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LUPUS HDL PROMOTES PRO-INFLAMMATORY RESPONSES IN MACROPHAGES THROUGH LOX1R BINDING AND ABROGATION OF ATF3 ACTIVITY

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Background Recent evidence indicates that high-density lipoprotein (HDL) exerts vasculoprotective activities by promoting activating transcription factor 3 (ATF3), leading to down-regulation of TLR-induced inflammatory responses. SLE is associated with increased cardiovascular disease (CVD) risk not explained by the Framingham risk score. Recent studies have indicated oxidised HDL as a possible contributor. We investigated the potential mechanisms by which lupus HDL may lose its anti-inflammatory effects and promote immune dysregulation.

Methods and results Compared to control HDL, SLE HDL activates NF κ B, promotes inflammatory cytokine production, and fails to block TLR-induced inflammation in control macrophages. This failure of lupus HDL to block inflammatory responses is due to an impaired ability to promote ATF3 synthesis and nuclear translocation. SLE HDL-induced pro-inflammatory responses in macrophages are dependent on its binding to lectin-like oxidised low-density lipoprotein receptor 1 (LOX1R), which promotes suppression of ATF3 activity in a ROCK1/2 kinase-dependent manner. This inflammation can be modulated *in vivo* as lupus-prone mice exposed to the HDL mimetic ETC-642 show improved ATF3 induction and significant abrogation of pro-inflammatory cytokines

Conclusions Lupus HDL promotes pro-inflammatory responses, increased NF κ B activity and decreased ATF3 synthesis and activity, in a LOX1R- and ROCK1/2 kinase-dependent manner. ETC-642 inhibited both *in vitro* and *in vivo* SLE HDL-induced inflammation.

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CELL-BASED THERAPY IN SYSTEMIC LUPUS ERYTHEMATOSUS (SLE): EFFECTS ON NEUTROPHIL NETTING

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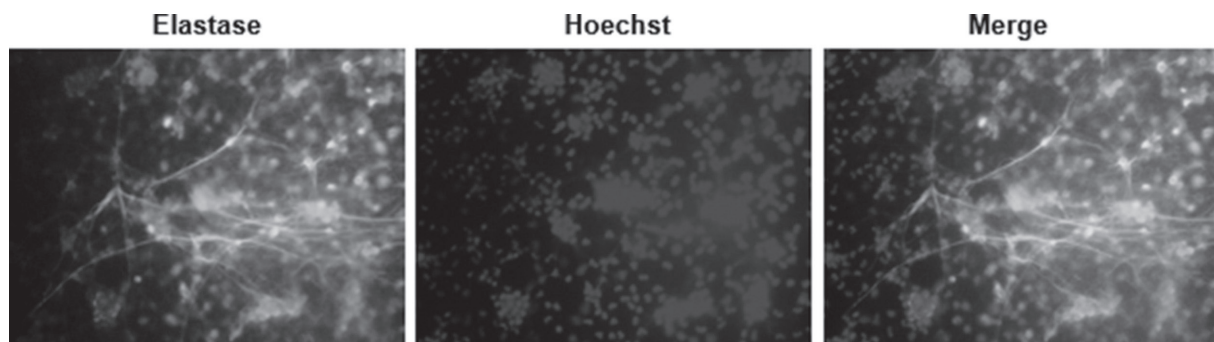
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Background Evidence that mesenchymal stem cells (MSCs) derived from bone marrow, fat and umbilical cord can be used to treat refractory SLE and SLE nephritis is growing. MSCs were originally described as cells from bone marrow that have the capacity to differentiate into bone, cartilage and fat. More recently it has been recognised that all MSCs are peri-cytes and that their greatest potential is because they are pleiotropic and can both sense and repair their environment. We hypothesised that MSCs can reduce neutrophil activation in SLE by inhibiting neutrophil netting thus reducing induction of T-helper follicular cells that promote the development of long-living plasmablasts that can secrete autoantibodies.

Materials and methods We studied neutrophils derived from healthy donors and patients with SLE. Neutrophils were isolated using MACSxpress™ Neutrophil Isolation Kit (Miltenyi) and onto coverslips in 24-well plates and incubated for 1–2 hours with conditioned medium derived from MSCs or control medium. Netting was induced by culture *ex vivo* with 20 nM PMA for 2 hours. Coverslips were fixed in 4% paraformaldehyde and NETs were quantified using anti-human antibody directed against neutrophil elastase colocalizing with extracellular DNA using Hoechst 33342.

Results To date we have optimised the conditions of our assay. Studies are ongoing to determine the effect of MSCs and/or their products on neutrophil netting. Figure 1: seen below are netting neutrophils induced as described above. Assays are underway to determine the effect of MSCs on SLE netting neutrophils *ex vivo*. **Conclusion** The possibility that MSCs and/or their products could act both on innate and adaptive immune responses in SLE is appealing. Demonstration of the effect of MSCs on neutrophils is critical in understanding the potential therapeutic role of MSCs in SLE and SLE related organ damage.

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Abstract II-18 Figure 1 Neutrophil Elastase colocalized with extracellular DNA. Control neutrophils were isolated from peripheral blood and stimulated with 10nM PMA for 2 hr at 370C. Cells were fixed and stained for detection of neutrophil Elastase (green) and DNA was labeled with Hoechst 33342 (blue).

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ELEVATED PLASMA CELL-FREE MITOCHONDRIAL DNA DEFINES A SUBGROUP OF LUPUS PATIENTS WITH MEMBRANOUS LUPUS NEPHRITIS

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Background Systemic lupus erythematosus (SLE) is an autoimmune disease with protean manifestations, characterised by production of antibodies against nucleic acids and upregulation of type I interferon-inducible genes in a majority of SLE patients. The principal drivers of this interferon signature are still not well understood. Recent work has shown that oxidised mitochondrial DNA released by neutrophils can stimulate plasmacytoid dendritic cells to produce interferon- α .¹ We hypothesised that cell-free mitochondrial DNA might contribute to type I interferon production in SLE, and we sought to examine whether cell-free mtDNA levels were increased in SLE patients relative to controls, or during disease flares.

Materials and methods A retrospective analysis was performed using banked plasma samples from 164 patients in our SLE cohort along with 57 banked plasma samples from healthy donors. DNA was isolated from the plasma samples and real-time quantitative PCR was performed, amplifying a target sequence in the mitochondrially-encoded gene NADH dehydrogenase I, as previously published.² In-depth clinical phenotyping of SLE patients in the cohort was performed and used to define subgroups of SLE patients, as well as the specific disease manifestations present during flares.

Results No significant difference was seen in cell-free mtDNA in plasma from SLE patients versus healthy donors (HD – 3060.3 copies/uL, N = 57, SLE - 3845.5 copies/uL, N = 164, p = 0.22). However, cell-free mtDNA levels were elevated in a subset of SLE patients with a history of membranous lupus nephritis, including those with a component of proliferative nephritis (WHO class V/III+V/IV+V), relative to patients with proliferative nephritis alone (WHO class III or class IV) (5313.9 copies/uL, N = 34 vs. 2062.5 copies/uL, N = 17, p = 0.02). A subset of 70 patients had multiple samples collected at visits before, during, and after flares of disease activity. Cell-free mtDNA levels rose at the peak of disease activity as assessed by SLEDAI score in 11/16 flares of class V/III+V/IV+V nephritis (p = 0.04), while it only did so in 4/11 of the remaining nephritis flares. In contrast, cell-free mtDNA rose at the peak of disease activity in only 4/20 flares where alopecia was present (p = 0.02).

Conclusions Cell-free mtDNA levels are elevated in a subset of SLE patients with a history of membranous nephritis, and were more likely to rise during flares of membranous nephritis versus other types of disease flares.

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RELEVANCE OF MOUSE LUPUS MODELS OF LUPUS NEPHRITIS TO PROGRESSION OF CKD

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Background Lupus nephritis progresses to chronic kidney disease (CKD) in an unacceptably high proportion of affected individuals. It is difficult to predict who is at risk for this complication and may therefore need more intensive therapy. Risk for progression of CKD in humans is associated with an interstitial molecular signature containing 68 genes (Ju W *et al.*, *Sci Transl Med*, 2015). Of these, a decrease in renal expression of EGF with a concomitant increase in urinary EGF improves the ability to predict CKD expression.

Materials and methods To determine whether these 68 genes can be used in pre-clinical studies to model disease and therapeutic responses, we analysed microarray data of kidneys from three mouse lupus strains at various disease stages and after remission induction. Renal macrophage gene expression was assessed using RNASeq.

Results 64/68 genes have mouse gene IDs and 61/64 are represented on the mouse microarray chip. Of these 49 (80.3%) were regulated in the same direction as in humans in at least one strain (38 in NZM2410 mice, 44 in NZW/BXSB mice, 33 in NZB/W mice and 28 common to all three strains). Few genes were unique to a single strain: of these, collagen genes were uniquely expressed in nephritic NZW/BXSB mice. To determine which genes could distinguish early from established disease we compared the profiles of newly proteinuric NZB/W mice to those of NZB/W mice with established disease. 9 genes, including EGF and TIMP1 only became abnormally regulated during established disease, confirming their association with CKD progression. Most of the CKD associated genes normalised after immunosuppression but tended to drift back to their abnormal values during impending relapse. Some of the abnormally regulated genes are derived from macrophages/DCs. Using RNASeq analysis of isolated renal macrophages from NZB/W mice we showed that macrophage restricted C1qa has a similarly high expression level in young and nephritic renal macrophages. Therefore the increased renal expression of this gene can be used as a biomarker of increased macrophage infiltration, a known poor prognostic feature in human lupus nephritis.

Conclusions Mice with lupus nephritis have a strikingly similar pattern of CKD-related gene expression to humans and these genes can be used to track therapeutic responses. Downregulation of EGF and upregulation of TIMP1 are markers of progressive disease in mice as in humans and C1qa can be used as a marker of macrophage infiltration. The fibrosis signature is best modelled in NZW/BXSB mice.