for IgG in humans, we included transgenic mice expressing FcyRIIA to our in vivo mechanistic investigations. Platelet activation and mitochondrial release were monitored upon intravenous injection of synthetic ICs in wild type (WT) and transgenic (Tg) FcyRIIA mice.

Results We found that on activation, platelets relocate their mitochondria toward the cell membrane and then release respiratory-competent mitochondria into the extracellular milieu, both as free organelles and encapsulated within vesicles called microparticles (MP). Extracellular mitochondria were internalised by bystander leukocytes and, importantly, induced leukocytes rolling and adhesion to the blood vessel wall, suggesting that neutrophils and/or endothelial cells are activated by extracellular mitochondria. Activated platelets were more abundant in SLE patients than control subjects, and were associated with IgG. Extracellular mitochondria, both encapsulated in platelet MPs or naked, were also observed in blood circulation in SLE, and were frequently associated with IgG. Mechanistically, ICs present in blood induce profound cell activation, which is dependent on platelet FcyRIIA and its signalling cascade.

Conclusions Platelets represent an important source of mitochondria, which release in blood upon stimulation of FcyRIIA, might promote systemic inflammation in SLE. Whether the blockade of FcyRIIA might represent an attractive avenue in SLE research, and whether platelet activation markers and extracellular mitochondria might be utilised as potential biomarkers for the stratification of lupus patients needs to be further considered.

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II-12 CSF-1 AND IL-34: DISTINCT POTENTIAL BIOMARKERS FOR LUPUS

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Background A noninvasive means to predict the onset and recurrence of lupus is needed to optimise and individualise treatment. Macrophages (Mø) are prominent in inflamed tissues targeted for destruction in SLE. We hypothesised that the principal molecules required for Mø survival and proliferation are biomarkers for SLE. CSF-1 and IL-34 are promising candidates as both (i) bind to cFMS expressed by Mø, thereby promoting Mø survival and proliferation and (ii) promote destructive inflammation. However, IL-34 and CSF-1 have differing functions, which may be related to IL-34, not CSF-1, binding to a second receptor and distinct spatial temporal expressions.

Materials and methods We analysed serum and urine CSF-1 and IL-34 levels in SLE patients with nephritis (LN), arthritis (LA), cutaneous and serositis compared with healthy controls in two large cohorts (ELISA). While serum and urine CSF-1 expression is elevated in each manifestation, CSF-1 is notably higher in LN. In contrast, serum IL-34 expression is dramatically higher in LA, not LN. Thus, we probed for CSF-1 and IL-34 expression in LN (kidney) and LA (synovium). Moreover, we longitudinally tracked serum CSF-1 and IL-34 prior to LN (biopsy proven), with disease activity including flares and during LA in comparison to disease activity

Results

- LN. CSF-1 and IL-34 are expressed in the same and different renal tubular epithelial cells in LN. Elevated serum or urine CSF-1, not IL-34, levels correlate with increasing intra-renal CSF-1 expression and histopathology index. Longitudinally tracking serum CSF-1, not IL-34, levels heralds the initial onset of nephritis and a rise in serum or urine CSF-1 predicts LN recurrences before clinical evidence of renal dysfunction and conventional serologic measures.
- LA. IL-34, not CSF-1, expression is higher in synovial fluid and synovium in LA compared to osteoarthritis and healthy controls and correlates with magnitude of intra-synovial leukocytes. Moreover, intra-synovial IL-34 expression is similar in LA and rheumatoid arthritis. Longitudinally monitoring serum IL-34, not CSF-1, levels track with clinical disease activity in LA and RA.

Conclusions Serial monitoring a rise in serum or urine CSF-1, not IL-34, in SLE reflects renal histopathology and clinical disease activity and the onset and reoccurrences of LN more accurately than conventional laboratory measures. While serial monitoring a rise in serum IL-34, not CSF-1, reflects clinical disease activity in LA. Thus, CSF-1 and IL-34 are inexpensive and accurate potential biomarkers for LN and LA, respectively.

II-13 SUPPRESSION OF SYSTEMIC AUTOIMMUNITY BY THE INNATE IMMUNE ADAPTOR STING

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Background Cytosolic DNA-sensing pathways that signal via the adaptor Stimulator of Interferon Genes (STING) mediate immunity to pathogens and have also been known to promote autoimmune pathology in DNAseII/III-deficient mice. However, the role of these pathways in systemic models of autoimmunity is unexplored. We hypothesised that cytosolic DNA sensing pathways contribute to the pathogenesis of autoimmune disease. Surprisingly, we report here that STING *potently* suppresses inflammation in several models of systemic lupus erythematosus (SLE).

Materials and methods A controlled F2 intercross between heterozygote STING$^{\pm}$ lpr$^{\pm}$littermates generated STING-deficient lupus-prone mice homozygous for deficiency in Fas as well as STING (STING/lpr, n ≥10) or wild type for STING (WT/lpr, n ≥10). Mice were analysed at 16 wk of age. A similar F2 cross was set up for IRF3$^{-/-}$ and MRL/lpr mice as well as STING$^{-/-}$ and C57Bl/6$^{lpr/lpr}$ and analysed as above for STING/lpr mice (n ≥10 per group). C57BL/6, cGAS$^{-/-}$, Unc93b$^{3d/3d}$, and STING$^{-/-}$ mice were injected i.p. with TMPD and evaluated at day 14 and 6 months post injection.

Results Lymphoid hypertrophy, autoantibody production, serum cytokine levels, and other indicators of immune activation were markedly increased in STING$^{-/-}$ autoimmune-prone mice compared to STING$^{+/+}$ littermates. As a result, STING$^{-/-}$ autoimmune-prone mice had significantly shorter lifespans than controls. TLR-dependent systemic inflammation during TMPD-mediated peritonitis was similarly aggravated in STING$^{-/-}$ and cGAS$^{-/-}$ mice. Mechanistically, cGAS and STING-deficient macrophages failed to express negative regulators of immune activation, and thus were hyper-responsive to TLR ligands. This hyper-reactivity corresponds to dramatically elevated numbers of inflammatory macrophages and granulocytes *in vivo*.

Conclusions Our findings reveal an unexpected negative regulatory role for STING during chronic inflammation. While the dysregulation of TLR7/9 signalling is a recurrent theme in systemic autoimmune, numerous studies have now revealed a *protective role* for TLR9 in SLE. Importantly, the exacerbated disease we observed in STING/lpr mice resembles that reported for TLR9/lpr mice and implies common protective mechanisms originating from STING and TLR9. Although the precise mechanism remains an open question, it is clear that cGAS/STING-dependent pathways maintain a threshold of negative regulators. We propose a similar setting of thresholds from TLR-dependent pathways and further suggest that such coordinated induction of cell-intrinsic thresholds of negative regulators is key in offsetting inflammation. Our data raise a cautionary note regarding the use of newly developed STING-directed therapeutics in systemic disease, because they may have unintended consequences and perturb a carefully orchestrated balance between cytosolic and endosomal signalling cascades.

II-14 DISTURBED CLEARANCE OF APOPTOTIC DEBRIS IN PRISTANE-TREATED TLR9KO MICE LEADS TO ACCUMULATION OF A UNIQUE MACROPHAGE POPULATION

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Background Nucleic acid binding TLRs have been found to play a critical role in the production of autoantibodies and disease development in animal models of SLE. Intriguingly, TLR9 appears to play both a protective and disease promoting role. While TLR9 is required for the production of anti-dsDNA autoantibodies, TLR9^{KO} autoimmune-prone mice develop more severe disease than their TLR9-sufficient counterparts. Studies from our group and others have pointed to B cell expression of TLR9 as a key determining factor. However, our recent studies point to an additional role for TLR9in myeloid lineage cells.

Materials and methods Pristane injected BALB/c wildtype (WT) and TLR9^{KO} mice were analysed for disease severity at 5 months. Kidney sections were stained for IgG deposition and Ly6G positive cells. Single cell suspensions of different tissues were analysed using flow cytometry. Mixed BM chimaeras (50% WT: 50% TLR9^{KO}) were injected with pristane and myeloid populations were analysed. For apoptotic cell clearance, WT and TLR9^{KO} bone marrow derived macrophages (BMDM) were stimulated with CFSE labelled apoptotic cells and analysed 24 hours later by confocal microscopy.

Results We have found that TLR9-deficiency dramatically exacerbates the onset of renal disease resulting in decreased survival. Increased levels of IgG accumulate in pristane treated TLR9^{KO} glomeruli compared to WT glomeruli, and the increased IgG deposits are associated with an increased myeloid infiltrate. Moreover, this myeloid infiltrate contained an increased frequency of granulocytes a well as an unusual CD11b^{+} Ly6C^{int} Ly6G^{int} (Ly6CG^{int}) subset. To better understand the origin of these populations, the myeloid subsets of pristane-treated mixed (TLR9^{WT} + TLR9^{KO}) BM chimaeras were analysed. Remarkably, the Ly6CG^{int} population was entirely derived from the TLR9^{KO} stem cells. Morphologic analysis revealed that the Ly6CG^{int} population are macrophages containing large lipid droplets, suggesting a role for TLR9 in degradation of pristane. Further *in vitro* analysis of BMDMs stimulated with apoptotic cells showed that most WT BMDMs cleared apoptotic cells by 24 h. However, a large fraction of TLR9-deficient BMDMs still had un-degraded apoptotic cells in the lysosomal compartment, suggesting a role for TLR9 in clearance.

Conclusions These data demonstrate a direct effect of TLR9-deficiency on the expansion of a unique CD11b^{+} population, and further suggest that these cells play a major and direct role in the accelerated disease characteristic in TLR9^{KO} mice. Furthermore, a specific role for TLR9 in the clearance of apoptotic cells may be the underlying cause for the accumulation of this CD11b + subset.

II-15 INHIBITION OF TLR RECOGNITION OF SELF NUCLEIC ACIDS BY PLASMACYTOID DENDRITIC CELLS USING OLIGONUCLEOTIDE-BASED INHIBITORS IN LUPUS

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Background SLE is an autoimmune disease where the immune tolerance to self-nucleic acids is broken with devastating consequences. The hallmark of the disease is an increased IFN-α signature in the blood which is accompanied with high levels of autoantibodies and disease activity. Self-nucleic acid recognition by Toll-like receptors (TLR)7 and TLR9 on B cells and plasmacytoid dendritic cells (PDC) is believed to be key in the pathogenesis of SLE promoting immune complexes (IC) and the production of type I IFN, both of which are associated with the severity of the disease.

Results We have generated and described oligonucleotide-based bi-functional inhibitors of TLR7&9 (called ImmunoRegulatory Sequences, IRS) and have shown that these can block IFN production by PDC as well as B cell activation. In addition, IRS are active in vivo and treatment of lupus-prone mice lead to reduced disease symptoms and end-organ damage. SLE patient are often treated with glucocorticoids (GC) but under maintenance levels often suffer from disease flares that necessitate high dose pulse therapy. We have shown that PDC were significantly more resistant to GC induced death in lupus-prone mice, a phenomenon that was completely reversed by pre-treatment with TLR7&9 inhibitor. These data provide a new understanding of the role of self-recognition of DNA and RNA by TLR as an important parameter during inflammatory response. These data also stress the potential utilisation of TLR7&9 specific inhibitors as corticosparing drugs which would be open new possibilities with respect