, Lindberg MR, Kennett MJ, Xiong N, Wang Y. Pad4 is essential for antibacterial innate immunity mediated by neutrophil extracellular traps. *The Journal of experimental medicine*. 2010;207:1853-1862

230. Tessarz P, Kouzarides T. Histone core modifications regulating nucleosome structure and dynamics. *Nature reviews. Molecular cell biology*. 2014;15:703-708

231. Denny MF, Yalavarthi S, Zhao W, Thacker SG, Anderson M, Sandy AR, McCune WJ, Kaplan MJ. A distinct subset of proinflammatory neutrophils isolated from patients with systemic lupus erythematosus induces vascular damage and synthesizes type i ifns. *Journal of immunology (Baltimore, Md. : 1950)*. 2010;184:3284-3297

232. Carmona-Rivera C, Kaplan MJ. Low-density granulocytes: A distinct class of neutrophils in systemic autoimmunity. *Seminars in immunopathology*. 2013;35:455-463

233. Liu CL, Tangsombatvisit S, Rosenberg JM, Mandelbaum G, Gillespie EC, Gozani OP, Alizadeh AA, Utz PJ. Specific post-translational histone modifications of neutrophil extracellular traps as immunogens and potential targets of lupus autoantibodies. *Arthritis research & therapy*. 2012;14:R25

234. Dwivedi N, Neeli I, Schall N, Wan H, Desiderio DM, Csernok E, Thompson PR, Dali H, Briand JP, Muller S, Radic M. Deimination of linker histones links neutrophil extracellular trap release with autoantibodies in systemic autoimmunity. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*. 2014;28:2840-2851

235. Pieterse E, Hofstra J, Berden J, Herrmann M, Dieker J, van der Vlag J. Acetylated histones contribute to the immunostimulatory potential of neutrophil extracellular traps in systemic lupus erythematosus. *Clinical and experimental immunology*. 2015;179:68-74

236. Knight JS, Carmona-Rivera C, Kaplan MJ. Proteins derived from neutrophil extracellular traps may serve as self-antigens and mediate organ damage in autoimmune diseases. *Frontiers in immunology*. 2012;3:380

237. Seredkina N, Rekvig OP. Acquired loss of renal nuclease activity is restricted to dnasei and is an organ-selective feature in murine lupus nephritis. *The American journal of pathology*. 2011;179:1120-1128

238. Fuhrman B, Gantman A, Aviram M. Paraoxonase 1 (pon1) deficiency in mice is associated with reduced expression of macrophage sr-bi and consequently the loss of hdl cytoprotection against apoptosis. *Atherosclerosis*. 2010;211:61-68

239. McMahon M, Grossman J, Skaggs B, Fitzgerald J, Sahakian L, Ragavendra N, Charles-Schoeman C, Watson K, Wong WK, Volkmann E, Chen W, Gorn A, Karpouzas G, Weisman M, Wallace DJ, Hahn BH. Dysfunctional proinflammatory high-density lipoproteins confer increased risk of atherosclerosis in women with systemic lupus erythematosus. *Arthritis and rheumatism*. 2009;60:2428-2437

240. Nuttall SL, Heaton S, Piper MK, Martin U, Gordon C. Cardiovascular risk in systemic lupus erythematosus--evidence of increased oxidative stress and dyslipidaemia. *Rheumatology (Oxford, England)*. 2003;42:758-762

241. Frostegard J, Svenungsson E, Wu R, Gunnarsson I, Lundberg IE, Klareskog L, Horkko S, Witztum JL. Lipid peroxidation is enhanced in patients with systemic lupus erythematosus and is associated with arterial and renal disease manifestations. *Arthritis and rheumatism*. 2005;52:192-200

242. Alves JD, Grima B. Oxidative stress in systemic lupus erythematosus and antiphospholipid syndrome: A gateway to atherosclerosis. *Current rheumatology reports*. 2003;5:383-390

243. Ames PR, Alves J, Murat I, Isenberg DA, Nourooz-Zadeh J. Oxidative stress in systemic lupus erythematosus and allied conditions with vascular involvement. *Rheumatology (Oxford, England)*. 1999;38:529-534

244. Delgado Alves J, Ames PR, Donohue S, Stanyer L, Nourooz-Zadeh J, Ravirajan C, Isenberg DA. Antibodies to high-density lipoprotein and beta2-glycoprotein i are inversely correlated with paraoxonase activity in systemic lupus erythematosus and primary antiphospholipid syndrome. *Arthritis and rheumatism*. 2002;46:2686-2694

245. Delgado Alves J, Mason LJ, Ames PR, Chen PP, Rauch J, Levine JS, Subang R, Isenberg DA. Antiphospholipid antibodies are associated with enhanced oxidative stress, decreased plasma nitric oxide and paraoxonase activity in an experimental mouse model. *Rheumatology (Oxford, England)*. 2005;44:1238-1244

246. Huang WN, Tso TK, Huang HY. Enhanced oxidative status but not corresponding elevated antioxidative status by anticardiolipin antibody and disease activity in patients with systemic lupus erythematosus. *Rheumatology international*. 2007;27:453-458

247. Srivastava R, Yu S, Parks BW, Black LL, Kabarowski JH. Autoimmune-mediated reduction of high-density lipoprotein-cholesterol and paraoxonase 1 activity in systemic lupus erythematosus-prone gld mice. *Arthritis and rheumatism*. 2011;63:201-211

248. Batuca JR, Ames PR, Isenberg DA, Alves JD. Antibodies toward high-density lipoprotein components inhibit paraoxonase activity in patients with systemic lupus erythematosus. *Annals of the New York Academy of Sciences*. 2007;1108:137-146

249. Lee HT, Lin CS, Lee CS, Tsai CY, Wei YH. Increased 8-hydroxy-2'-deoxyguanosine in plasma and decreased mrna expression of human 8-oxoguanine DNA glycosylase 1, anti-oxidant enzymes, mitochondrial biogenesis-related proteins and glycolytic enzymes in leucocytes in patients with systemic lupus erythematosus. *Clinical and experimental immunology*. 2014;176:66-77

250. Marsillach J, Becker JO, Vaisar T, Hahn BH, Brunzell JD, Furlong CE, de Boer IH, McMahon MA, Hoofnagle AN. Paraoxonase-3 is depleted from the high-density lipoproteins of autoimmune disease patients with subclinical atherosclerosis. *Journal of proteome research*. 2015;14:2046-2054

251. Buyon JP, Cohen P, Merrill JT, Gilkeson G, Kaplan M, James J, McCune WJ, Bernatsky S, Elkon K. A highlight from the lupus 2014 meeting: Eight great ideas. *Lupus science & medicine*. 2015;2:e000087

252. Barzilai O, Ram M, Shoenfeld Y. Viral infection can induce the production of autoantibodies. *Current opinion in rheumatology*. 2007;19:636-643

253. Ramos-Casals M, Cuadrado MJ, Alba P, Sanna G, Brito-Zeron P, Bertolaccini L, Babini A, Moreno A, D'Cruz D, Khamashta MA. Acute viral infections in patients with systemic lupus erythematosus: Description of 23 cases and review of the literature. *Medicine*. 2008;87:311-318

254. Stratton MA. Drug-induced systemic lupus erythematosus. *Clinical pharmacy*. 1985;4:657-663

255. Courtney PA, Crockard AD, Williamson K, Irvine AE, Kennedy RJ, Bell AL. Increased apoptotic peripheral blood neutrophils in systemic lupus erythematosus: Relations with disease activity, antibodies to double stranded DNA, and neutropenia. *Ann Rheum Dis*. 1999;58:309-314

256. Ohsako S, Hara M, Harigai M, Fukasawa C, Kashiwazaki S. Expression and function of fas antigen and bcl-2 in human systemic lupus erythematosus lymphocytes. *Clinical immunology and immunopathology*. 1994;73:109-114

257. Kuhn A, Sonntag M, Richter-Hintz D, Oslislo C, Megahed M, Ruzicka T, Lehmann P. Phototesting in lupus erythematosus: A 15-year experience. *Journal of the American Academy of Dermatology*. 2001;45:86-95

258. Wang B, Dong X, Yuan Z, Zuo Y, Wang J. Ssa/ro antigen expressed on membrane of uvb-induced apoptotic keratinocytes is pathogenic but not detectable in supernatant of cell culture. *Chinese medical journal*. 1999;112:512-515

259. Kahlenberg JM, Kaplan MJ. Mechanisms of premature atherosclerosis in rheumatoid arthritis and lupus. *Annu Rev Med*. 2013;64:249-263

260. Squatrito D, Emmi G, Silvestri E, Ciucciarelli L, D'Elios MM, Prisco D, Emmi L. Pathogenesis and potential therapeutic targets in systemic lupus erythematosus: From bench to bedside. *Auto- immunity highlights*. 2014;5:33-45

261. Ettinger WH, Goldberg AP, Applebaum-Bowden D, Hazzard WR. Dyslipoproteinemia in systemic lupus erythematosus. Effect of corticosteroids. *The American journal of medicine*. 1987;83:503-508

262. Wallace DJ, Metzger AL, Stecher VJ, Turnbull BA, Kern PA. Cholesterol-lowering effect of hydroxychloroquine in patients with rheumatic disease: Reversal of deleterious effects of steroids on lipids. *The American journal of medicine*. 1990;89:322-326

263. Zonana-Nacach A, Barr SG, Magder LS, Petri M. Damage in systemic lupus erythematosus and its association with corticosteroids. *Arthritis and rheumatism*. 2000;43:1801-1808

264. Mok CC. Epidemiology and survival of systemic lupus erythematosus in hong kong chinese. *Lupus*. 2011;20:767-771

265. Halpern WG, Lappin P, Zanardi T, Cai W, Corcoran M, Zhong J, Baker KP. Chronic administration of belimumab, a blys antagonist, decreases tissue and peripheral blood b-lymphocyte populations in cynomolgus monkeys: Pharmacokinetic, pharmacodynamic,

and toxicologic effects. *Toxicological sciences : an official journal of the Society of Toxicology*. 2006;91:586-599

266. Furie R, Nicholls K, Cheng TT, Houssiau F, Burgos-Vargas R, Chen SL, Hillson JL, Meadows-Shropshire S, Kinaszczuk M, Merrill JT. Efficacy and safety of abatacept in lupus nephritis: A twelve-month, randomized, double-blind study. *Arthritis & rheumatology (Hoboken, N.J.)*. 2014;66:379-389

267. Kalunian KC, Merrill JT, Maciuca R, McBride JM, Townsend MJ, Wei X, Davis JC, Jr., Kennedy WP. A phase ii study of the efficacy and safety of rontalizumab (rhumab interferon-alpha) in patients with systemic lupus erythematosus (rose). *Annals of the rheumatic diseases*. 2015

268. Petri M, Wallace DJ, Spindler A, Chindalore V, Kalunian K, Mysler E, Neuwelt CM, Robbie G, White WI, Higgs BW, Yao Y, Wang L, Ethgen D, Greth W. Sifalimumab, a human anti-interferon-alpha monoclonal antibody, in systemic lupus erythematosus: A phase i randomized, controlled, dose-escalation study. *Arthritis and rheumatism*. 2013;65:1011-1021

269. Fatemi A, Moosavi M, Sayedbonakdar Z, Farajzadegan Z, Kazemi M, Smiley A. Atorvastatin effect on systemic lupus erythematosus disease activity: A double-blind randomized clinical trial. *Clinical rheumatology*. 2014;33:1273-1278

270. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, Abraham J, Adair T, Aggarwal R, Ahn SY, Alvarado M, Anderson HR, Anderson LM, Andrews KG, Atkinson C, Baddour LM, Barker-Collo S, Bartels DH, Bell ML, Benjamin EJ, Bennett D, Bhalla K, Bikbov B, Bin Abdulhak A, Birbeck G, Blyth F, Bolliger I, Boufous S, Bucello C, Burch M, Burney P, Carapetis J, Chen H, Chou D, Chugh SS, Coffeng LE,

Colan SD, Colquhoun S, Colson KE, Condon J, Connor MD, Cooper LT, Corriere M, Cortinovis M, de Vaccaro KC, Couser W, Cowie BC, Criqui MH, Cross M, Dabhadkar KC, Dahodwala N, De Leo D, Degenhardt L, Delossantos A, Denenberg J, Des Jarlais DC, Dharmaratne SD, Dorsey ER, Driscoll T, Duber H, Ebel B, Erwin PJ, Espindola P, Ezzati M, Feigin V, Flaxman AD, Forouzanfar MH, Fowkes FG, Franklin R, Fransen M, Freeman MK, Gabriel SE, Gakidou E, Gaspari F, Gillum RF, Gonzalez-Medina D, Halasa YA, Haring D, Harrison JE, Havmoeller R, Hay RJ, Hoen B, Hotez PJ, Hoy D, Jacobsen KH, James SL, Jasrasaria R, Jayaraman S, Johns N, Karthikeyan G, Kassebaum N, Keren A, Khoo JP, Knowlton LM, Kobusingye O, Koranteng A, Krishnamurthi R, Lipnick M, Lipshultz SE, Ohno SL, Mabweijano J, MacIntyre MF, Mallinger L, March L, Marks GB, Marks R, Matsumori A, Matzopoulos R, Mayosi BM, McAnulty JH, McDermott MM, McGrath J, Mensah GA, Merriman TR, Michaud C, Miller M, Miller TR, Mock C, Mocumbi AO, Mokdad AA, Moran A, Mulholland K, Nair MN, Naldi L, Narayan KM, Nasseri K, Norman P, O'Donnell M, Omer SB, Ortblad K, Osborne R, Ozgediz D, Pahari B, Pandian JD, Rivero AP, Padilla RP, Perez-Ruiz F, Perico N, Phillips D, Pierce K, Pope CA, 3rd, Porrini E, Pourmalek F, Raju M, Ranganathan D, Rehm JT, Rein DB, Remuzzi G, Rivara FP, Roberts T, De Leon FR, Rosenfeld LC, Rushton L, Sacco RL, Salomon JA, Sampson U, Sanman E, Schwebel DC, Segui-Gomez M, Shepard DS, Singh D, Singleton J, Sliwa K, Smith E, Steer A, Taylor JA, Thomas B, Tleyjeh IM, Towbin JA, Truelsen T, Undurraga EA, Venketasubramanian N, Vijayakumar L, Vos T, Wagner GR, Wang M, Wang W, Watt K, Weinstock MA, Weintraub R, Wilkinson JD, Woolf AD, Wulf S, Yeh PH, Yip P, Zabetian A, Zheng ZJ, Lopez AD, Murray CJ, AlMazroa MA, Memish ZA. Global and regional mortality from

235 causes of death for 20 age groups in 1990 and 2010: A systematic analysis for the global burden of disease study 2010. *Lancet*. 2012;380:2095-2128

271. National Heart Lung aBI. Bethesda, md: Us department of health and human services, national institutes of health; 2012. *Morbidity and Mortality: 2012 Chart Book on Cardiovascular, Lung, and Blood Diseases*.

272. Dawber TR, Meadors GF, Moore FE, Jr. Epidemiological approaches to heart disease: The framingham study. *American journal of public health and the nation's health*. 1951;41:279-281

273. Kannel WB, Dawber TR, Kagan A, Revotskie N, Stokes J, 3rd. Factors of risk in the development of coronary heart disease--six year follow-up experience. The framingham study. *Annals of internal medicine*. 1961;55:33-50

274. Greenland P, Knoll MD, Stamler J, Neaton JD, Dyer AR, Garside DB, Wilson PW. Major risk factors as antecedents of fatal and nonfatal coronary heart disease events. *Jama*. 2003;290:891-897

275. Executive summary of the third report of the national cholesterol education program (ncep) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel iii). *Jama*. 2001;285:2486-2497

276. Lamon-Fava S, Wilson PW, Schaefer EJ. Impact of body mass index on coronary heart disease risk factors in men and women. The framingham offspring study. *Arteriosclerosis, thrombosis, and vascular biology*. 1996;16:1509-1515

277. Manzi S, Selzer F, Sutton-Tyrrell K, Fitzgerald SG, Rairie JE, Tracy RP, Kuller LH. Prevalence and risk factors of carotid plaque in women with systemic lupus erythematosus. *Arthritis and rheumatism*. 1999;42:51-60

278. Parish S, Offer A, Clarke R, Hopewell JC, Hill MR, Otvos JD, Armitage J, Collins R. Lipids and lipoproteins and risk of different vascular events in the mrc/bhf heart protection study. *Circulation*. 2012;125:2469-2478

279. Ingelsson E, Schaefer EJ, Contois JH, McNamara JR, Sullivan L, Keyes MJ, Pencina MJ, Schoonmaker C, Wilson PW, D'Agostino RB, Vasan RS. Clinical utility of different lipid measures for prediction of coronary heart disease in men and women. *Jama*. 2007;298:776-785

280. Ockene IS. Deja vu all over again. *Circulation*. 2012;125:2412-2413

281. Ridker PM. Clinical application of c-reactive protein for cardiovascular disease detection and prevention. *Circulation*. 2003;107:363-369

282. Ajeganova S, Ehrnfelt C, Alizadeh R, Rohani M, Jogestrand T, Hafstrom I, Frostegard J. Longitudinal levels of apolipoproteins and antibodies against phosphorylcholine are independently associated with carotid artery atherosclerosis 5 years after rheumatoid arthritis onset--a prospective cohort study. *Rheumatology (Oxford, England)*. 2011;50:1785-1793

283. Holvoet P, Vanhaecke J, Janssens S, Van de Werf F, Collen D. Oxidized ldl and malondialdehyde-modified ldl in patients with acute coronary syndromes and stable coronary artery disease. *Circulation*. 1998;98:1487-1494

284. Toshima S, Hasegawa A, Kurabayashi M, Itabe H, Takano T, Sugano J, Shimamura K, Kimura J, Michishita I, Suzuki T, Nagai R. Circulating oxidized low density lipoprotein levels. A biochemical risk marker for coronary heart disease. *Arteriosclerosis, thrombosis, and vascular biology*. 2000;20:2243-2247

285. Lee KW, Lip GY, Tayebjee M, Foster W, Blann AD. Circulating endothelial cells, von willebrand factor, interleukin-6, and prognosis in patients with acute coronary syndromes. *Blood*. 2005;105:526-532

286. Getz GS, Reardon CA. Animal models of atherosclerosis. *Arteriosclerosis, thrombosis, and vascular biology*. 2012;32:1104-1115

287. Murray PJ, Wynn TA. Obstacles and opportunities for understanding macrophage polarization. *Journal of leukocyte biology*. 2011;89:557-563

288. Rausch PG, Moore TG. Granule enzymes of polymorphonuclear neutrophils: A phylogenetic comparison. *Blood*. 1975;46:913-919

289. Podrez EA, Abu-Soud HM, Hazen SL. Myeloperoxidase-generated oxidants and atherosclerosis. *Free radical biology & medicine*. 2000;28:1717-1725

290. Surwit RS, Feinglos MN, Rodin J, Sutherland A, Petro AE, Opara EC, Kuhn CM, Rebuffe-Scrive M. Differential effects of fat and sucrose on the development of obesity and diabetes in c57bl/6j and a/j mice. *Metabolism: clinical and experimental*. 1995;44:645-651

291. Buettner R, Scholmerich J, Bollheimer LC. High-fat diets: Modeling the metabolic disorders of human obesity in rodents. *Obesity (Silver Spring, Md.)*. 2007;15:798-808

292. Kiely DK, Wolf PA, Cupples LA, Beiser AS, Myers RH. Familial aggregation of stroke. The framingham study. *Stroke; a journal of cerebral circulation*. 1993;24:1366-1371

293. Lloyd-Jones DM, Nam BH, D'Agostino RB, Sr., Levy D, Murabito JM, Wang TJ, Wilson PW, O'Donnell CJ. Parental cardiovascular disease as a risk factor for cardiovascular disease in middle-aged adults: A prospective study of parents and offspring. *Jama*. 2004;291:2204-2211

294. Bellenguez C, Bevan S, Gschwendtner A, Spencer CC, Burgess AI, Pirinen M, Jackson CA, Traylor M, Strange A, Su Z, Band G, Syme PD, Malik R, Pera J, Norrving B, Lemmens R, Freeman C, Schanz R, James T, Poole D, Murphy L, Segal H, Cortellini L, Cheng YC, Woo D, Nalls MA, Muller-Myhsok B, Meisinger C, Seedorf U, Ross-Adams H, Boonen S, Wloch-Kopec D, Valant V, Slark J, Furie K, Delavaran H, Langford C, Deloukas P, Edkins S, Hunt S, Gray E, Dronov S, Peltonen L, Gretarsdottir S, Thorleifsson G, Thorsteinsdottir U, Stefansson K, Boncoraglio GB, Parati EA, Attia J, Holliday E, Levi C, Franzosi MG, Goel A, Helgadottir A, Blackwell JM, Bramon E, Brown MA, Casas JP, Corvin A, Duncanson A, Jankowski J, Mathew CG, Palmer CN, Plomin R, Rautanen A, Sawcer SJ, Trembath RC, Viswanathan AC, Wood NW, Worrall BB, Kittner SJ, Mitchell BD, Kissela B, Meschia JF, Thijs V, Lindgren A, Macleod MJ, Slowik A, Walters M, Rosand J, Sharma P, Farrall M, Sudlow CL, Rothwell PM, Dichgans M, Donnelly P, Markus HS. Genome-wide association study identifies a variant in hdac9 associated with large vessel ischemic stroke. *Nature genetics*. 2012;44:328-333

295. Harrison SC, Smith AJ, Jones GT, Swerdlow DI, Rampuri R, Bown MJ, Folkersen L, Baas AF, de Borst GJ, Blankensteijn JD, Price JF, van der Graaf Y, McLachlan S, Agu O, Hofman A, Uitterlinden AG, Franco-Cereceda A, Ruigrok YM, van't Hof FN, Powell JT, van Rij AM, Casas JP, Eriksson P, Holmes MV, Asselbergs FW, Hingorani AD, Humphries SE. Interleukin-6 receptor pathways in abdominal aortic aneurysm. *European heart journal*. 2013;34:3707-3716

296. Traylor M, Makela KM, Kilarski LL, Holliday EG, Devan WJ, Nalls MA, Wiggins KL, Zhao W, Cheng YC, Achterberg S, Malik R, Sudlow C, Bevan S, Raitoharju E, Oksala N, Thijs V, Lemmens R, Lindgren A, Slowik A, Maguire JM, Walters M, Algra A,

Sharma P, Attia JR, Boncoraglio GB, Rothwell PM, de Bakker PI, Bis JC, Saleheen D, Kittner SJ, Mitchell BD, Rosand J, Meschia JF, Levi C, Dichgans M, Lehtimaki T, Lewis CM, Markus HS. A novel mmp12 locus is associated with large artery atherosclerotic stroke using a genome-wide age-at-onset informed approach. *PLoS genetics*. 2014;10:e1004469

297. Thanassoulis G, Campbell CY, Owens DS, Smith JG, Smith AV, Peloso GM, Kerr KF, Pechlivanis S, Budoff MJ, Harris TB, Malhotra R, O'Brien KD, Kamstrup PR, Nordestgaard BG, Tybjaerg-Hansen A, Allison MA, Aspelund T, Criqui MH, Heckbert SR, Hwang SJ, Liu Y, Sjogren M, van der Pals J, Kalsch H, Muhleisen TW, Nothen MM, Cupples LA, Caslake M, Di Angelantonio E, Danesh J, Rotter JI, Sigurdsson S, Wong Q, Erbel R, Kathiresan S, Melander O, Gudnason V, O'Donnell CJ, Post WS. Genetic associations with valvular calcification and aortic stenosis. *The New England journal of medicine*. 2013;368:503-512

298. Ortlepp JR, Hoffmann R, Ohme F, Lauscher J, Bleckmann F, Hanrath P. The vitamin d receptor genotype predisposes to the development of calcific aortic valve stenosis. *Heart (British Cardiac Society)*. 2001;85:635-638

299. Large-scale gene-centric analysis identifies novel variants for coronary artery disease. *PLoS genetics*. 2011;7:e1002260

300. Kilarski LL, Achterberg S, Devan WJ, Traylor M, Malik R, Lindgren A, Pare G, Sharma P, Slowik A, Thijs V, Walters M, Worrall BB, Sale MM, Algra A, Kappelle LJ, Wijmenga C, Norrving B, Sandling JK, Ronnblom L, Goris A, Franke A, Sudlow C, Rothwell PM, Levi C, Holliday EG, Fornage M, Psaty B, Gretarsdottir S, Thorsteinsdottir U, Seshadri S, Mitchell BD, Kittner S, Clarke R, Hopewell JC, Bis JC, Boncoraglio GB,

Meschia J, Ikram MA, Hansen BM, Montaner J, Thorleifsson G, Stefanson K, Rosand J, de Bakker PI, Farrall M, Dichgans M, Markus HS, Bevan S. Meta-analysis in more than 17,900 cases of ischemic stroke reveals a novel association at 12q24.12. *Neurology*. 2014;83:678-685

301. Gustafsen C, Kjolby M, Nyegaard M, Mattheisen M, Lundhede J, Buttenschon H, Mors O, Bentzon JF, Madsen P, Nykjaer A, Glerup S. The hypercholesterolemia-risk gene sort1 facilitates pcsk9 secretion. *Cell metabolism*. 2014;19:310-318

302. Gretarsdottir S, Baas AF, Thorleifsson G, Holm H, den Heijer M, de Vries JP, Kranendonk SE, Zeebregts CJ, van Sterkenburg SM, Geelkerken RH, van Rij AM, Williams MJ, Boll AP, Kostic JP, Jonasdottir A, Jonasdottir A, Walters GB, Masson G, Sulem P, Saemundsdottir J, Mouy M, Magnusson KP, Tromp G, Elmore JR, Sakalihasan N, Limet R, Defraigne JO, Ferrell RE, Ronkainen A, Ruigrok YM, Wijmenga C, Grobbee DE, Shah SH, Granger CB, Quyyumi AA, Vaccarino V, Patel RS, Zafari AM, Levey AI, Austin H, Girelli D, Pignatti PF, Olivieri O, Martinelli N, Malerba G, Trabetti E, Becker LC, Becker DM, Reilly MP, Rader DJ, Mueller T, Dieplinger B, Haltmayer M, Urbonavicius S, Lindblad B, Gottsater A, Gaetani E, Pola R, Wells P, Rodger M, Forgie M, Langlois N, Corral J, Vicente V, Fontcuberta J, Espana F, Grarup N, Jorgensen T, Witte DR, Hansen T, Pedersen O, Aben KK, de Graaf J, Holewijn S, Folkersen L, Franco-Cereceda A, Eriksson P, Collier DA, Stefansson H, Steinthorsdottir V, Rafnar T, Valdimarsson EM, Magnadottir HB, Sveinbjornsdottir S, Olafsson I, Magnusson MK, Palmason R, Haraldsdottir V, Andersen K, Onundarson PT, Thorgeirsson G, Kiemeney LA, Powell JT, Carey DJ, Kuivaniemi H, Lindholt JS, Jones GT, Kong A, Blankensteijn JD, Matthiasson SE, Thorsteinsdottir U, Stefansson K. Genome-wide association study

identifies a sequence variant within the dab2ip gene conferring susceptibility to abdominal aortic aneurysm. *Nature genetics*. 2010;42:692-697

303. Bis JC, Kavousi M, Franceschini N, Isaacs A, Abecasis GR, Schminke U, Post WS, Smith AV, Cupples LA, Markus HS, Schmidt R, Huffman JE, Lehtimaki T, Baumert J, Munzel T, Heckbert SR, Dehghan A, North K, Oostra B, Bevan S, Stoegerer EM, Hayward C, Raitakari O, Meisinger C, Schillert A, Sanna S, Volzke H, Cheng YC, Thorsson B, Fox CS, Rice K, Rivadeneira F, Nambi V, Halperin E, Petrovic KE, Peltonen L, Wichmann HE, Schnabel RB, Dorr M, Parsa A, Aspelund T, Demissie S, Kathiresan S, Reilly MP, Taylor K, Uitterlinden A, Couper DJ, Sitzer M, Kahonen M, Illig T, Wild PS, Orru M, Ludemann J, Shuldiner AR, Eiriksdottir G, White CC, Rotter JI, Hofman A, Seissler J, Zeller T, Usala G, Ernst F, Launer LJ, D'Agostino RB, Sr., O'Leary DH, Ballantyne C, Thiery J, Ziegler A, Lakatta EG, Chilukoti RK, Harris TB, Wolf PA, Psaty BM, Polak JF, Li X, Rathmann W, Uda M, Boerwinkle E, Klopp N, Schmidt H, Wilson JF, Viikari J, Koenig W, Blankenberg S, Newman AB, Witteman J, Heiss G, Duijn C, Scuteri A, Homuth G, Mitchell BD, Gudnason V, O'Donnell CJ. Meta-analysis of genome-wide association studies from the charge consortium identifies common variants associated with carotid intima media thickness and plaque. *Nature genetics*. 2011;43:940-947

304. Deloukas P, Kanoni S, Willenborg C, Farrall M, Assimes TL, Thompson JR, Ingelsson E, Saleheen D, Erdmann J, Goldstein BA, Stirrups K, Konig IR, Cazier JB, Johansson A, Hall AS, Lee JY, Willer CJ, Chambers JC, Esko T, Folkersen L, Goel A, Grundberg E, Havulinna AS, Ho WK, Hopewell JC, Eriksson N, Kleber ME, Kristiansson K, Lundmark P, Lyytikainen LP, Rafelt S, Shungin D, Strawbridge RJ, Thorleifsson G,

Tikkanen E, Van Zuydam N, Voight BF, Waite LL, Zhang W, Ziegler A, Absher D, Altshuler D, Balmforth AJ, Barroso I, Braund PS, Burgdorf C, Claudi-Boehm S, Cox D, Dimitriou M, Do R, Doney AS, El Mokhtari N, Eriksson P, Fischer K, Fontanillas P, Franco-Cereceda A, Gigante B, Groop L, Gustafsson S, Hager J, Hallmans G, Han BG, Hunt SE, Kang HM, Illig T, Kessler T, Knowles JW, Kolovou G, Kuusisto J, Langenberg C, Langford C, Leander K, Lokki ML, Lundmark A, McCarthy MI, Meisinger C, Melander O, Mihailov E, Maouche S, Morris AD, Muller-Nurasyid M, Nikus K, Peden JF, Rayner NW, Rasheed A, Rosinger S, Rubin D, Rumpf MP, Schafer A, Sivananthan M, Song C, Stewart AF, Tan ST, Thorgeirsson G, van der Schoot CE, Wagner PJ, Wells GA, Wild PS, Yang TP, Amouyel P, Arveiler D, Basart H, Boehnke M, Boerwinkle E, Brambilla P, Cambien F, Cupples AL, de Faire U, Dehghan A, Diemert P, Epstein SE, Evans A, Ferrario MM, Ferrieres J, Gauguier D, Go AS, Goodall AH, Gudnason V, Hazen SL, Holm H, Iribarren C, Jang Y, Kahonen M, Kee F, Kim HS, Klopp N, Koenig W, Kratzer W, Kuulasmaa K, Laakso M, Laaksonen R, Lee JY, Lind L, Ouwehand WH, Parish S, Park JE, Pedersen NL, Peters A, Quertermous T, Rader DJ, Salomaa V, Schadt E, Shah SH, Sinisalo J, Stark K, Stefansson K, Tregouet DA, Virtamo J, Wallentin L, Wareham N, Zimmermann ME, Nieminen MS, Hengstenberg C, Sandhu MS, Pastinen T, Syvanen AC, Hovingh GK, Dedoussis G, Franks PW, Lehtimaki T, Metspalu A, Zalloua PA, Siegbahn A, Schreiber S, Ripatti S, Blankenberg SS, Perola M, Clarke R, Boehm BO, O'Donnell C, Reilly MP, Marz W, Collins R, Kathiresan S, Hamsten A, Kooner JS, Thorsteinsdottir U, Danesh J, Palmer CN, Roberts R, Watkins H, Schunkert H, Samani NJ. Large-scale association analysis identifies new risk loci for coronary artery disease. *Nature genetics*. 2013;45:25-33

305. Helgadottir A, Thorleifsson G, Magnusson KP, Gretarsdottir S, Steinthorsdottir V, Manolescu A, Jones GT, Rinkel GJ, Blankensteijn JD, Ronkainen A, Jaaskelainen JE, Kyo Y, Lenk GM, Sakalihasan N, Kostulas K, Gottsater A, Flex A, Stefansson H, Hansen T, Andersen G, Weinsheimer S, Borch-Johnsen K, Jorgensen T, Shah SH, Quyyumi AA, Granger CB, Reilly MP, Austin H, Levey AI, Vaccarino V, Palsdottir E, Walters GB, Jonsdottir T, Snorradottir S, Magnusdottir D, Gudmundsson G, Ferrell RE, Sveinbjornsdottir S, Hernesniemi J, Niemela M, Limet R, Andersen K, Sigurdsson G, Benediktsson R, Verhoeven EL, Teijink JA, Grobbee DE, Rader DJ, Collier DA, Pedersen O, Pola R, Hillert J, Lindblad B, Valdimarsson EM, Magnadottir HB, Wijmenga C, Tromp G, Baas AF, Ruigrok YM, van Rij AM, Kuivaniemi H, Powell JT, Matthiasson SE, Gulcher JR, Thorgeirsson G, Kong A, Thorsteinsdottir U, Stefansson K. The same sequence variant on 9p21 associates with myocardial infarction, abdominal aortic aneurysm and intracranial aneurysm. *Nature genetics*. 2008;40:217-224

306. O'Donnell CJ, Kavousi M, Smith AV, Kardia SL, Feitosa MF, Hwang SJ, Sun YV, Province MA, Aspelund T, Dehghan A, Hoffmann U, Bielak LF, Zhang Q, Eiriksdottir G, van Duijn CM, Fox CS, de Andrade M, Kraja AT, Sigurdsson S, Elias-Smale SE, Murabito JM, Launer LJ, van der Lugt A, Kathiresan S, Krestin GP, Herrington DM, Howard TD, Liu Y, Post W, Mitchell BD, O'Connell JR, Shen H, Shuldiner AR, Altshuler D, Elosua R, Salomaa V, Schwartz SM, Siscovick DS, Voight BF, Bis JC, Glazer NL, Psaty BM, Boerwinkle E, Heiss G, Blankenberg S, Zeller T, Wild PS, Schnabel RB, Schillert A, Ziegler A, Munzel TF, White CC, Rotter JI, Nalls M, Oudkerk M, Johnson AD, Newman AB, Uitterlinden AG, Massaro JM, Cunningham J, Harris TB, Hofman A, Peyser PA, Borecki IB, Cupples LA, Gudnason V, Witteman JC. Genome-

wide association study for coronary artery calcification with follow-up in myocardial infarction. *Circulation*. 2011;124:2855-2864

307. Ken-Dror G, Talmud PJ, Humphries SE, Drenos F. Apoe/c1/c4/c2 gene cluster genotypes, haplotypes and lipid levels in prospective coronary heart disease risk among uk healthy men. *Molecular medicine (Cambridge, Mass.)*. 2010;16:389-399

308. Lilja HE, Soro A, Ylitalo K, Nuotio I, Viikari JS, Salomaa V, Vartiainen E, Taskinen MR, Peltonen L, Pajukanta P. A candidate gene study in low hdl-cholesterol families provides evidence for the involvement of the apoa2 gene and the apoa1c3a4 gene cluster. *Atherosclerosis*. 2002;164:103-111

309. Kuningas M, Magi R, Westendorp RG, Slagboom PE, Remm M, van Heemst D. Haplotypes in the human foxo1a and foxo3a genes; impact on disease and mortality at old age. *European journal of human genetics : EJHG*. 2007;15:294-301

310. Ellinor PT, Lunetta KL, Albert CM, Glazer NL, Ritchie MD, Smith AV, Arking DE, Muller-Nurasyid M, Krijthe BP, Lubitz SA, Bis JC, Chung MK, Dorr M, Ozaki K, Roberts JD, Smith JG, Pfeufer A, Sinner MF, Lohman K, Ding J, Smith NL, Smith JD, Rienstra M, Rice KM, Van Wagoner DR, Magnani JW, Wakili R, Clauss S, Rotter JI, Steinbeck G, Launer LJ, Davies RW, Borkovich M, Harris TB, Lin H, Volker U, Volzke H, Milan DJ, Hofman A, Boerwinkle E, Chen LY, Soliman EZ, Voight BF, Li G, Chakravarti A, Kubo M, Tedrow UB, Rose LM, Ridker PM, Conen D, Tsunoda T, Furukawa T, Sotoodehnia N, Xu S, Kamatani N, Levy D, Nakamura Y, Parvez B, Mahida S, Furie KL, Rosand J, Muhammad R, Psaty BM, Meitinger T, Perz S, Wichmann HE, Witteman JC, Kao WH, Kathiresan S, Roden DM, Uitterlinden AG, Rivadeneira F, McKnight B, Sjogren M, Newman AB, Liu Y, Gollob MH, Melander O,

Tanaka T, Stricker BH, Felix SB, Alonso A, Darbar D, Barnard J, Chasman DI, Heckbert SR, Benjamin EJ, Gudnason V, Kaab S. Meta-analysis identifies six new susceptibility loci for atrial fibrillation. *Nature genetics*. 2012;44:670-675

311. Villard E, Perret C, Gary F, Proust C, Dilanian G, Hengstenberg C, Ruppert V, Arbustini E, Wichter T, Germain M, Dubourg O, Tavazzi L, Aumont MC, DeGroote P, Fauchier L, Trochu JN, Gibelin P, Aupetit JF, Stark K, Erdmann J, Hetzer R, Roberts AM, Barton PJ, Regitz-Zagrosek V, Aslam U, Duboscq-Bidot L, Meyborg M, Maisch B, Madeira H, Waldenstrom A, Galve E, Cleland JG, Dorent R, Roizes G, Zeller T, Blankenberg S, Goodall AH, Cook S, Tregouet DA, Tiret L, Isnard R, Komajda M, Charron P, Cambien F. A genome-wide association study identifies two loci associated with heart failure due to dilated cardiomyopathy. *European heart journal*. 2011;32:1065-1076

312. Stark K, Esslinger UB, Reinhard W, Petrov G, Winkler T, Komajda M, Isnard R, Charron P, Villard E, Cambien F, Tiret L, Aumont MC, Dubourg O, Trochu JN, Fauchier L, Degroote P, Richter A, Maisch B, Wichter T, Zollbrecht C, Grassl M, Schunkert H, Linsel-Nitschke P, Erdmann J, Baumert J, Illig T, Klopp N, Wichmann HE, Meisinger C, Koenig W, Lichtner P, Meitinger T, Schillert A, Konig IR, Hetzer R, Heid IM, Regitz-Zagrosek V, Hengstenberg C. Genetic association study identifies hspb7 as a risk gene for idiopathic dilated cardiomyopathy. *PLoS genetics*. 2010;6:e1001167

313. Traylor M, Farrall M, Holliday EG, Sudlow C, Hopewell JC, Cheng YC, Fornage M, Ikram MA, Malik R, Bevan S, Thorsteinsdottir U, Nalls MA, Longstreth W, Wiggins KL, Yadav S, Parati EA, Destefano AL, Worrall BB, Kittner SJ, Khan MS, Reiner AP, Helgadottir A, Achterberg S, Fernandez-Cadenas I, Abboud S, Schmidt R, Walters M, Chen WM, Ringelstein EB, O'Donnell M, Ho WK, Pera J, Lemmens R, Norrving B,

Higgins P, Benn M, Sale M, Kuhlenbaumer G, Doney AS, Vicente AM, Delavaran H, Algra A, Davies G, Oliveira SA, Palmer CN, Deary I, Schmidt H, Pandolfo M, Montaner J, Carty C, de Bakker PI, Kostulas K, Ferro JM, van Zuydam NR, Valdimarsson E, Nordestgaard BG, Lindgren A, Thijs V, Slowik A, Saleheen D, Pare G, Berger K, Thorleifsson G, Hofman A, Mosley TH, Mitchell BD, Furie K, Clarke R, Levi C, Seshadri S, Gschwendtner A, Boncoraglio GB, Sharma P, Bis JC, Gretarsdottir S, Psaty BM, Rothwell PM, Rosand J, Meschia JF, Stefansson K, Dichgans M, Markus HS. Genetic risk factors for ischaemic stroke and its subtypes (the metastroke collaboration): A meta-analysis of genome-wide association studies. *The Lancet. Neurology*. 2012;11:951-962

314. Smith JG, Newton-Cheh C. Genome-wide association studies of late-onset cardiovascular disease. *Journal of molecular and cellular cardiology*. 2015;83:131-141

315. Gudbjartsson DF, Holm H, Gretarsdottir S, Thorleifsson G, Walters GB, Thorgeirsson G, Gulcher J, Mathiesen EB, Njolstad I, Nyrnes A, Wilsgaard T, Hald EM, Hveem K, Stoltenberg C, Kucera G, Stubblefield T, Carter S, Roden D, Ng MC, Baum L, So WY, Wong KS, Chan JC, Gieger C, Wichmann HE, Gschwendtner A, Dichgans M, Kuhlenbaumer G, Berger K, Ringelstein EB, Bevan S, Markus HS, Kostulas K, Hillert J, Sveinbjornsdottir S, Valdimarsson EM, Lochen ML, Ma RC, Darbar D, Kong A, Arnar DO, Thorsteinsdottir U, Stefansson K. A sequence variant in zfhx3 on 16q22 associates with atrial fibrillation and ischemic stroke. *Nature genetics*. 2009;41:876-878

316. Bown MJ, Jones GT, Harrison SC, Wright BJ, Bumpstead S, Baas AF, Gretarsdottir S, Badger SA, Bradley DT, Burnand K, Child AH, Clough RE, Cockerill G, Hafez H, Scott DJ, Futers S, Johnson A, Sohrabi S, Smith A, Thompson MM, van Bockxmeer FM,

Waltham M, Matthiasson SE, Thorleifsson G, Thorsteinsdottir U, Blankensteijn JD, Teijink JA, Wijmenga C, de Graaf J, Kiemeney LA, Assimes TL, McPherson R, Folkersen L, Franco-Cereceda A, Palmen J, Smith AJ, Sylvius N, Wild JB, Refstrup M, Edkins S, Gwilliam R, Hunt SE, Potter S, Lindholt JS, Frikke-Schmidt R, Tybjaerg-Hansen A, Hughes AE, Golledge J, Norman PE, van Rij A, Powell JT, Eriksson P, Stefansson K, Thompson JR, Humphries SE, Sayers RD, Deloukas P, Samani NJ. Abdominal aortic aneurysm is associated with a variant in low-density lipoprotein receptor-related protein 1. *American journal of human genetics*. 2011;89:619-627

317. Mommersteeg MT, Brown NA, Prall OW, de Gier-de Vries C, Harvey RP, Moorman AF, Christoffels VM. Pitx2c and nkx2-5 are required for the formation and identity of the pulmonary myocardium. *Circulation research*. 2007;101:902-909

318. Arking DE, Junttila MJ, Goyette P, Huertas-Vazquez A, Eijgelsheim M, Blom MT, Newton-Cheh C, Reinier K, Teodorescu C, Uy-Evanado A, Carter-Monroe N, Kaikkonen KS, Kortelainen ML, Boucher G, Lagace C, Moes A, Zhao X, Kolodgie F, Rivadeneira F, Hofman A, Witteman JC, Uitterlinden AG, Marsman RF, Pazoki R, Bardai A, Koster RW, Dehghan A, Hwang SJ, Bhatnagar P, Post W, Hilton G, Prineas RJ, Li M, Kottgen A, Ehret G, Boerwinkle E, Coresh J, Kao WH, Psaty BM, Tomaselli GF, Sotoodehnia N, Siscovick DS, Burke GL, Marban E, Spooner PM, Cupples LA, Jui J, Gunson K, Kesaniemi YA, Wilde AA, Tardif JC, O'Donnell CJ, Bezzina CR, Virmani R, Stricker BH, Tan HL, Albert CM, Chakravarti A, Rioux JD, Huikuri HV, Chugh SS. Identification of a sudden cardiac death susceptibility locus at 2q24.2 through genome-wide association in european ancestry individuals. *PLoS genetics*. 2011;7:e1002158

319. Gordon T, Castelli WP, Hjortland MC, Kannel WB, Dawber TR. High density lipoprotein as a protective factor against coronary heart disease. The framingham study. *The American journal of medicine*. 1977;62:707-714

320. Braunwald E, Bonow RO. *Braunwald's heart disease : A textbook of cardiovascular medicine*. Philadelphia: Saunders; 2012.

321. Walker KH, Hall WD, Hurst JW. Clinical methods, 3rd edition: The history, physical, and laboratory examinations. In: Walker HK, Hall WD, Hurst JW, eds. *Clinical methods: The history, physical, and laboratory examinations*. Boston: Butterworth Publishers, a division of Reed Publishing; 1990.

322. O'Connell BJ, Genest J, Jr. High-density lipoproteins and endothelial function. *Circulation*. 2001;104:1978-1983

323. Khera AV, Cuchel M, de la Llera-Moya M, Rodrigues A, Burke MF, Jafri K, French BC, Phillips JA, Mucksavage ML, Wilensky RL, Mohler ER, Rothblat GH, Rader DJ. Cholesterol efflux capacity, high-density lipoprotein function, and atherosclerosis. *The New England journal of medicine*. 2011;364:127-135

324. de Souza JA, Vindis C, Negre-Salvayre A, Rye KA, Couturier M, Therond P, Chantepie S, Salvayre R, Chapman MJ, Kontush A. Small, dense hdl 3 particles attenuate apoptosis in endothelial cells: Pivotal role of apolipoprotein a-i. *Journal of cellular and molecular medicine*. 2010;14:608-620

325. Seetharam D, Mineo C, Gormley AK, Gibson LL, Vongpatanasin W, Chambliss KL, Hahner LD, Cummings ML, Kitchens RL, Marcel YL, Rader DJ, Shaul PW. High-density lipoprotein promotes endothelial cell migration and reendothelialization via scavenger receptor-b type i. *Circulation research*. 2006;98:63-72

326. Yvan-Charvet L, Pagler TA, Seimon TA, Thorp E, Welch CL, Witztum JL, Tabas I, Tall AR. Abca1 and abcg1 protect against oxidative stress-induced macrophage apoptosis during efferocytosis. *Circulation research*. 2010;106:1861-1869

327. Vaisar T, Pennathur S, Green PS, Gharib SA, Hoofnagle AN, Cheung MC, Byun J, Vuletic S, Kassim S, Singh P, Chea H, Knopp RH, Brunzell J, Geary R, Chait A, Zhao XQ, Elkon K, Marcovina S, Ridker P, Oram JF, Heinecke JW. Shotgun proteomics implicates protease inhibition and complement activation in the antiinflammatory properties of hdl. *The Journal of clinical investigation*. 2007;117:746-756

328. Murata N, Sato K, Kon J, Tomura H, Yanagita M, Kuwabara A, Ui M, Okajima F. Interaction of sphingosine 1-phosphate with plasma components, including lipoproteins, regulates the lipid receptor-mediated actions. *The Biochemical journal*. 2000;352 Pt 3:809-815

329. Kontush A, Lindahl M, Lhomme M, Calabresi L, Chapman MJ, Davidson WS. Structure of hdl: Particle subclasses and molecular components. *Handbook of experimental pharmacology*. 2015;224:3-51

330. Kontush A, Therond P, Zerrad A, Couturier M, Negre-Salvayre A, de Souza JA, Chantepie S, Chapman MJ. Preferential sphingosine-1-phosphate enrichment and sphingomyelin depletion are key features of small dense hdl3 particles: Relevance to antiapoptotic and antioxidative activities. *Arteriosclerosis, thrombosis, and vascular biology*. 2007;27:1843-1849

331. Argraves KM, Argraves WS. Hdl serves as a s1p signaling platform mediating a multitude of cardiovascular effects. *Journal of lipid research*. 2007;48:2325-2333

332. Al Gadban MM, German J, Truman JP, Soodavar F, Riemer EC, Twal WO, Smith KJ, Heller D, Hofbauer AF, Oates JC, Hammad SM. Lack of nitric oxide synthases increases lipoprotein immune complex deposition in the aorta and elevates plasma sphingolipid levels in lupus. *Cellular immunology*. 2012;276:42-51

333. Heinecke JW. Lipoprotein oxidation in cardiovascular disease: Chief culprit or innocent bystander? *The Journal of experimental medicine*. 2006;203:813-816

334. Mungrue IN, Bredt DS, Stewart DJ, Husain M. From molecules to mammals: What's nos got to do with it? *Acta Physiol Scand*. 2003;179:123-135

335. Tsutsui M, Shimokawa H, Otsuji Y, Yanagihara N. Pathophysiological relevance of no signaling in the cardiovascular system: Novel insight from mice lacking all no synthases. *Pharmacol Ther*. 2010;128:499-508

336. Harry BL, Sanders JM, Feaver RE, Lansey M, Deem TL, Zarbock A, Bruce AC, Pryor AW, Gelfand BD, Blackman BR, Schwartz MA, Ley K. Endothelial cell pecam-1 promotes atherosclerotic lesions in areas of disturbed flow in apoe-deficient mice. *Arteriosclerosis, thrombosis, and vascular biology*. 2008;28:2003-2008

337. Kaplanski G, Marin V, Fabrigoule M, Boulay V, Benoliel AM, Bongrand P, Kaplanski S, Farnarier C. Thrombin-activated human endothelial cells support monocyte adhesion in vitro following expression of intercellular adhesion molecule-1 (icam-1; cd54) and vascular cell adhesion molecule-1 (vcam-1; cd106). *Blood*. 1998;92:1259-1267

338. Viola J, Soehnlein O. Atherosclerosis - a matter of unresolved inflammation. *Seminars in immunology*. 2015

339. Weber C, Noels H. Atherosclerosis: Current pathogenesis and therapeutic options. *Nature medicine*. 2011;17:1410-1422

340. Schwenke DC, Carew TE. Initiation of atherosclerotic lesions in cholesterol-fed rabbits. Ii. Selective retention of ldl vs. Selective increases in ldl permeability in susceptible sites of arteries. *Arteriosclerosis (Dallas, Tex.)*. 1989;9:908-918

341. Olin-Lewis K, Krauss RM, La Belle M, Blanche PJ, Barrett PH, Wight TN, Chait A. Apoc-iii content of apob-containing lipoproteins is associated with binding to the vascular proteoglycan biglycan. *Journal of lipid research*. 2002;43:1969-1977

342. Olsson U, Camejo G, Hurt-Camejo E, Elfsber K, Wiklund O, Bondjers G. Possible functional interactions of apolipoprotein b-100 segments that associate with cell proteoglycans and the apob/e receptor. *Arteriosclerosis, thrombosis, and vascular biology*. 1997;17:149-155

343. Zaguri R, Verbovetski I, Atallah M, Trahtemberg U, Krispin A, Nahari E, Leitersdorf E, Mevorach D. 'Danger' effect of low-density lipoprotein (ldl) and oxidized ldl on human immature dendritic cells. *Clinical and experimental immunology*. 2007;149:543-552

344. Loomans CJ, Wan H, de Crom R, van Haperen R, de Boer HC, Leenen PJ, Drexhage HA, Rabelink TJ, van Zonneveld AJ, Staal FJ. Angiogenic murine endothelial progenitor cells are derived from a myeloid bone marrow fraction and can be identified by endothelial no synthase expression. *Arteriosclerosis, thrombosis, and vascular biology*. 2006;26:1760-1767

345. Alessio AM, Beltrame MP, Nascimento MC, Vicente CP, de Godoy JA, Silva JC, Bittar LF, Lorand-Metze I, de Paula EV, Annichino-Bizzacchi JM. Circulating progenitor and mature endothelial cells in deep vein thrombosis. *International journal of medical sciences*. 2013;10:1746-1754

346. Shintani S, Murohara T, Ikeda H, Ueno T, Honma T, Katoh A, Sasaki K, Shimada T, Oike Y, Imaizumi T. Mobilization of endothelial progenitor cells in patients with acute myocardial infarction. *Circulation*. 2001;103:2776-2779

347. Petoumenos V, Nickenig G, Werner N. High-density lipoprotein exerts vasculoprotection via endothelial progenitor cells. *Journal of cellular and molecular medicine*. 2009;13:4623-4635

348. Noor R, Shuaib U, Wang CX, Todd K, Ghani U, Schwindt B, Shuaib A. High-density lipoprotein cholesterol regulates endothelial progenitor cells by increasing enos and preventing apoptosis. *Atherosclerosis*. 2007;192:92-99

349. Gonzalez-Pecchi V, Valdes S, Pons V, Honorato P, Martinez LO, Lamperti L, Aguayo C, Radojkovic C. Apolipoprotein a-i enhances proliferation of human endothelial progenitor cells and promotes angiogenesis through the cell surface atp synthase. *Microvascular research*. 2015;98:9-15

350. Goldstein JL, Brown MS. Binding and degradation of low density lipoproteins by cultured human fibroblasts. Comparison of cells from a normal subject and from a patient with homozygous familial hypercholesterolemia. *The Journal of biological chemistry*. 1974;249:5153-5162

351. Goldstein JL, Ho YK, Basu SK, Brown MS. Binding site on macrophages that mediates uptake and degradation of acetylated low density lipoprotein, producing massive cholesterol deposition. *Proceedings of the National Academy of Sciences of the United States of America*. 1979;76:333-337

352. Laurat E, Poirier B, Tupin E, Caligiuri G, Hansson GK, Bariety J, Nicoletti A. In vivo downregulation of t helper cell 1 immune responses reduces atherogenesis in apolipoprotein e-knockout mice. *Circulation*. 2001;104:197-202

353. Fadini GP, Simoni F, Cappellari R, Vitturi N, Galasso S, Vigili de Kreutzenberg S, Previato L, Avogaro A. Pro-inflammatory monocyte-macrophage polarization imbalance in human hypercholesterolemia and atherosclerosis. *Atherosclerosis*. 2014;237:805-808

354. Martinez FO, Gordon S, Locati M, Mantovani A. Transcriptional profiling of the human monocyte-to-macrophage differentiation and polarization: New molecules and patterns of gene expression. *Journal of immunology (Baltimore, Md. : 1950)*. 2006;177:7303-7311

355. Edwards JP, Zhang X, Frauwirth KA, Mosser DM. Biochemical and functional characterization of three activated macrophage populations. *Journal of leukocyte biology*. 2006;80:1298-1307

356. Feig JE, Rong JX, Shamir R, Sanson M, Vengrenyuk Y, Liu J, Rayner K, Moore K, Garabedian M, Fisher EA. Hdl promotes rapid atherosclerosis regression in mice and alters inflammatory properties of plaque monocyte-derived cells. *Proceedings of the National Academy of Sciences of the United States of America*. 2011;108:7166-7171

357. Abramson SL, Gallin JI. Il-4 inhibits superoxide production by human mononuclear phagocytes. *Journal of immunology (Baltimore, Md. : 1950)*. 1990;144:625-630

358. Martinez FO, Helming L, Gordon S. Alternative activation of macrophages: An immunologic functional perspective. *Annual review of immunology*. 2009;27:451-483

359. Pinto AR, Paolicelli R, Salimova E, Gospocic J, Slonimsky E, Bilbao-Cortes D, Godwin JW, Rosenthal NA. An abundant tissue macrophage population in the adult murine heart with a distinct alternatively-activated macrophage profile. *PloS one*. 2012;7:e36814

360. Suc I, Escargueil-Blanc I, Troly M, Salvayre R, Negre-Salvayre A. Hdl and apoa prevent cell death of endothelial cells induced by oxidized ldl. *Arteriosclerosis, thrombosis, and vascular biology*. 1997;17:2158-2166

361. Mineo C, Yuhanna IS, Quon MJ, Shaul PW. High density lipoprotein-induced endothelial nitric-oxide synthase activation is mediated by akt and map kinases. *The Journal of biological chemistry*. 2003;278:9142-9149

362. Baker PW, Rye KA, Gamble JR, Vadas MA, Barter PJ. Ability of reconstituted high density lipoproteins to inhibit cytokine-induced expression of vascular cell adhesion molecule-1 in human umbilical vein endothelial cells. *Journal of lipid research*. 1999;40:345-353

363. Yvan-Charvet L, Welch C, Pagler TA, Ranalletta M, Lamkanfi M, Han S, Ishibashi M, Li R, Wang N, Tall AR. Increased inflammatory gene expression in abc transporter-deficient macrophages: Free cholesterol accumulation, increased signaling via toll-like receptors, and neutrophil infiltration of atherosclerotic lesions. *Circulation*. 2008;118:1837-1847

364. Soran H, Hama S, Yadav R, Durrington PN. Hdl functionality. *Current opinion in lipidology*. 2012;23:353-366

365. Gilchrist M, Thorsson V, Li B, Rust AG, Korb M, Roach JC, Kennedy K, Hai T, Bolouri H, Aderem A. Systems biology approaches identify atf3 as a negative regulator of toll-like receptor 4. *Nature*. 2006;441:173-178

366. Li HF, Cheng CF, Liao WJ, Lin H, Yang RB. Atf3-mediated epigenetic regulation protects against acute kidney injury. *Journal of the American Society of Nephrology : JASN*. 2010;21:1003-1013

367. De Nardo D, Labzin LI, Kono H, Seki R, Schmidt SV, Beyer M, Xu D, Zimmer S, Lahrmann C, Schildberg FA, Vogelhuber J, Kraut M, Ulas T, Kerksiek A, Krebs W, Bode N, Grebe A, Fitzgerald ML, Hernandez NJ, Williams BR, Knolle P, Kneilling M, Rocken M, Lutjohann D, Wright SD, Schultze JL, Latz E. High-density lipoprotein mediates anti-inflammatory reprogramming of macrophages via the transcriptional regulator atf3. *Nature immunology*. 2014;15:152-160

368. Suzuki M, Pritchard DK, Becker L, Hoofnagle AN, Tanimura N, Bammler TK, Beyer RP, Bumgarner R, Vaisar T, de Beer MC, de Beer FC, Miyake K, Oram JF, Heinecke JW. High-density lipoprotein suppresses the type i interferon response, a family of potent antiviral immunoregulators, in macrophages challenged with lipopolysaccharide. *Circulation*. 2010;122:1919-1927

369. Hyka N, Dayer JM, Modoux C, Kohno T, Edwards CK, 3rd, Roux-Lombard P, Burger D. Apolipoprotein a-i inhibits the production of interleukin-1beta and tumor necrosis factor-alpha by blocking contact-mediated activation of monocytes by t lymphocytes. *Blood*. 2001;97:2381-2389

370. Passlick B, Flieger D, Ziegler-Heitbrock HW. Identification and characterization of a novel monocyte subpopulation in human peripheral blood. *Blood*. 1989;74:2527-2534

371. Robbins CS, Chudnovskiy A, Rauch PJ, Figueiredo JL, Iwamoto Y, Gorbatov R, Etzrodt M, Weber GF, Ueno T, van Rooijen N, Mulligan-Kehoe MJ, Libby P, Nahrendorf M, Pittet MJ, Weissleder R, Swirski FK. Extramedullary hematopoiesis generates ly-6c(high) monocytes that infiltrate atherosclerotic lesions. *Circulation*. 2012;125:364-374

372. Rogacev KS, Cremers B, Zawada AM, Seiler S, Binder N, Ege P, Grosse-Dunker G, Heisel I, Hornof F, Jeken J, Rebling NM, Ulrich C, Scheller B, Bohm M, Fliser D, Heine

GH. Cd14++cd16+ monocytes independently predict cardiovascular events: A cohort study of 951 patients referred for elective coronary angiography. *Journal of the American College of Cardiology*. 2012;60:1512-1520

373. Wrigley BJ, Shantsila E, Tapp LD, Lip GY. Cd14++cd16+ monocytes in patients with acute ischaemic heart failure. *European journal of clinical investigation*. 2013;43:121-130

374. Rogacev KS, Ulrich C, Blomer L, Hornof F, Oster K, Ziegelin M, Cremers B, Grenner Y, Geisel J, Schlitt A, Kohler H, Fliser D, Girndt M, Heine GH. Monocyte heterogeneity in obesity and subclinical atherosclerosis. *European heart journal*. 2010;31:369-376

375. Zawada AM, Rogacev KS, Rotter B, Winter P, Marell RR, Fliser D, Heine GH. Supersage evidence for cd14++cd16+ monocytes as a third monocyte subset. *Blood*. 2011;118:e50-61

376. Zawada AM, Rogacev KS, Schirmer SH, Sester M, Bohm M, Fliser D, Heine GH. Monocyte heterogeneity in human cardiovascular disease. *Immunobiology*. 2012;217:1273-1284

377. Combadiere C, Potteaux S, Rodero M, Simon T, Pezard A, Esposito B, Merval R, Proudfoot A, Tedgui A, Mallat Z. Combined inhibition of ccl2, cx3cr1, and ccr5 abrogates ly6c(hi) and ly6c(lo) monocytosis and almost abolishes atherosclerosis in hypercholesterolemic mice. *Circulation*. 2008;117:1649-1657

378. Schlitt A, Heine GH, Blankenberg S, Espinola-Klein C, Dopheide JF, Bickel C, Lackner KJ, Iz M, Meyer J, Darius H, Rupprecht HJ. Cd14+cd16+ monocytes in coronary artery disease and their relationship to serum tnf-alpha levels. *Thrombosis and haemostasis*. 2004;92:419-424

379. Tapp LD, Shantsila E, Wrigley BJ, Pamukcu B, Lip GY. The cd14++cd16+ monocyte subset and monocyte-platelet interactions in patients with st-elevation myocardial infarction. *Journal of thrombosis and haemostasis : JTH*. 2012;10:1231-1241

380. Tsujioka H, Imanishi T, Ikejima H, Kuroi A, Takarada S, Tanimoto T, Kitabata H, Okochi K, Arita Y, Ishibashi K, Komukai K, Kataiwa H, Nakamura N, Hirata K, Tanaka A, Akasaka T. Impact of heterogeneity of human peripheral blood monocyte subsets on myocardial salvage in patients with primary acute myocardial infarction. *Journal of the American College of Cardiology*. 2009;54:130-138

381. Ancuta P, Liu KY, Misra V, Wacleche VS, Gosselin A, Zhou X, Gabuzda D. Transcriptional profiling reveals developmental relationship and distinct biological functions of cd16+ and cd16- monocyte subsets. *BMC genomics*. 2009;10:403

382. Cho KY, Miyoshi H, Kuroda S, Yasuda H, Kamiyama K, Nakagawara J, Takigami M, Kondo T, Atsumi T. The phenotype of infiltrating macrophages influences arteriosclerotic plaque vulnerability in the carotid artery. *Journal of stroke and cerebrovascular diseases : the official journal of National Stroke Association*. 2013;22:910-918

383. Xu XX, Tabas I. Sphingomyelinase enhances low density lipoprotein uptake and ability to induce cholesteryl ester accumulation in macrophages. *The Journal of biological chemistry*. 1991;266:24849-24858

384. Podrez EA, Schmitt D, Hoff HF, Hazen SL. Myeloperoxidase-generated reactive nitrogen species convert ldl into an atherogenic form in vitro. *The Journal of clinical investigation*. 1999;103:1547-1560

385. Funk CD, Cyrus T. 12/15-lipoxygenase, oxidative modification of ldl and atherogenesis. *Trends in cardiovascular medicine*. 2001;11:116-124

386. Sparrow CP, Parthasarathy S, Steinberg D. Enzymatic modification of low density lipoprotein by purified lipoxygenase plus phospholipase a2 mimics cell-mediated oxidative modification. *Journal of lipid research*. 1988;29:745-753

387. Upston JM, Neuzil J, Stocker R. Oxidation of ldl by recombinant human 15-lipoxygenase: Evidence for alpha-tocopherol-dependent oxidation of esterified core and surface lipids. *Journal of lipid research*. 1996;37:2650-2661

388. Upston JM, Neuzil J, Witting PK, Alleva R, Stocker R. Oxidation of free fatty acids in low density lipoprotein by 15-lipoxygenase stimulates nonenzymic, alpha-tocopherol-mediated peroxidation of cholesteryl esters. *The Journal of biological chemistry*. 1997;272:30067-30074

389. Huo Y, Zhao L, Hyman MC, Shashkin P, Harry BL, Burcin T, Forlow SB, Stark MA, Smith DF, Clarke S, Srinivasan S, Hedrick CC, Pratico D, Witztum JL, Nadler JL, Funk CD, Ley K. Critical role of macrophage 12/15-lipoxygenase for atherosclerosis in apolipoprotein e-deficient mice. *Circulation*. 2004;110:2024-2031

390. Lordan S, O'Callaghan YC, O'Brien NM. Death-signaling pathways in human myeloid cells by oxldl and its cytotoxic components 7beta-hydroxycholesterol and cholesterol-5beta,6beta-epoxide. *Journal of biochemical and molecular toxicology*. 2007;21:362-372

391. Mohiuddin I, Chai H, Lin PH, Lumsden AB, Yao Q, Chen C. Nitrotyrosine and chlorotyrosine: Clinical significance and biological functions in the vascular system. *The Journal of surgical research*. 2006;133:143-149

392. Yla-Herttuala S, Palinski W, Rosenfeld ME, Parthasarathy S, Carew TE, Butler S, Witztum JL, Steinberg D. Evidence for the presence of oxidatively modified low density lipoprotein in atherosclerotic lesions of rabbit and man. *The Journal of clinical investigation*. 1989;84:1086-1095

393. Cushing SD, Berliner JA, Valente AJ, Territo MC, Navab M, Parhami F, Gerrity R, Schwartz CJ, Fogelman AM. Minimally modified low density lipoprotein induces monocyte chemotactic protein 1 in human endothelial cells and smooth muscle cells. *Proceedings of the National Academy of Sciences of the United States of America*. 1990;87:5134-5138

394. Tiwari RL, Singh V, Singh A, Rana M, Verma A, Kothari N, Kohli M, Bogra J, Dikshit M, Barthwal MK. Pkcdelta-irak1 axis regulates oxidized ldl-induced il-1beta production in monocytes. *Journal of lipid research*. 2014;55:1226-1244

395. Undurti A, Huang Y, Lupica JA, Smith JD, DiDonato JA, Hazen SL. Modification of high density lipoprotein by myeloperoxidase generates a pro-inflammatory particle. *The Journal of biological chemistry*. 2009;284:30825-30835

396. Vaisar T, Mayer P, Nilsson E, Zhao XQ, Knopp R, Prazen BJ. Hdl in humans with cardiovascular disease exhibits a proteomic signature. *Clinica chimica acta; international journal of clinical chemistry*. 2010;411:972-979

397. Panzenboeck U, Raitmayer S, Reicher H, Lindner H, Glatter O, Malle E, Sattler W. Effects of reagent and enzymatically generated hypochlorite on physicochemical and metabolic properties of high density lipoproteins. *The Journal of biological chemistry*. 1997;272:29711-29720

398. Xia Y, Zweier JL. Superoxide and peroxynitrite generation from inducible nitric oxide synthase in macrophages. *Proceedings of the National Academy of Sciences of the United States of America*. 1997;94:6954-6958

399. Shao B, Pennathur S, Pagani I, Oda MN, Witztum JL, Oram JF, Heinecke JW. Modifying apolipoprotein a-i by malondialdehyde, but not by an array of other reactive carbonyls, blocks cholesterol efflux by the abca1 pathway. *The Journal of biological chemistry*. 2010;285:18473-18484

400. Garner B, Witting PK, Waldeck AR, Christison JK, Raftery M, Stocker R. Oxidation of high density lipoproteins. I. Formation of methionine sulfoxide in apolipoproteins ai and aii is an early event that accompanies lipid peroxidation and can be enhanced by alpha-tocopherol. *The Journal of biological chemistry*. 1998;273:6080-6087

401. Pennathur S, Bergt C, Shao B, Byun J, Kassim SY, Singh P, Green PS, McDonald TO, Brunzell J, Chait A, Oram JF, O'Brien K, Geary RL, Heinecke JW. Human atherosclerotic intima and blood of patients with established coronary artery disease contain high density lipoprotein damaged by reactive nitrogen species. *The Journal of biological chemistry*. 2004;279:42977-42983

402. Wu J, He Z, Gao X, Wu F, Ding R, Ren Y, Jiang Q, Fan M, Liang C, Wu Z. Oxidized high-density lipoprotein impairs endothelial progenitor cells' function by activation of cd36-mapk-tsp-1 pathways. *Antioxidants & redox signaling*. 2015;22:308-324

403. Bergt C, Pennathur S, Fu X, Byun J, O'Brien K, McDonald TO, Singh P, Anantharamaiah GM, Chait A, Brunzell J, Geary RL, Oram JF, Heinecke JW. The myeloperoxidase product hypochlorous acid oxidizes hdl in the human artery wall and

impairs abca1-dependent cholesterol transport. *Proceedings of the National Academy of Sciences of the United States of America*. 2004;101:13032-13037

404. Shao B. Site-specific oxidation of apolipoprotein a-i impairs cholesterol export by abca1, a key cardioprotective function of hdl. *Biochimica et biophysica acta*. 2012;1821:490-501

405. Smith JD. Myeloperoxidase, inflammation, and dysfunctional high-density lipoprotein. *Journal of clinical lipidology*. 2010;4:382-388

406. Bergt C, Marsche G, Panzenboeck U, Heinecke JW, Malle E, Sattler W. Human neutrophils employ the myeloperoxidase/hydrogen peroxide/chloride system to oxidatively damage apolipoprotein a-i. *Eur J Biochem*. 2001;268:3523-3531

407. Fu X, Kassim SY, Parks WC, Heinecke JW. Hypochlorous acid oxygenates the cysteine switch domain of pro-matrilysin (mmp-7). A mechanism for matrix metalloproteinase activation and atherosclerotic plaque rupture by myeloperoxidase. *The Journal of biological chemistry*. 2001;276:41279-41287

408. Shao B, Bergt C, Fu X, Green P, Voss JC, Oda MN, Oram JF, Heinecke JW. Tyrosine 192 in apolipoprotein a-i is the major site of nitration and chlorination by myeloperoxidase, but only chlorination markedly impairs abca1-dependent cholesterol transport. *The Journal of biological chemistry*. 2005;280:5983-5993

409. Shao B, Pennathur S, Heinecke JW. Myeloperoxidase targets apolipoprotein a-i, the major high density lipoprotein protein, for site-specific oxidation in human atherosclerotic lesions. *The Journal of biological chemistry*. 2012;287:6375-6386

410. Zhang R, Brennan ML, Fu X, Aviles RJ, Pearce GL, Penn MS, Topol EJ, Sprecher DL, Hazen SL. Association between myeloperoxidase levels and risk of coronary artery disease. *Jama*. 2001;286:2136-2142

411. Yin K, Liao DF, Tang CK. Atp-binding membrane cassette transporter a1 (abca1): A possible link between inflammation and reverse cholesterol transport. *Molecular medicine (Cambridge, Mass.)*. 2010;16:438-449

412. Yvan-Charvet L, Pagler T, Gautier EL, Avagyan S, Siry RL, Han S, Welch CL, Wang N, Randolph GJ, Snoeck HW, Tall AR. Atp-binding cassette transporters and hdl suppress hematopoietic stem cell proliferation. *Science (New York, N.Y.)*. 2010;328:1689-1693

413. Koseki M, Hirano K, Masuda D, Ikegami C, Tanaka M, Ota A, Sandoval JC, Nakagawa-Toyama Y, Sato SB, Kobayashi T, Shimada Y, Ohno-Iwashita Y, Matsuura F, Shimomura I, Yamashita S. Increased lipid rafts and accelerated lipopolysaccharide-induced tumor necrosis factor-alpha secretion in abca1-deficient macrophages. *Journal of lipid research*. 2007;48:299-306

414. Eiserich JP, Hristova M, Cross CE, Jones AD, Freeman BA, Halliwell B, van der Vliet A. Formation of nitric oxide-derived inflammatory oxidants by myeloperoxidase in neutrophils. *Nature*. 1998;391:393-397

415. Karakas M, Koenig W. Myeloperoxidase production by macrophage and risk of atherosclerosis. *Curr Atheroscler Rep*. 2012;14:277-283

416. van der Vliet A, Eiserich JP, Halliwell B, Cross CE. Formation of reactive nitrogen species during peroxidase-catalyzed oxidation of nitrite. A potential additional mechanism of nitric oxide-dependent toxicity. *The Journal of biological chemistry*. 1997;272:7617-7625

417. Thomson L. 3-nitrotyrosine modified proteins in atherosclerosis. *Disease markers*. 2015;2015:708282

418. Shigenaga MK, Lee HH, Blount BC, Christen S, Shigeno ET, Yip H, Ames BN. Inflammation and no(x)-induced nitration: Assay for 3-nitrotyrosine by hplc with electrochemical detection. *Proceedings of the National Academy of Sciences of the United States of America*. 1997;94:3211-3216

419. Giugliano G, Brevetti G, Lanero S, Schiano V, Laurenzano E, Chiariello M. Leukocyte count in peripheral arterial disease: A simple, reliable, inexpensive approach to cardiovascular risk prediction. *Atherosclerosis*. 2010;210:288-293

420. Guasti L, Dentali F, Castiglioni L, Maroni L, Marino F, Squizzato A, Ageno W, Gianni M, Gaudio G, Grandi AM, Cosentino M, Venco A. Neutrophils and clinical outcomes in patients with acute coronary syndromes and/or cardiac revascularisation. A systematic review on more than 34,000 subjects. *Thrombosis and haemostasis*. 2011;106:591-599

421. Ionita MG, van den Borne P, Catanzariti LM, Moll FL, de Vries JP, Pasterkamp G, Vink A, de Kleijn DP. High neutrophil numbers in human carotid atherosclerotic plaques are associated with characteristics of rupture-prone lesions. *Arteriosclerosis, thrombosis, and vascular biology*. 2010;30:1842-1848

422. Drechsler M, Megens RT, van Zandvoort M, Weber C, Soehnlein O. Hyperlipidemia-triggered neutrophilia promotes early atherosclerosis. *Circulation*. 2010;122:1837-1845

423. Van Lenten BJ, Wagner AC, Navab M, Anantharamaiah GM, Hui EK, Nayak DP, Fogelman AM. D-4f, an apolipoprotein a-i mimetic peptide, inhibits the inflammatory response induced by influenza a infection of human type ii pneumocytes. *Circulation*. 2004;110:3252-3258

424. Rotzius P, Thams S, Soehnlein O, Kenne E, Tseng CN, Bjorkstrom NK, Malmberg KJ, Lindbom L, Eriksson EE. Distinct infiltration of neutrophils in lesion shoulders in apoe-/- mice. *The American journal of pathology*. 2010;177:493-500

425. Knight JS, Luo W, O'Dell AA, Yalavarthi S, Zhao W, Subramanian V, Guo C, Grenn RC, Thompson PR, Eitzman DT, Kaplan MJ. Peptidylarginine deiminase inhibition reduces vascular damage and modulates innate immune responses in murine models of atherosclerosis. *Circulation research*. 2014;114:947-956

426. Fuchs TA, Brill A, Duerschmied D, Schatzberg D, Monestier M, Myers DD, Jr., Wrobleski SK, Wakefield TW, Hartwig JH, Wagner DD. Extracellular DNA traps promote thrombosis. *Proceedings of the National Academy of Sciences of the United States of America*. 2010;107:15880-15885

427. von Bruhl ML, Stark K, Steinhart A, Chandraratne S, Konrad I, Lorenz M, Khandoga A, Tirniceriu A, Coletti R, Kollnberger M, Byrne RA, Laitinen I, Walch A, Brill A, Pfeiler S, Manukyan D, Braun S, Lange P, Riegger J, Ware J, Eckart A, Haidari S, Rudelius M, Schulz C, Echtler K, Brinkmann V, Schwaiger M, Preissner KT, Wagner DD, Mackman N, Engelmann B, Massberg S. Monocytes, neutrophils, and platelets cooperate to initiate and propagate venous thrombosis in mice in vivo. *The Journal of experimental medicine*. 2012;209:819-835

428. Maugeri N, Campana L, Gavina M, Covino C, De Metrio M, Panciroli C, Maiuri L, Maseri A, D'Angelo A, Bianchi ME, Rovere-Querini P, Manfredi AA. Activated platelets present high mobility group box 1 to neutrophils, inducing autophagy and promoting the extrusion of neutrophil extracellular traps. *Journal of thrombosis and haemostasis : JTH*. 2014;12:2074-2088

429. Clark SR, Ma AC, Tavener SA, McDonald B, Goodarzi Z, Kelly MM, Patel KD, Chakrabarti S, McAvoy E, Sinclair GD, Keys EM, Allen-Vercoe E, Devinney R, Doig CJ, Green FH, Kubes P. Platelet tlr4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. *Nature medicine*. 2007;13:463-469

430. Warnatsch A, Ioannou M, Wang Q, Papayannopoulos V. Inflammation. Neutrophil extracellular traps license macrophages for cytokine production in atherosclerosis. *Science (New York, N.Y.)*. 2015;349:316-320

431. Jimenez-Alcazar M, Napirei M, Panda R, Kohler EC, Kremer Hovinga JA, Mannherz HG, Peine S, Renne T, Lammle B, Fuchs TA. Impaired dnase1-mediated degradation of neutrophil extracellular traps is associated with acute thrombotic microangiopathies. *Journal of thrombosis and haemostasis : JTH*. 2015;13:732-742

432. Ge L, Zhou X, Ji WJ, Lu RY, Zhang Y, Zhang YD, Ma YQ, Zhao JH, Li YM. Neutrophil extracellular traps in ischemia-reperfusion injury-induced myocardial no-reflow: Therapeutic potential of dnase-based reperfusion strategy. *American journal of physiology. Heart and circulatory physiology*. 2015;308:H500-509

433. Martinod K, Demers M, Fuchs TA, Wong SL, Brill A, Gallant M, Hu J, Wang Y, Wagner DD. Neutrophil histone modification by peptidylarginine deiminase 4 is critical for deep vein thrombosis in mice. *Proceedings of the National Academy of Sciences of the United States of America*. 2013;110:8674-8679

434. Lewis HD, Liddle J, Coote JE, Atkinson SJ, Barker MD, Bax BD, Bicker KL, Bingham RP, Campbell M, Chen YH, Chung CW, Craggs PD, Davis RP, Eberhard D, Joberty G, Lind KE, Locke K, Maller C, Martinod K, Patten C, Polyakova O, Rise CE, Rudiger M, Sheppard RJ, Slade DJ, Thomas P, Thorpe J, Yao G, Drewes G, Wagner DD, Thompson

PR, Prinjha RK, Wilson DM. Inhibition of pad4 activity is sufficient to disrupt mouse and human net formation. *Nature chemical biology*. 2015;11:189-191

435. Hazen SL, Hsu FF, Mueller DM, Crowley JR, Heinecke JW. Human neutrophils employ chlorine gas as an oxidant during phagocytosis. *The Journal of clinical investigation*. 1996;98:1283-1289

436. Jiang Q, Hurst JK. Relative chlorinating, nitrating, and oxidizing capabilities of neutrophils determined with phagocytosable probes. *The Journal of biological chemistry*. 1997;272:32767-32772

437. Hazen SL, Heinecke JW. 3-chlorotyrosine, a specific marker of myeloperoxidase-catalyzed oxidation, is markedly elevated in low density lipoprotein isolated from human atherosclerotic intima. *The Journal of clinical investigation*. 1997;99:2075-2081

438. Rajamaki K, Lappalainen J, Oorni K, Valimaki E, Matikainen S, Kovanen PT, Eklund KK. Cholesterol crystals activate the nlrp3 inflammasome in human macrophages: A novel link between cholesterol metabolism and inflammation. *PloS one*. 2010;5:e11765

439. Nymo S, Niyonzima N, Espevik T, Mollnes TE. Cholesterol crystal-induced endothelial cell activation is complement-dependent and mediated by tnf. *Immunobiology*. 2014;219:786-792

440. Bui MN, Sack MN, Moutsatsos G, Lu DY, Katz P, McCown R, Breall JA, Rackley CE. Autoantibody titers to oxidized low-density lipoprotein in patients with coronary atherosclerosis. *American heart journal*. 1996;131:663-667

441. Stemme S, Faber B, Holm J, Wiklund O, Witztum JL, Hansson GK. T lymphocytes from human atherosclerotic plaques recognize oxidized low density lipoprotein. *Proceedings of the National Academy of Sciences of the United States of America*. 1995;92:3893-3897

442. Salonen JT, Yla-Herttuala S, Yamamoto R, Butler S, Korpela H, Salonen R, Nyyssonen K, Palinski W, Witztum JL. Autoantibody against oxidised ldl and progression of carotid atherosclerosis. *Lancet*. 1992;339:883-887

443. Faria-Neto JR, Chyu KY, Li X, Dimayuga PC, Ferreira C, Yano J, Cercek B, Shah PK. Passive immunization with monoclonal igm antibodies against phosphorylcholine reduces accelerated vein graft atherosclerosis in apolipoprotein e-null mice. *Atherosclerosis*. 2006;189:83-90

444. Che J, Li G, Wang W, Li Q, Liu H, Chen K, Liu T. Serum autoantibodies against human oxidized low-density lipoproteins are inversely associated with severity of coronary stenotic lesions calculated by gensini score. *Cardiology journal*. 2011;18:364-370

445. de Faire U, Frostegard J. Natural antibodies against phosphorylcholine in cardiovascular disease. *Annals of the New York Academy of Sciences*. 2009;1173:292-300

446. Karvonen J, Paivansalo M, Kesaniemi YA, Horkko S. Immunoglobulin m type of autoantibodies to oxidized low-density lipoprotein has an inverse relation to carotid artery atherosclerosis. *Circulation*. 2003;108:2107-2112

447. Nilsson I, Shibuya M, Wennstrom S. Differential activation of vascular genes by hypoxia in primary endothelial cells. *Experimental cell research*. 2004;299:476-485

448. Minta JO, Yun JJ, Kabiawu O, Jones J. Mrna differential display identification of vascular smooth muscle early response genes regulated by pdgf. *Molecular and cellular biochemistry*. 2006;281:63-75

449. Leclercq A, Houard X, Philippe M, Ollivier V, Sebbag U, Meilhac O, Michel JB. Involvement of intraplaque hemorrhage in atherothrombosis evolution via neutrophil protease enrichment. *Journal of leukocyte biology*. 2007;82:1420-1429

450. Moreno JA, Ortega-Gomez A, Delbosc S, Beaufort N, Sorbets E, Louedec L, Esposito-Farese M, Tubach F, Nicoletti A, Steg PG, Michel JB, Feldman L, Meilhac O. In vitro and in vivo evidence for the role of elastase shedding of cd163 in human atherothrombosis. *European heart journal*. 2012;33:252-263

451. Carmona-Rivera C, Zhao W, Yalavarthi S, Kaplan MJ. Neutrophil extracellular traps induce endothelial dysfunction in systemic lupus erythematosus through the activation of matrix metalloproteinase-2. *Annals of the rheumatic diseases*. 2015;74:1417-1424

452. Clarke MC, Figg N, Maguire JJ, Davenport AP, Goddard M, Littlewood TD, Bennett MR. Apoptosis of vascular smooth muscle cells induces features of plaque vulnerability in atherosclerosis. *Nature medicine*. 2006;12:1075-1080

453. Llorente-Cortes V, Otero-Vinas M, Berrozpe M, Badimon L. Intracellular lipid accumulation, low-density lipoprotein receptor-related protein expression, and cell survival in vascular smooth muscle cells derived from normal and atherosclerotic human coronaries. *European journal of clinical investigation*. 2004;34:182-190

454. Muller JE, Tofler GH. Triggering and hourly variation of onset of arterial thrombosis. *Annals of epidemiology*. 1992;2:393-405

455. Hooks JJ, Moutsopoulos HM, Geis SA, Stahl NI, Decker JL, Notkins AL. Immune interferon in the circulation of patients with autoimmune disease. *The New England journal of medicine*. 1979;301:5-8

456. Mohan IK, Das UN. Oxidant stress, anti-oxidants and essential fatty acids in systemic lupus erythematosus. *Prostaglandins, leukotrienes, and essential fatty acids*. 1997;56:193-198

457. Ara J, Ali R. Polynucleotide specificity of anti-reactive oxygen species (ros) DNA antibodies. *Clinical and experimental immunology*. 1993;94:134-139

458. Njoku C, Self SE, Ruiz P, Hofbauer AF, Gilkeson GS, Oates JC. Inducible nitric oxide synthase inhibitor sd-3651 reduces proteinuria in mrl/lpr mice deficient in the nos2 gene. *Journal of investigative medicine : the official publication of the American Federation for Clinical Research*. 2008;56:911-919

459. Perazzio SF, Salomao R, Silva NP, Andrade LE. Increased neutrophil oxidative burst metabolism in systemic lupus erythematosus. *Lupus*. 2012;21:1543-1551

460. Torres A, Askari AD, Malemud CJ. Cardiovascular disease complications in systemic lupus erythematosus. *Biomarkers in medicine*. 2009;3:239-252

461. Cates AM, Holden VI, Myers EM, Smith CK, Kaplan MJ, Kahlenberg JM. Interleukin 10 hampers endothelial cell differentiation and enhances the effects of interferon alpha on lupus endothelial cell progenitors. *Rheumatology (Oxford, England)*. 2015;54:1114-1123

462. Albini A, Marchisone C, Del Grosso F, Benelli R, Masiello L, Tacchetti C, Bono M, Ferrantini M, Rozera C, Truini M, Belardelli F, Santi L, Noonan DM. Inhibition of angiogenesis and vascular tumor growth by interferon-producing cells: A gene therapy approach. *The American journal of pathology*. 2000;156:1381-1393

463. Denny MF, Thacker S, Mehta H, Somers EC, Dodick T, Barrat FJ, McCune WJ, Kaplan MJ. Interferon-alpha promotes abnormal vasculogenesis in lupus: A potential pathway for premature atherosclerosis. *Blood*. 2007;110:2907-2915

464. Doring Y, Manthey HD, Drechsler M, Lievens D, Megens RT, Soehnlein O, Busch M, Manca M, Koenen RR, Pelisek J, Daemen MJ, Lutgens E, Zenke M, Binder CJ, Weber

C, Zernecke A. Auto-antigenic protein-DNA complexes stimulate plasmacytoid dendritic cells to promote atherosclerosis. *Circulation*. 2012;125:1673-1683

465. Mok MY, Yiu KH, Wong CY, Qiuwaxi J, Lai WH, Wong WS, Tse HF, Lau CS. Low circulating level of cd133+kdr+cells in patients with systemic sclerosis. *Clinical and experimental rheumatology*. 2010;28:S19-25

466. Castejon R, Jimenez-Ortiz C, Valero-Gonzalez S, Rosado S, Mellor S, Yebra-Bango M. Decreased circulating endothelial progenitor cells as an early risk factor of subclinical atherosclerosis in systemic lupus erythematosus. *Rheumatology (Oxford, England)*. 2014;53:631-638

467. Moonen JR, de Leeuw K, van Seijen XJ, Kallenberg CG, van Luyn MJ, Bijl M, Harmsen MC. Reduced number and impaired function of circulating progenitor cells in patients with systemic lupus erythematosus. *Arthritis research & therapy*. 2007;9:R84

468. Thacker SG, Duquaine D, Park J, Kaplan MJ. Lupus-prone new zealand black/new zealand white f1 mice display endothelial dysfunction and abnormal phenotype and function of endothelial progenitor cells. *Lupus*. 2010;19:288-299

469. Rajagopalan S, Somers EC, Brook RD, Kehrer C, Pfenninger D, Lewis E, Chakrabarti A, Richardson BC, Shelden E, McCune WJ, Kaplan MJ. Endothelial cell apoptosis in systemic lupus erythematosus: A common pathway for abnormal vascular function and thrombosis propensity. *Blood*. 2004;103:3677-3683

470. Li J, Fu Q, Cui H, Qu B, Pan W, Shen N, Bao C. Interferon-alpha priming promotes lipid uptake and macrophage-derived foam cell formation: A novel link between interferon-alpha and atherosclerosis in lupus. *Arthritis and rheumatism*. 2011;63:492-502

471. Goossens P, Gijbels MJ, Zernecke A, Eijgelaar W, Vergouwe MN, van der Made I, Vanderlocht J, Beckers L, Buurman WA, Daemen MJ, Kalinke U, Weber C, Lutgens E, de Winther MP. Myeloid type i interferon signaling promotes atherosclerosis by stimulating macrophage recruitment to lesions. *Cell metabolism*. 2010;12:142-153

472. Somers EC, Zhao W, Lewis EE, Wang L, Wing JJ, Sundaram B, Kazerooni EA, McCune WJ, Kaplan MJ. Type i interferons are associated with subclinical markers of cardiovascular disease in a cohort of systemic lupus erythematosus patients. *PloS one*. 2012;7:e37000

473. Reiss AB, Awadallah NW, Malhotra S, Montesinos MC, Chan ES, Javitt NB, Cronstein BN. Immune complexes and ifn-gamma decrease cholesterol 27-hydroxylase in human arterial endothelium and macrophages. *Journal of lipid research*. 2001;42:1913-1922

474. Schattner M, Lazzari M, Trevani AS, Malchiodi E, Kempfer AC, Isturiz MA, Geffner JR. Activation of human platelets by immune complexes prepared with cationized human igg. *Blood*. 1993;82:3045-3051

475. Meyer T, Robles-Carrillo L, Robson T, Langer F, Desai H, Davila M, Amaya M, Francis JL, Amirkhosravi A. Bevacizumab immune complexes activate platelets and induce thrombosis in fcgr2a transgenic mice. *Journal of thrombosis and haemostasis : JTH*. 2009;7:171-181

476. Megens RT, Vijayan S, Lievens D, Doring Y, van Zandvoort MA, Grommes J, Weber C, Soehnlein O. Presence of luminal neutrophil extracellular traps in atherosclerosis. *Thrombosis and haemostasis*. 2012;107:597-598

477. Fuchs TA, Brill A, Wagner DD. Neutrophil extracellular trap (net) impact on deep vein thrombosis. *Arteriosclerosis, thrombosis, and vascular biology*. 2012;32:1777-1783

478. Macritchie N, Grassia G, Sabir SR, Maddaluno M, Welsh P, Sattar N, Ialenti A, Kurowska-Stolarska M, McInnes IB, Brewer JM, Garside P, Maffia P. Plasmacytoid dendritic cells play a key role in promoting atherosclerosis in apolipoprotein e-deficient mice. *Arteriosclerosis, thrombosis, and vascular biology*. 2012;32:2569-2579

479. Clement M, Charles N, Escoubet B, Guedj K, Chauveheid MP, Caligiuri G, Nicoletti A, Papo T, Sacre K. Cd4+cxcr3+ t cells and plasmacytoid dendritic cells drive accelerated atherosclerosis associated with systemic lupus erythematosus. *Journal of autoimmunity*. 2015

480. Fujii J, Kurahashi T, Konno T, Homma T, Iuchi Y. Oxidative stress as a potential causal factor for autoimmune hemolytic anemia and systemic lupus erythematosus. *World journal of nephrology*. 2015;4:213-222

481. Toller-Kawahisa JE, Canicoba NC, Venancio VP, Kawahisa R, Antunes LM, Cunha TM, Marzocchi-Machado CM. Systemic lupus erythematosus onset in lupus-prone b6.Mrl/lpr mice is influenced by weight gain and is preceded by an increase in neutrophil oxidative burst activity. *Free radical biology & medicine*. 2015;86:362-373

482. Deguchi H, Fernandez JA, Hackeng TM, Banka CL, Griffin JH. Cardiolipin is a normal component of human plasma lipoproteins. *Proceedings of the National Academy of Sciences of the United States of America*. 2000;97:1743-1748

483. Batuca JR, Ames PR, Amaral M, Favas C, Isenberg DA, Delgado Alves J. Anti-atherogenic and anti-inflammatory properties of high-density lipoprotein are affected by specific antibodies in systemic lupus erythematosus. *Rheumatology (Oxford, England)*. 2009;48:26-31

484. de Carvalho JF, Borba EF, Viana VS, Bueno C, Leon EP, Bonfa E. Anti-lipoprotein lipase antibodies: A new player in the complex atherosclerotic process in systemic lupus erythematosus? *Arthritis and rheumatism*. 2004;50:3610-3615

485. Koskenmies S, Vaarala O, Widen E, Kere J, Palosuo T, Julkunen H. The association of antibodies to cardiolipin, beta 2-glycoprotein i, prothrombin, and oxidized low-density lipoprotein with thrombosis in 292 patients with familial and sporadic systemic lupus erythematosus. *Scandinavian journal of rheumatology*. 2004;33:246-252

486. Dinu AR, Merrill JT, Shen C, Antonov IV, Myones BL, Lahita RG. Frequency of antibodies to the cholesterol transport protein apolipoprotein a1 in patients with sle. *Lupus*. 1998;7:355-360

487. O'Neill SG, Giles I, Lambrianides A, Manson J, D'Cruz D, Schrieber L, March LM, Latchman DS, Isenberg DA, Rahman A. Antibodies to apolipoprotein a-i, high-density lipoprotein, and c-reactive protein are associated with disease activity in patients with systemic lupus erythematosus. *Arthritis and rheumatism*. 2010;62:845-854

488. Winfield JB, Shaw M, Taylor RP, Eisenberg RA. Nature of double-stranded DNA binding activity in seropositive rheumatoid arthritis: Formation of low avidity DNA/rheumatoid factor/igg/low density lipoprotein complexes. *Journal of immunology (Baltimore, Md. : 1950)*. 1981;126:1596-1602

489. Vaarala O, Alfthan G, Jauhiainen M, Leirisalo-Repo M, Aho K, Palosuo T. Crossreaction between antibodies to oxidised low-density lipoprotein and to cardiolipin in systemic lupus erythematosus. *Lancet*. 1993;341:923-925

490. Kobayashi K, Lopez LR, Shoenfeld Y, Matsuura E. The role of innate and adaptive immunity to oxidized low-density lipoprotein in the development of atherosclerosis. *Annals of the New York Academy of Sciences*. 2005;1051:442-454

491. Kobayashi K, Tada K, Itabe H, Ueno T, Liu PH, Tsutsumi A, Kuwana M, Yasuda T, Shoenfeld Y, de Groot PG, Matsuura E. Distinguished effects of antiphospholipid antibodies and anti-oxidized ldl antibodies on oxidized ldl uptake by macrophages. *Lupus*. 2007;16:929-938

492. Reiss AB, Anwar K, Merrill JT, Chan ES, Awadallah NW, Cronstein BN, Michael Belmont H, Belilos E, Rosenblum G, Belostocki K, Bonetti L, Hasneen K, Carsons SE. Plasma from systemic lupus patients compromises cholesterol homeostasis: A potential mechanism linking autoimmunity to atherosclerotic cardiovascular disease. *Rheumatology international*. 2010;30:591-598

493. Reiss AB, Wan DW, Anwar K, Merrill JT, Wirkowski PA, Shah N, Cronstein BN, Chan ES, Carsons SE. Enhanced cd36 scavenger receptor expression in thp-1 human monocytes in the presence of lupus plasma: Linking autoimmunity and atherosclerosis. *Experimental biology and medicine (Maywood, N.J.)*. 2009;234:354-360

494. Hasunuma Y, Matsuura E, Makita Z, Katahira T, Nishi S, Koike T. Involvement of beta 2-glycoprotein i and anticardiolipin antibodies in oxidatively modified low-density lipoprotein uptake by macrophages. *Clinical and experimental immunology*. 1997;107:569-573

495. Yassin LM, Londono J, Montoya G, De Sanctis JB, Rojas M, Ramirez LA, Garcia LF, Vasquez G. Atherosclerosis development in sle patients is not determined by monocytes ability to bind/endocytose ox-ldl. *Autoimmunity*. 2011;44:201-210

496. Kabakov AE, Saenko VA, Poverenny AM. Ldl-mediated interaction of DNA and DNA-anti-DNA immune complexes with cell surface. *Clinical and experimental immunology*. 1991;83:359-363

497. Kabakov AE, Tertov VV, Saenko VA, Poverenny AM, Orekhov AN. The atherogenic effect of lupus sera: Systemic lupus erythematosus-derived immune complexes stimulate the accumulation of cholesterol in cultured smooth muscle cells from human aorta. *Clinical immunology and immunopathology*. 1992;63:214-220

498. Skaggs BJ, Hahn BH, Sahakian L, Grossman J, McMahon M. Dysfunctional, pro-inflammatory hdl directly upregulates monocyte pdgfrbeta, chemotaxis and tnfalpha production. *Clinical immunology (Orlando, Fla.)*. 2010;137:147-156

499. Woo JM, Lin Z, Navab M, Van Dyck C, Trejo-Lopez Y, Woo KM, Li H, Castellani LW, Wang X, Iikuni N, Rullo OJ, Wu H, La Cava A, Fogelman AM, Lusis AJ, Tsao BP. Treatment with apolipoprotein a-1 mimetic peptide reduces lupus-like manifestations in a murine lupus model of accelerated atherosclerosis. *Arthritis research & therapy*. 2010;12:R93

500. Murphy AJ, Woollard KJ, Suhartoyo A, Stirzaker RA, Shaw J, Sviridov D, Chin-Dusting JP. Neutrophil activation is attenuated by high-density lipoprotein and apolipoprotein a-i in in vitro and in vivo models of inflammation. *Arteriosclerosis, thrombosis, and vascular biology*. 2011;31:1333-1341

501. McMahon M, Grossman J, FitzGerald J, Dahlin-Lee E, Wallace DJ, Thong BY, Badsha H, Kalunian K, Charles C, Navab M, Fogelman AM, Hahn BH. Proinflammatory high-density lipoprotein as a biomarker for atherosclerosis in patients with systemic lupus erythematosus and rheumatoid arthritis. *Arthritis and rheumatism*. 2006;54:2541-2549

502. Marsillach J, Becker JO, Vaisar T, Hahn BH, Brunzell JD, Furlong CE, de Boer IH, McMahon MA, Hoofnagle AN, Group DER. Paraoxonase-3 is depleted from the high-density lipoproteins of autoimmune disease patients with subclinical atherosclerosis. *Journal of proteome research*. 2015;14:2046-2054

503. Ronda N, Favari E, Borghi MO, Ingegnoli F, Gerosa M, Chighizola C, Zimetti F, Adorni MP, Bernini F, Meroni PL. Impaired serum cholesterol efflux capacity in rheumatoid arthritis and systemic lupus erythematosus. *Annals of the rheumatic diseases*. 2014;73:609-615

504. Ardoin SP, Sandborg C, Schanberg LE. Management of dyslipidemia in children and adolescents with systemic lupus erythematosus. *Lupus*. 2007;16:618-626

505. Ouimet M, Franklin V, Mak E, Liao X, Tabas I, Marcel YL. Autophagy regulates cholesterol efflux from macrophage foam cells via lysosomal acid lipase. *Cell metabolism*. 2011;13:655-667

506. Amuro H, Ito T, Miyamoto R, Sugimoto H, Torii Y, Son Y, Nakamichi N, Yamazaki C, Hoshino K, Kaisho T, Ozaki Y, Inaba M, Amakawa R, Fukuhara S. Statins, inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme a reductase, function as inhibitors of cellular and molecular components involved in type i interferon production. *Arthritis and rheumatism*. 2010;62:2073-2085

507. Mora S, Ridker PM. Justification for the use of statins in primary prevention: An intervention trial evaluating rosuvastatin (jupiter)--can c-reactive protein be used to target statin therapy in primary prevention? *The American journal of cardiology*. 2006;97:33A-41A

508. Ascer E, Bertolami MC, Venturinelli ML, Buccheri V, Souza J, Nicolau JC, Ramires JA, Serrano CV, Jr. Atorvastatin reduces proinflammatory markers in hypercholesterolemic patients. *Atherosclerosis*. 2004;177:161-166

509. Yoshimura K, Nagasawa A, Kudo J, Onoda M, Morikage N, Furutani A, Aoki H, Hamano K. Inhibitory effect of statins on inflammation-related pathways in human abdominal aortic aneurysm tissue. *International journal of molecular sciences*. 2015;16:11213-11228

510. Baigent C, Keech A, Kearney PM, Blackwell L, Buck G, Pollicino C, Kirby A, Sourjina T, Peto R, Collins R, Simes R. Efficacy and safety of cholesterol-lowering treatment: Prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. *Lancet*. 2005;366:1267-1278

511. Barter PJ, Caulfield M, Eriksson M, Grundy SM, Kastelein JJ, Komajda M, Lopez-Sendon J, Mosca L, Tardif JC, Waters DD, Shear CL, Revkin JH, Buhr KA, Fisher MR, Tall AR, Brewer B. Effects of torcetrapib in patients at high risk for coronary events. *The New England journal of medicine*. 2007;357:2109-2122

512. Di Bartolo BA, Nicholls SJ, Bao S, Rye KA, Heather AK, Barter PJ, Bursill C. The apolipoprotein a-i mimetic peptide etc-642 exhibits anti-inflammatory properties that are comparable to high density lipoproteins. *Atherosclerosis*. 2011;217:395-400

513. Navab M, Shechter I, Anantharamaiah GM, Reddy ST, Van Lenten BJ, Fogelman AM. Structure and function of hdl mimetics. *Arteriosclerosis, thrombosis, and vascular biology*. 2010;30:164-168

514. Bayes M, Rabasseda X, Prous JR. Gateways to clinical trials. *Methods and findings in experimental and clinical pharmacology*. 2004;26:473-503

515. Eiserich JP, Cross CE, Jones AD, Halliwell B, van der Vliet A. Formation of nitrating and chlorinating species by reaction of nitrite with hypochlorous acid. A novel mechanism for nitric oxide-mediated protein modification. *The Journal of biological chemistry*. 1996;271:19199-19208

516. McCord JM, Fridovich I. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). *The Journal of biological chemistry*. 1969;244:6049-6055

517. Palmer RM, Ashton DS, Moncada S. Vascular endothelial cells synthesize nitric oxide from l-arginine. *Nature*. 1988;333:664-666

518. Huie RE, Padmaja S. The reaction of no with superoxide. *Free radical research communications*. 1993;18:195-199

519. Sato R, Nishibayashi H, Omura T. Naphthoquinone-dependent oxidation of reduced triphosphopyridine nucleotide by liver microsomes. *Biochimica et biophysica acta*. 1962;63:550-552

520. Iyer GYN, Islam MF, Quastel JH. Biochemical aspects of phagocytosis. *Nature*. 1961;192:535-541

521. Vivekanandan-Giri A, Slocum JL, Byun J, Tang C, Sands RL, Gillespie BW, Heinecke JW, Saran R, Kaplan MJ, Pennathur S. High density lipoprotein is targeted for oxidation by myeloperoxidase in rheumatoid arthritis. *Ann Rheum Dis*. 2013

522. Vivekanandan-Giri A, Byun J, Pennathur S. Quantitative analysis of amino acid oxidation markers by tandem mass spectrometry. *Methods Enzymol*. 2011;491:73-89

523. Gordon SM, Deng J, Lu LJ, Davidson WS. Proteomic characterization of human plasma high density lipoprotein fractionated by gel filtration chromatography. *Journal of proteome research*. 2010;9:5239-5249

524. Munafo DB, Johnson JL, Brzezinska AA, Ellis BA, Wood MR, Catz SD. Dnase i inhibits a late phase of reactive oxygen species production in neutrophils. *Journal of innate immunity*. 2009;1:527-542

525. Kirchner T, Moller S, Klinger M, Solbach W, Laskay T, Behnen M. The impact of various reactive oxygen species on the formation of neutrophil extracellular traps. *Mediators of inflammation*. 2012;2012:849136

526. Goud AP, Goud PT, Diamond MP, Gonik B, Abu-Soud HM. Activation of the cgmp signaling pathway is essential in delaying oocyte aging in diabetes mellitus. *Biochemistry*. 2006;45:11366-11378

527. Furst DE. Pharmacokinetics of hydroxychloroquine and chloroquine during treatment of rheumatic diseases. *Lupus*. 1996;5 Suppl 1:S11-15

528. Causey CP, Jones JE, Slack JL, Kamei D, Jones LE, Subramanian V, Knuckley B, Ebrahimi P, Chumanevich AA, Luo Y, Hashimoto H, Sato M, Hofseth LJ, Thompson PR. The development of n-alpha-(2-carboxyl)benzoyl-n(5)-(2-fluoro-1-iminoethyl)-l-ornithine amide (o-f-amidine) and n-alpha-(2-carboxyl)benzoyl-n(5)-(2-chloro-1-iminoethyl)-l-ornithine amide (o-cl-amidine) as second generation protein arginine deiminase (pad) inhibitors. *J Med Chem*. 2011;54:6919-6935

529. Vivekanandan-Giri A, Slocum JL, Byun J, Tang C, Sands RL, Gillespie BW, Heinecke JW, Saran R, Kaplan MJ, Pennathur S. High density lipoprotein is targeted for oxidation by myeloperoxidase in rheumatoid arthritis. *Annals of the rheumatic diseases*. 2013;72:1725-1731

530. Ikeda Y, Imai Y, Kumagai H, Nosaka T, Morikawa Y, Hisaoka T, Manabe I, Maemura K, Nakaoka T, Imamura T, Miyazono K, Komuro I, Nagai R, Kitamura T. Vasorin, a

transforming growth factor beta-binding protein expressed in vascular smooth muscle cells, modulates the arterial response to injury in vivo. *Proceedings of the National Academy of Sciences of the United States of America*. 2004;101:10732-10737

531. Malapeira J, Esselens C, Bech-Serra JJ, Canals F, Arribas J. Adam17 (tace) regulates tgfbeta signaling through the cleavage of vasorin. *Oncogene*. 2011;30:1912-1922

532. Huang A, Dong J, Li S, Wang C, Ding H, Li H, Su X, Ge X, Sun L, Bai C, Shen X, Fang T, Li J, Shao N. Exosomal transfer of vasorin expressed in hepatocellular carcinoma cells promotes migration of human umbilical vein endothelial cells. *International journal of biological sciences*. 2015;11:961-969

533. McMillen TS, Heinecke JW, LeBoeuf RC. Expression of human myeloperoxidase by macrophages promotes atherosclerosis in mice. *Circulation*. 2005;111:2798-2804

534. Telles RW, Ferreira GA, da Silva NP, Sato EI. Increased plasma myeloperoxidase levels in systemic lupus erythematosus. *Rheumatology international*. 2010;30:779-784

535. Brennan ML, Anderson MM, Shih DM, Qu XD, Wang X, Mehta AC, Lim LL, Shi W, Hazen SL, Jacob JS, Crowley JR, Heinecke JW, Lusis AJ. Increased atherosclerosis in myeloperoxidase-deficient mice. *The Journal of clinical investigation*. 2001;107:419-430

536. Leshner M, Wang S, Lewis C, Zheng H, Chen XA, Santy L, Wang Y. Pad4 mediated histone hypercitrullination induces heterochromatin decondensation and chromatin unfolding to form neutrophil extracellular trap-like structures. *Frontiers in immunology*. 2012;3:307

537. Carmona-Rivera C, Zhao W, Yalavarthi S, Kaplan MJ. Neutrophil extracellular traps induce endothelial dysfunction in systemic lupus erythematosus through the activation of matrix metalloproteinase-2. *Ann Rheum Dis*. 2014

538. Soehnlein O. Multiple roles for neutrophils in atherosclerosis. *Circulation research*. 2012;110:875-888

539. Dorweiler B, Torzewski M, Dahm M, Kirkpatrick CJ, Lackner KJ, Vahl CF. Subendothelial infiltration of neutrophil granulocytes and liberation of matrix-destabilizing enzymes in an experimental model of human neo-intima. *Thromb Haemost*. 2008;99:373-381

540. Kessenbrock K, Krumbholz M, Schonermarck U, Back W, Gross WL, Werb Z, Grone HJ, Brinkmann V, Jenne DE. Netting neutrophils in autoimmune small-vessel vasculitis. *Nature medicine*. 2009;15:623-625

541. Nofer JR. Signal transduction by hdl: Agonists, receptors, and signaling cascades. *Handbook of experimental pharmacology*. 2015;224:229-256

542. Mao J, Liu W, Wang Y. Apolipoprotein a-i expression suppresses cox-2 expression by reducing reactive oxygen species in hepatocytes. *Biochemical and biophysical research communications*. 2014;454:359-363

543. Smith CK, Vivekanandan-Giri A, Tang C, Knight JS, Mathew A, Padilla RL, Gillespie BW, Carmona-Rivera C, Liu X, Subramanian V, Hasni S, Thompson PR, Heinecke JW, Saran R, Pennathur S, Kaplan MJ. Neutrophil extracellular trap-derived enzymes oxidize high-density lipoprotein: An additional proatherogenic mechanism in systemic lupus erythematosus. *Arthritis & rheumatology (Hoboken, N.J.)*. 2014;66:2532-2544

544. Jaguin M, Houlbert N, Fardel O, Lecureur V. Polarization profiles of human m-csf-generated macrophages and comparison of m1-markers in classically activated macrophages from gm-csf and m-csf origin. *Cellular immunology*. 2013;281:51-61

545. Di Bartolo BA, Vanags LZ, Tan JT, Bao S, Rye KA, Barter PJ, Bursill CA. The apolipoprotein a-i mimetic peptide, etc-642, reduces chronic vascular inflammation in the rabbit. *Lipids in health and disease*. 2011;10:224

546. Li D, Gordon S, Schwendeman A, Remaley AT. Apolipoprotein mimetic peptides for stimulating cholesterol efflux. *Apolipoprotein mimetics in management of human disease*. 2015:29-42

547. Becker AM, Dao KH, Han BK, Kornu R, Lakhanpal S, Mobley AB, Li QZ, Lian Y, Wu T, Reimold AM, Olsen NJ, Karp DR, Chowdhury FZ, Farrar JD, Satterthwaite AB, Mohan C, Lipsky PE, Wakeland EK, Davis LS. Sle peripheral blood b cell, t cell and myeloid cell transcriptomes display unique profiles and each subset contributes to the interferon signature. *PloS one*. 2013;8:e67003

548. Tabas I. Macrophage death and defective inflammation resolution in atherosclerosis. *Nature reviews. Immunology*. 2010;10:36-46

549. Hai TW, Liu F, Coukos WJ, Green MR. Transcription factor atf cdna clones: An extensive family of leucine zipper proteins able to selectively form DNA-binding heterodimers. *Genes & development*. 1989;3:2083-2090

550. Hai T, Wolfgang CD, Marsee DK, Allen AE, Sivaprasad U. Atf3 and stress responses. *Gene expression*. 1999;7:321-335

551. Chen BP, Liang G, Whelan J, Hai T. Atf3 and atf3 delta zip. Transcriptional repression versus activation by alternatively spliced isoforms. *The Journal of biological chemistry*. 1994;269:15819-15826

552. Lai PF, Cheng CF, Lin H, Tseng TL, Chen HH, Chen SH. Atf3 protects against lps-induced inflammation in mice via inhibiting hmgb1 expression. *Evidence-based complementary and alternative medicine : eCAM*. 2013;2013:716481

553. Besler C, Heinrich K, Rohrer L, Doerries C, Riwanto M, Shih DM, Chroni A, Yonekawa K, Stein S, Schaefer N, Mueller M, Akhmedov A, Daniil G, Manes C, Templin C, Wyss C, Maier W, Tanner FC, Matter CM, Corti R, Furlong C, Lusis AJ, von Eckardstein A, Fogelman AM, Luscher TF, Landmesser U. Mechanisms underlying adverse effects of hdl on enos-activating pathways in patients with coronary artery disease. *The Journal of clinical investigation*. 2011;121:2693-2708

554. Marsche G, Levak-Frank S, Quehenberger O, Heller R, Sattler W, Malle E. Identification of the human analog of sr-bi and lox-1 as receptors for hypochlorite-modified high density lipoprotein on human umbilical venous endothelial cells. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*. 2001;15:1095-1097

555. Zhang M, Gao X, Wu J, Liu D, Cai H, Fu L, Mei C. Oxidized high-density lipoprotein enhances inflammatory activity in rat mesangial cells. *Diabetes/metabolism research and reviews*. 2010;26:455-463

556. Li L, Sawamura T, Renier G. Glucose enhances human macrophage lox-1 expression: Role for lox-1 in glucose-induced macrophage foam cell formation. *Circulation research*. 2004;94:892-901

557. Lee HY, Kim SD, Baek SH, Choi JH, Cho KH, Zabel BA, Bae YS. Serum amyloid a stimulates macrophage foam cell formation via lectin-like oxidized low-density

lipoprotein receptor 1 upregulation. *Biochemical and biophysical research communications*. 2013;433:18-23

558. Crucet M, Wust SJ, Spielmann P, Luscher TF, Wenger RH, Matter CM. Hypoxia enhances lipid uptake in macrophages: Role of the scavenger receptors lox1, sra, and cd36. *Atherosclerosis*. 2013;229:110-117

559. Iwata A, Miura S, Zhang B, Imaizumi S, Uehara Y, Shiomi M, Saku K. Antiatherogenic effects of newly developed apolipoprotein a-i mimetic peptide/phospholipid complexes against aortic plaque burden in watanabe-heritable hyperlipidemic rabbits. *Atherosclerosis*. 2011;218:300-307

560. Joshi PH, Toth PP, Lirette ST, Griswold ME, Massaro JM, Martin SS, Blaha MJ, Kulkarni KR, Khokhar AA, Correa A, D'Agustino RB, Sr., Jones SR. Association of high-density lipoprotein subclasses and incident coronary heart disease: The jackson heart and framingham offspring cohort studies. *European journal of preventive cardiology*. 2014

561. Martin SS, Khokhar AA, May HT, Kulkarni KR, Blaha MJ, Joshi PH, Toth PP, Muhlestein JB, Anderson JL, Knight S, Li Y, Spertus JA, Jones SR. Hdl cholesterol subclasses, myocardial infarction, and mortality in secondary prevention: The lipoprotein investigators collaborative. *European heart journal*. 2015;36:22-30

562. Park SJ, Lee KP, Kang S, Lee J, Sato K, Chung HY, Okajima F, Im DS. Sphingosine 1-phosphate induced anti-atherogenic and atheroprotective m2 macrophage polarization through il-4. *Cellular signalling*. 2014;26:2249-2258

563. Christoffersen C, Obinata H, Kumaraswamy SB, Galvani S, Ahnstrom J, Sevvana M, Egerer-Sieber C, Muller YA, Hla T, Nielsen LB, Dahlback B. Endothelium-protective

sphingosine-1-phosphate provided by hdl-associated apolipoprotein m. *Proceedings of the National Academy of Sciences of the United States of America*. 2011;108:9613-9618

564. Argraves KM, Sethi AA, Gazzolo PJ, Wilkerson BA, Remaley AT, Tybjaerg-Hansen A, Nordestgaard BG, Yeatts SD, Nicholas KS, Barth JL, Argraves WS. S1p, dihydro-s1p and c24:1-ceramide levels in the hdl-containing fraction of serum inversely correlate with occurrence of ischemic heart disease. *Lipids in health and disease*. 2011;10:70

565. Sattler KJ, Elbasan S, Keul P, Elter-Schulz M, Bode C, Graler MH, Brocker-Preuss M, Budde T, Erbel R, Heusch G, Levkau B. Sphingosine 1-phosphate levels in plasma and hdl are altered in coronary artery disease. *Basic research in cardiology*. 2010;105:821-832

566. Kwon YG, Min JK, Kim KM, Lee DJ, Billiar TR, Kim YM. Sphingosine 1-phosphate protects human umbilical vein endothelial cells from serum-deprived apoptosis by nitric oxide production. *The Journal of biological chemistry*. 2001;276:10627-10633

567. Clark KL, Reed TJ, Wolf SJ, Lowe L, Hodgin JB, Kahlenberg JM. Epidermal injury promotes nephritis flare in lupus-prone mice. *Journal of autoimmunity*. 2015

568. Ghoreishi M, Dutz JP. Cutaneous lupus erythematosus: Recent lessons from animal models. *Lupus*. 2010;19:1029-1035

569. Ni JQ, Ouyang Q, Lin L, Huang Z, Lu H, Chen X, Lin H, Wang Z, Xu D, Zhang Y. Role of toll-like receptor 4 on lupus lung injury and atherosclerosis in lps-challenge apoe(-)/(-) mice. *Clinical & developmental immunology*. 2013;2013:476856

570. Mehta JL, Sanada N, Hu CP, Chen J, Dandapat A, Sugawara F, Satoh H, Inoue K, Kawase Y, Jishage K, Suzuki H, Takeya M, Schnackenberg L, Beger R, Hermonat PL,

Thomas M, Sawamura T. Deletion of lox-1 reduces atherogenesis in ldlr knockout mice fed high cholesterol diet. *Circulation research*. 2007;100:1634-1642

571. Ding Z, Mizeracki AM, Hu C, Mehta JL. Lox-1 deletion and macrophage trafficking in atherosclerosis. *Biochemical and biophysical research communications*. 2013;440:210-214

572. Takanabe-Mori R, Ono K, Sowa N, Wada H, Takaya T, Horie T, Satoh-Asahara N, Shimatsu A, Fujita M, Sawamura T, Hasegawa K. Lectin-like oxidized low-density lipoprotein receptor-1 is required for the adipose tissue expression of proinflammatory cytokines in high-fat diet-induced obese mice. *Biochemical and biophysical research communications*. 2010;398:576-580

573. Xu X, Gao X, Potter BJ, Cao JM, Zhang C. Anti-lox-1 rescues endothelial function in coronary arterioles in atherosclerotic apoe knockout mice. *Arteriosclerosis, thrombosis, and vascular biology*. 2007;27:871-877

574. McMahon M, Skaggs B. Pathogenesis and treatment of atherosclerosis in lupus. *Rheumatic diseases clinics of North America*. 2014;40:475-495, viii

575. Knight JS, Kaplan MJ. Cardiovascular disease in lupus: Insights and updates. *Current opinion in rheumatology*. 2013;25:597-605

576. Wang Y, Agerberth B, Lothgren A, Almstedt A, Johansson J. Apolipoprotein a-i binds and inhibits the human antibacterial/cytotoxic peptide ll-37. *The Journal of biological chemistry*. 1998;273:33115-33118

577. Sorensen O, Bratt T, Johnsen AH, Madsen MT, Borregaard N. The human antibacterial cathelicidin, hcap-18, is bound to lipoproteins in plasma. *The Journal of biological chemistry*. 1999;274:22445-22451

578. Francis GA. High density lipoprotein oxidation: In vitro susceptibility and potential in vivo consequences. *Biochimica et biophysica acta*. 2000;1483:217-235

579. Sharma N, Akhade AS, Qadri A. Sphingosine-1-phosphate suppresses tlr-induced cxcl8 secretion from human t cells. *Journal of leukocyte biology*. 2013;93:521-528

580. Awojoodu AO, Ogle ME, Sefcik LS, Bowers DT, Martin K, Brayman KL, Lynch KR, Peirce-Cottler SM, Botchwey E. Sphingosine 1-phosphate receptor 3 regulates recruitment of anti-inflammatory monocytes to microvessels during implant arteriogenesis. *Proceedings of the National Academy of Sciences of the United States of America*. 2013;110:13785-13790

581. Duenas AI, Aceves M, Fernandez-Pisonero I, Gomez C, Orduna A, Crespo MS, Garcia-Rodriguez C. Selective attenuation of toll-like receptor 2 signalling may explain the atheroprotective effect of sphingosine 1-phosphate. *Cardiovascular research*. 2008;79:537-544

582. Vickers KC, Palmisano BT, Shoucri BM, Shamburek RD, Remaley AT. Micrornas are transported in plasma and delivered to recipient cells by high-density lipoproteins. *Nature cell biology*. 2011;13:423-433

583. Wang G, Tam LS, Li EK, Kwan BC, Chow KM, Luk CC, Li PK, Szeto CC. Serum and urinary cell-free mir-146a and mir-155 in patients with systemic lupus erythematosus. *The Journal of rheumatology*. 2010;37:2516-2522

584. Zhang Y, Zhang M, Zhong M, Suo Q, Lv K. Expression profiles of mirnas in polarized macrophages. *International journal of molecular medicine*. 2013;31:797-802

585. Qu B, Shen N. Mirnas in the pathogenesis of systemic lupus erythematosus. *International journal of molecular sciences*. 2015;16:9557-9572

586. Fernandez-Hernando C, Suarez Y, Rayner KJ, Moore KJ. Micrornas in lipid metabolism. *Current opinion in lipidology*. 2011;22:86-92

587. Rayner KJ, Moore KJ. Microrna control of high-density lipoprotein metabolism and function. *Circulation research*. 2014;114:183-192

588. Chang L, Clifton P, Barter P, Mackinnon M. High density lipoprotein subpopulations in chronic liver disease. *Hepatology (Baltimore, Md.)*. 1986;6:46-49

589. Pirillo A, Catapano AL, Norata GD. Hdl in infectious diseases and sepsis. *Handbook of experimental pharmacology*. 2015;224:483-508

590. Salahuddin T, Natarajan B, Playford MP, Joshi AA, Teague H, Masmoudi Y, Selwaness M, Chen MY, Bluemke DA, Mehta NN. Cholesterol efflux capacity in humans with psoriasis is inversely related to non-calcified burden of coronary atherosclerosis. *European heart journal*. 2015

591. Okada T, Harada K, Okuni M. Serum hdl-cholesterol and lipoprotein fraction in kawasaki disease (acute mucocutaneous lymph node syndrome). *Japanese circulation journal*. 1982;46:1039-1044

592. Mitamura T, Ohno S, Ariga H, Ohsaka T, Iwasaki N, Matsuda H, Matsumiya H. Lipoprotein cholesterol concentrations in patients with behcet's disease. *Clinica chimica acta; international journal of clinical chemistry*. 1988;175:277-283

593. Lin AM, Rubin CJ, Khandpur R, Wang JY, Riblett M, Yalavarthi S, Villanueva EC, Shah P, Kaplan MJ, Bruce AT. Mast cells and neutrophils release il-17 through extracellular trap formation in psoriasis. *Journal of immunology (Baltimore, Md. : 1950)*. 2011;187:490-500