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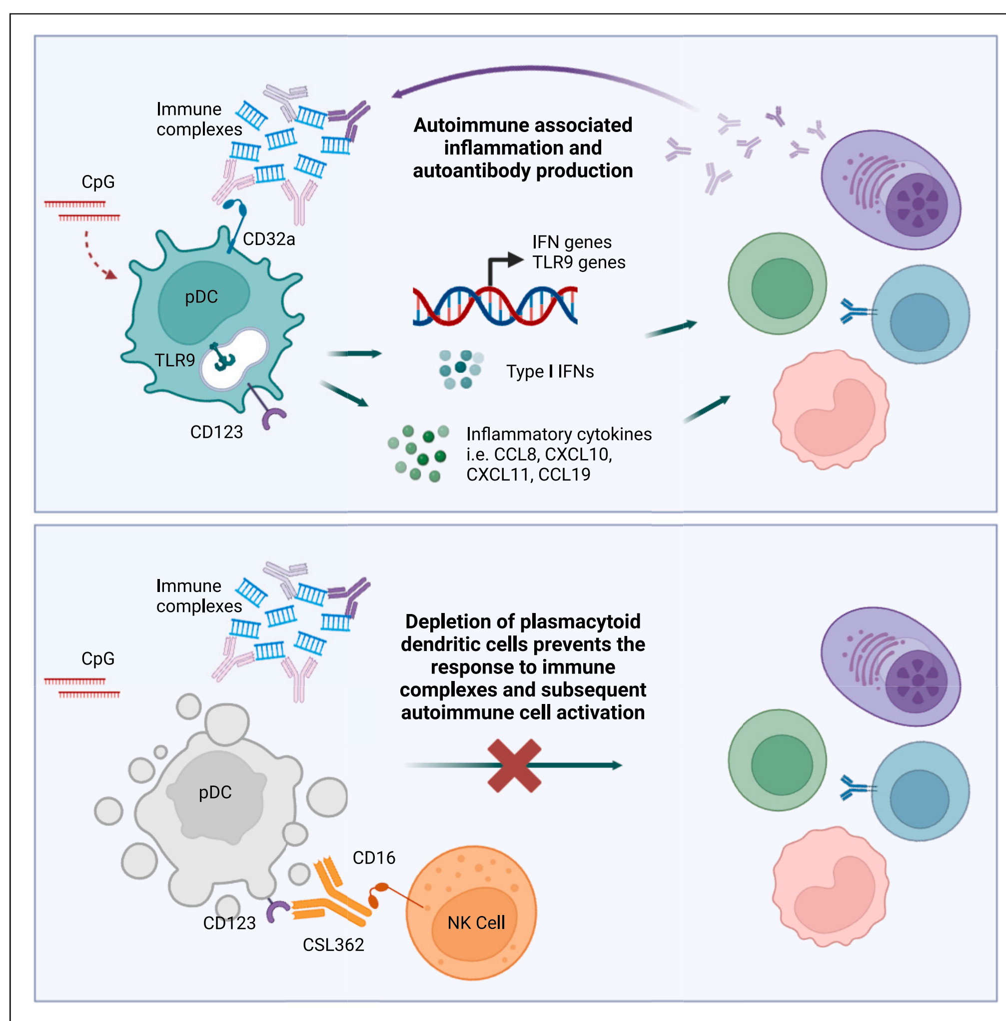

Katherine A. Monaghan, Alberta Hoi, Cristina Gamell, ..., Ian Wicks, Eric Morand, Nicholas Wilson

katherine.monaghan@csl.com.au

Highlights

CD123 is highly expressed on pDCs and basophils which are depleted with CSL362

CSL362 treatment reduced TLR and IC induced IFN production and gene expression

Found additional soluble factors pDCs produce that potentially contribute to SLE

Show evidence of a transcriptomic profile in SLE consistent with alteration of pDCs

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CSL362 potently and specifically depletes pDCs *in vitro* and ablates SLE-immune complex-induced IFN responses

Katherine A. Monaghan,[1,14,*] Alberta Hoi,[2,3] Cristina Gamell,[1] Tsin Yee Tai,[1,8] Bryan Linggi,[7,9] Jarrat Jordan,[7,10] Matteo Cesaroni,[7,11] Takahiro Sato,[7,12] Milica Ng,[1] Shereen Oon,[4,5,6] Jacqueline Benson,[7,13] Ian Wicks,[4,5,6] Eric Morand,[2,3] and Nicholas Wilson[1]

SUMMARY

Systemic lupus erythematosus (SLE) is an autoimmune disease with significant morbidity and mortality. Type I interferon (IFN) drives SLE pathology and plasmacytoid dendritic cells (pDCs) are potent producers of IFN; however, the specific effects of pDC depletion have not been demonstrated. We show CD123 was highly expressed on pDCs and the anti-CD123 antibody CSL362 potently depleted pDCs *in vitro*. CSL362 pre-treatment abrogated the induction of IFNα and IFN-induced gene transcription following stimulation with SLE patient-derived serum or immune complexes. RNA transcripts induced in pDCs by *ex vivo* stimulation with TLR ligands were reflected in gene expression profiles of SLE blood, and correlated with disease severity. TLR ligand-induced protein production by SLE patient peripheral mononuclear cells was abrogated by CSL362 pre-treatment including proteins over expressed in SLE patient serum. These findings implicate pDCs as key drivers in the cellular activation and production of soluble factors seen in SLE.

[1]Research and Development, CSL Limited, Melbourne, VIC 3010, Australia

[2]Centre for Inflammatory Disease, School of Clinical Sciences, Monash University, Melbourne, VIC 3168, Australia

[3]Monash Health, Clayton, VIC 3168, Australia

[4]The Walter and Eliza Hall Institute, Parkville, VIC 3052, Australia

[5]The Royal Melbourne Hospital, Parkville, VIC 3050, Australia

[6]The University of Melbourne Parkville, Parkville, VIC 3010, Australia

[7]Janssen Research and Development LLC, Spring House, PA 19477, USA

[8]Present address: Baker Heart and Diabetes Institute, Melbourne, 3004, Australia

[9]Present address: Alimentiv, Inc, 100 Dundas St, Suite 200, London, ON N6A 5B6, Canada

[10]Present address: Research and Development, Dragonfly Therapeutics, Waltham, MA 02451, USA

[11]Present address: Precision Oncology, Sanofi R&D, 94400, Vitry-sur-Seine, France

[12]Present address: Oncology Experimental Medicine Unit, GSK, PA 19426, USA

[13]Present address: Sorriso Pharmaceuticals, Sandy, UT 84094, USA

[14]Lead contact

*Correspondence: katherine.monaghan@csl.com.au

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INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disease with significant morbidity and mortality[1] despite current treatments. SLE is classically characterized by the presence of high levels of circulating autoantibodies and immune complexes (IC) which can activate the production of the type I interferons (IFN). Numerous studies in SLE patients have found elevated IFNα and IFN-induced chemokines in the serum[2–5] and the presence of an IFN gene signature in peripheral blood.[6,7] These data support a central role for type I IFN in SLE pathogenesis and provided a rationale for the inhibition of type I IFN in SLE. Several IFN blocking strategies have been evaluated in clinical trials,[8–12] with the anti-IFN receptor monoclonal antibody anifrolumab showing sufficient efficacy in two Phase 3 trials[13,14] to merit its approval in 2021 by regulators including the US Food and Drug Administration.

Plasmacytoid dendritic cells (pDCs) are specialized, bone-marrow-derived dendritic cells that are regarded as the primary producers of type I IFN.[15,16] pDCs produce large amounts of IFN following toll-like receptor (TLR) 7 and TLR9 activation by pathogen-associated-molecular-patterns or human-derived nucleic acids as reviewed by Guiducci et al.[17] In SLE, IC containing host-derived nucleic acids and a variety of autoantibodies are internalized by CD32a (FcγRIIa) expressed by pDCs and subsequently stimulate endosomal TLR7 and TLR9 to promote IFN production.[18–22] Direct evidence for a pathogenic role of pDCs has been shown in murine SLE models.[23,24] However, evidence implicating pDCs in human SLE has been largely indirect, with reports describing altered pDC numbers in peripheral blood,[25–28] abundant pDCs producing IFNα/β in the cutaneous lesions of lupus patients[25,29] and activation of pDCs *in vitro* by DNA-containing IC via TLR9.[22,30] Recently, two monoclonal antibodies BIIB059 and VIB7734 that specifically target pDCs have demonstrated clinical activity.[31–34] Compared to other cell types in peripheral blood, pDCs express high levels of CD123 (IL-3Rα).[29,35] CSL362, also known as talacotuzumab, is a fully humanized monoclonal antibody that binds to CD123, with the Fc region engineered to increase affinity for CD16 (FcγRIIIa), thereby enhancing antibody-dependent cell-mediated cytotoxicity (ADCC) which has been demonstrated to be mediated by NK cells.[36] CSL362 has enhanced ADCC activity against CD123+ acute myeloid leukemia (AML) and chronic myeloid leukemia

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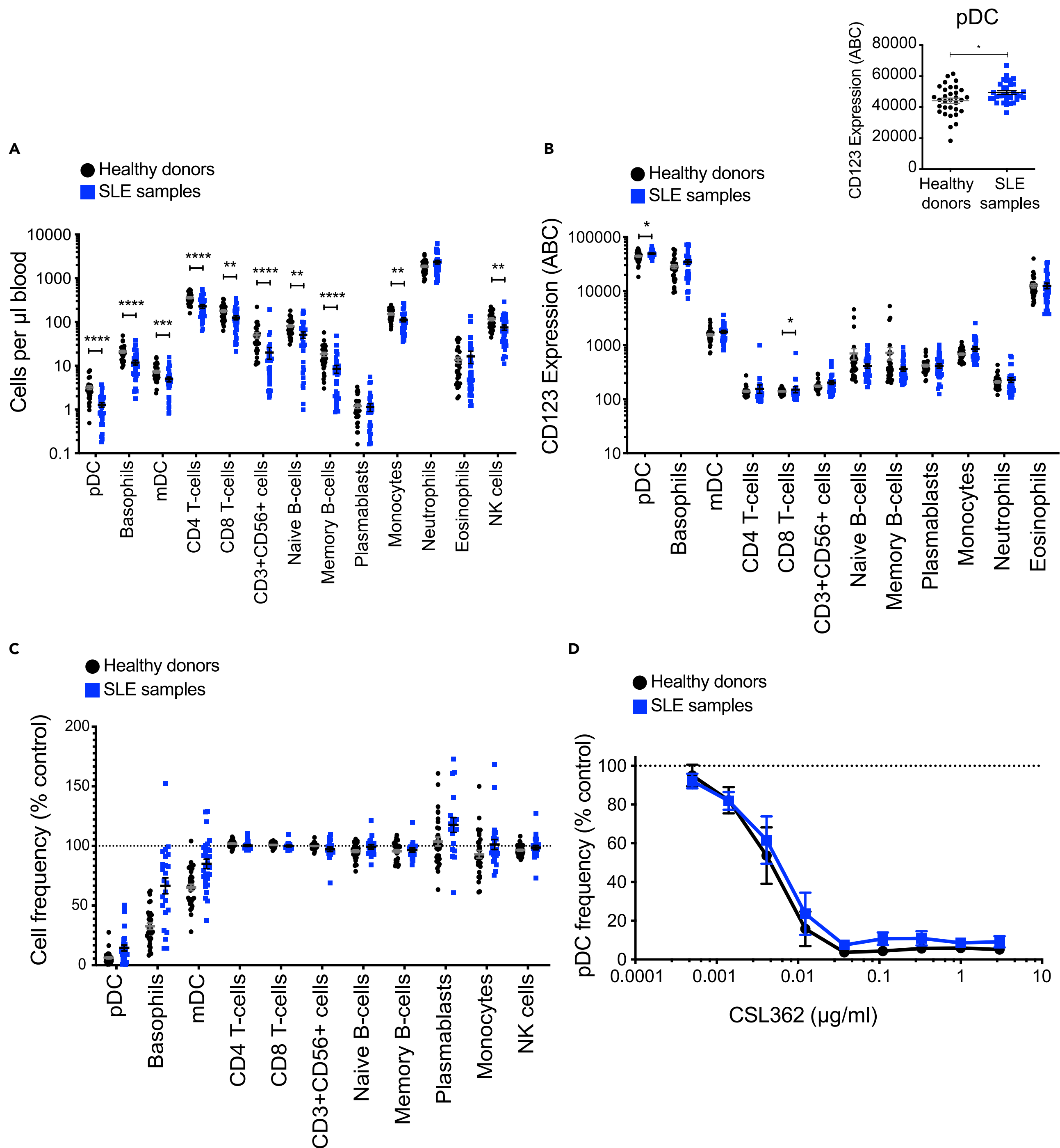


Figure 1. Characterization of cell subsets, CD123 expression and CSL362 mediated cell depletion in SLE patient peripheral blood and matched healthy controls

Whole blood from 32 healthy donors (HD) and 33 SLE patients was analyzed by flow cytometry to determine absolute counts and expression of CD123.
(A) SLE patients have significantly fewer pDCs, basophils, mDCs, CD4 T-cells, CD8 T-cells, NKT-cells, naive B-cells, memory B-cells, monocytes and NK cells.
(B) pDCs and basophils express the highest levels of CD123, mDCs express an intermediate amount while all other cell types have a mean receptor expression of less than 1500 ABC.
(C) PBMC from 19 to 29 SLE donors and 31–34 healthy controls were cultured with CSL362 (1 µg/mL) for 20 h before the proportion of each cell type was determined by flow cytometry and normalized to no antibody control.

Figure 1. *Continued*

(D) PBMC cultures from 6 healthy controls and 7 SLE patients were cultured with CSL362 (0.0005–3 μg/mL) for 20 h before the proportion of pDCs was determined by flow cytometry and normalized to no antibody control. ABC = Antibodies bound per cell as determined by BD quantibrite beads. Bars depict mean ± SEM (Mann-Whitney test ****p ≤ 0.0001, ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05 and n.s p > 0.05.
See also Figures S1 and S2.

(CML) blasts and leukemic stem cells *in vitro,* and reduces leukemic cell growth in murine xenograft models of human AML.[36–38] Phase I and II clinical trials in of CSL362 in AML - NCT01632852, NCT02472145 have[39,40] been completed. CSL362 also potently depletes both pDCs and basophils *in vivo* in non-human primates[35,36] and in AML patients.[39,40]

We have previously shown that CSL362 depletes pDCs from the blood of SLE donors *ex vivo* and prevents TLR7-and TLR9-induced IFNα production.[35] In this study, we confirm that CSL362 potently depletes pDCs in peripheral blood from both SLE and healthy donors (HD). Furthermore, we show that pDC depletion eliminates IFNα production and an IFN-gene signature induced by SLE-derived IC. In addition, we identify soluble factors produced by pDCs in response to TLR7 and TLR9-stimulation through RNA-seq of purified pDCs and show the concentrations of several of these proteins, including IP-10/CXCL10, ITAC/CXCL11 and MCP-2/CCL8, are elevated in SLE serum and are reduced by CSL362 *in vitro*. These data strongly support the notion that pDC depletion using CSL362 could be beneficial in the treatment of SLE by selective targeting of the most potent source of IFN.

RESULTS

SLE cohort clinical information

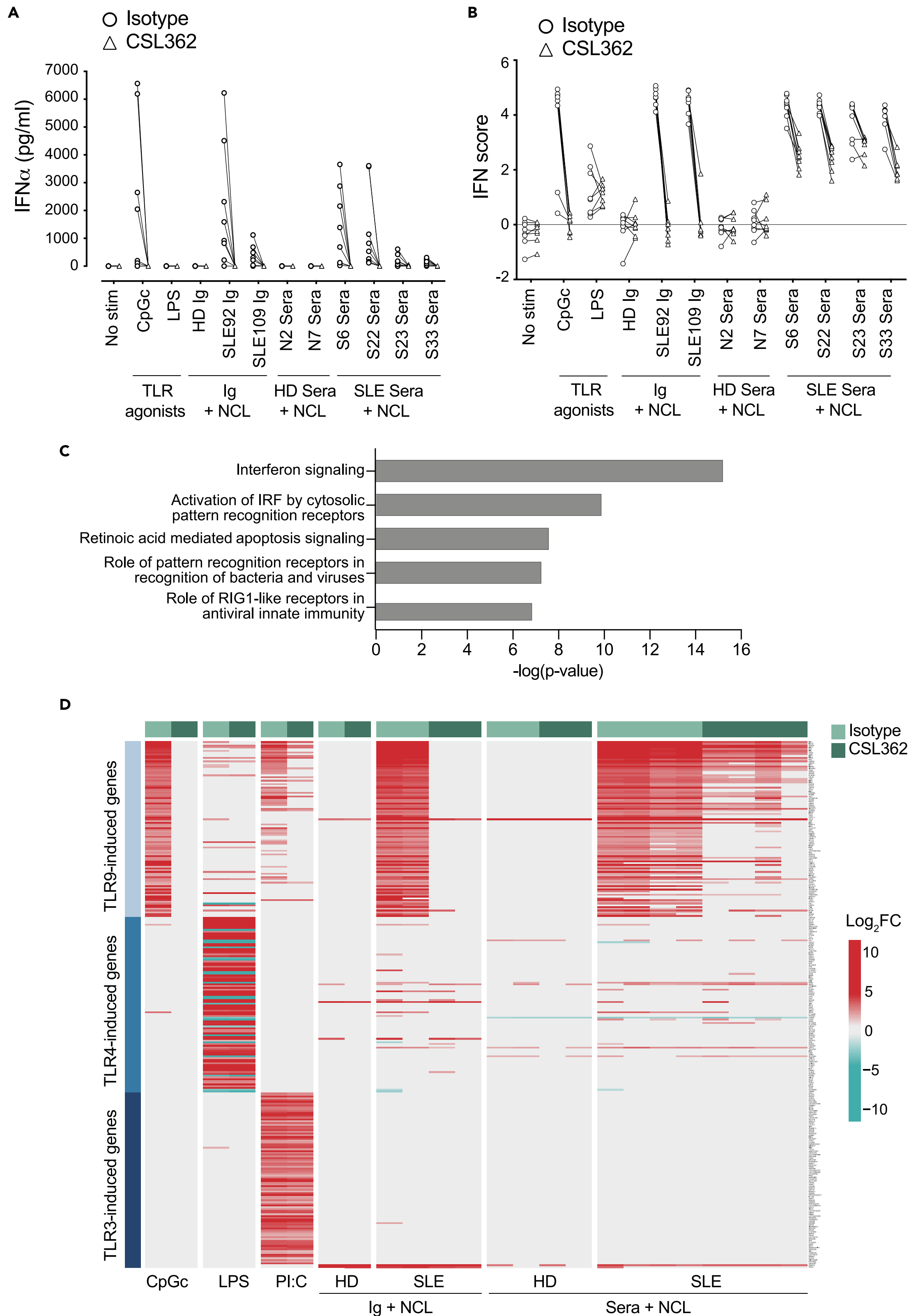
Our primary SLE cohort consists of patients who all fulfilled at least 4 of the American College of Rheumatology Classification criteria, with a mean (± standard error, SE) age of 44 (±2.66) years, and 91% female. Most patients have low to moderate disease activity level, with a mean (±SE) SLEDAI-2K score of 4.15 (±0.71). Only 2/33 (6%) had high disease activity at the time of sampling. 19/33 (57.6%) of the cohort had positive anti-dsDNA antibodies. 18/33 (54.5%) were being treated with corticosteroid and a similar proportion with immunosuppressant/s (Table S1). Our smaller secondary cohort represented a similar age range with a mean of 49.88 (±6.21) and proportion of females (87.5%), but tended to have lower disease activity with a mean SLEDAI-2K of 3 (±1.13) and only 2/8 (25%) participants having positive ds-DNA, and a higher proportion receiving corticosteroid (6/8, 75%) at the time of sampling (Table S2).

CSL362 selectively depletes pDCs from SLE and HD peripheral blood samples

Baseline levels of various blood cell subsets in SLE and HD samples were analyzed for absolute cell numbers of pDCs, mDCs, basophils, monocytes, $CD4^+$ T-cells, $CD8^+$ T-cells, $CD3^+CD56^+$ NKT-cells, naive B-cells, memory B-cells, plasmablasts, neutrophils, eosinophils and NK cells. Peripheral blood cell subsets were significantly different in SLE patients compared to HD. SLE patients had significantly fewer pDC (p = <0.0001) in peripheral blood (PB) compared to HD (Figure 1A). SLE patients also had significantly (p = <0.01) fewer basophils, NK cells, mDCs, monocytes, $CD4^+$ T-cells, $CD8^+$ T-Cells, $CD3^+CD56^+$ cells, naive B-cells and memory B-cells (Figure 1A), consistent with the lymphopenia typically observed in SLE. Our SLE cohort had equivalent levels of plasmablasts, neutrophils and eosinophils compared to HD.

The expression of CD123, which is the target of CSL362, was evaluated on PB cell subsets in SLE and HD (Figure 1B). CD123 was detected on various cell types, but was most prominently expressed on pDC and basophils and to a lesser extent mDC whereas all other cell types had <1500 receptors per cell (Figure 1B). There was significantly more CD123 expression on SLE patient pDC (p = 0.023) and CD8 T cells (p = 0.037) (Figure 1B).

The addition of CSL362 to peripheral blood mononuclear cell (PBMC) cultures resulted in degrees of cell depletion across different cell subsets which were concordant with CD123 receptor density (Figure 1C), with pDCs being most effectively and significantly depleted with CSL362, in comparison to depletion of basophils and mDCs (Figure S1). No significant depletion of any other cell subsets was observed. CSL362 potently depleted pDCs in a dose-dependent manner from both SLE and HD PBMC, with near complete depletion at concentrations above 0.0123 μg/mL (Figures 1D and S1A). CSL362 resulted in significant, although incomplete, depletion of basophils in SLE and HDs, with some depletion of mDCs also observed. Monocytes, which express lower levels of CD123, were not depleted by CSL362 (Figure S1).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IFNα (pg/ml)
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CpGc
LPS
HD Ig
SLE92 Ig
SLE109 Ig
N2 Sera
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S22 Sera
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TLR agonists
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Activation of IRF by cytosolic pattern recognition receptors
Retinoic acid mediated apoptosis signaling
Role of pattern recognition receptors in recognition of bacteria and viruses
Role of RIG1-like receptors in antiviral innate immunity
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TLR3-induced genes
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CpGc
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PI:C
HD
SLE
Ig + NCL
Sera + NCL

Figure 2. Effects of CSL362 on IFNα production and gene expression induced by TLR ligands, serum or purified IgG from SLE patients

PBMC from 8 SLE donors were treated with 1 μg/mL CSL362 or isotype control for 20 h before the cells were resuspended in various stimuli (0.25 μM CpGc, 10 μg/mL LPS, HD Ig + NCL, SLE Ig + NCL, SLE sera + NCL or HD sera + NCL) and cultured for 24 h.

(A) Supernatant was then collected and analyzed for IFNα production by ELISA. IFNα levels for each donor PBMC culture treated with isotype control (circles) or CSL362 (triangles) after stimulation are shown.

(B) RNA was also extracted from the cultured cells and analyzed by RNA-seq. Graph shows the IFN gene score for each treatment (isotype control circles, CSL362 triangles) and stimulation determined as the average log2 fold change compared to control (HD PBMC unstimulated and treated with isotype control) of 11 IFN genes (*IFI44L, IFIT1, IFIT3, IRF7, ISG15, MX1, MX2, OAS1, OAS2, SERPING1* and *XAF1*).

(C) The genes differentially expressed (absolute log2FC >1, FDR<0.05) between PBMCs stimulated with SLE immune complexes (SLE Ig + NCL) in the presence of CSL362 or isotype control were subjected to pathway analysis with Ingenuity (IPA). The top 5 altered processes, ranked by p value of overlap, are shown.

(D) Heatmap of the expression changes of TLR9, TLR4 and TLR3 genes in response to the different stimuli with and without CSL362 treatment. PI:C columns show the expression of n = 3 SLE donors only, stimulated with 10 μg/mL poly I:C (PI:C) all other columns show n = 8 SLE donors. NCL = Necrotic Cell Lysates used at 0.1 mg/mL.

See also Figure S3.

After confirming SLE patient NK cells are capable of inducing CSL362 mediated ADCC in culture we then investigated NK cell activation and CD16 expression in our study given their key effector role.[36] The proportion of NK cells expressing CD107a+, an activation marker, showed a dose-dependent increase in SLE and HD in response to CSL362 (Figure S2). CSL362 has a engineered Fc to enhance CD16 binding and therefore ADCC effectiveness.[36] Both HD and SLE patients NK cells showed a dose dependent decrease in CD16 expression in the presence of CSL362 with SLE patients expressing lower pre-treatment CD16 levels than HD (Figure S2).

CSL362 treatment specifically reduces IFN production and genes induced by TLR9 or SLE immune complex stimulation *in vitro*

Nucleic acid-containing immune complexes (IC) in SLE can induce IFN production by pDCs through stimulation of TLR7 and TLR9.[19] However, additional TLRs and cell types may also respond to IC to induce an IFN response. Using SLE sera as a source of autoantibodies and necrotic cell lysates (NCL) as a source of nucleic acids we prepared SLE IC, either with purified Ig isolated from SLE donor sera (SLE Ig + NCL), or with whole SLE sera (SLE sera + NCL), as previously described.[22,41] Stimulation of SLE donor-derived PBMC with CpGc, SLE Ig + NCL, SLE sera + NCL, but not HD Ig or sera + NCL, was able to induce IFNα secretion to varying degrees with the different stimuli (Figure 2A). IFNα induction by these stimuli was reduced to baseline by pre-treatment with CSL362 (Figure 2A). pDC depletion by CSL362 in these cultures was confirmed by flow cytometry (Figure S3). These stimuli also induced the upregulation of a previously described 11 gene IFN-signature,[35] which was also abrogated by pre-treatment with CSL362 (Figure 2B). Similar results were obtained with a small number of HD PBMC samples stimulated alongside the SLE PBMC (Figure S3).

We next determined global gene expression profiles induced by these stimuli to understand the specificity of the effects of CSL362 on these responses. To identify the most relevant biological processes altered by CSL362 in PBMCs stimulated with SLE Ig + NCL, we performed pathway analysis using IPA software. IFN signaling was identified as the top ranked significantly overrepresented canonical pathway inhibited by CSL362 (-log10 p value = 15.2), consistent with pDCs being the primary target of CSL362 (Figure 2C). This effect of CSL362 on IFN signaling is consistent with the reduction in IFNα levels and IFN gene score observed with CSL362 treatment (Figures 2A and 2B). Analysis of the transcriptomic response on CSL362 treatment also identified inhibition of pathways related to Pattern Recognition Receptors (Activation of IRF by Cytosolic Pattern Recognition Receptors, Role of Pattern of Recognition Receptors in recognition of Bacteria and Viruses, and Role of RIG-1-like Receptors in Antiviral Innate Immunity) (Figure 2C), consistent with CSL362 depleting pDCs and thus interfering with IFN production following TLR activation by pathogen-associated-molecular-patterns.

In light of these results, we then expanded our analysis on CSL362 effects on TLR-induced genes to include other stimuli. Stimulation of PBMC with the TLR agonists CpGc, LPS and poly I:C gave distinct gene expression profiles with very little overlap in their responses (Figure 2D). Both SLE Ig + NCL, and SLE sera + NCL gave gene expression profiles similar to CpGc, with little to no overlap with LPS- and poly I:C-induced gene expression profiles. HD Ig or sera + NCL induced very few genes that overlapped with those induced by any of the TLR agonists, suggesting that individually Ig, NCL or sera are not sufficient to induce the observed gene expression profiles in response to SLE Ig or sera + NCL. Depletion of the pDCs via pre-treatment of

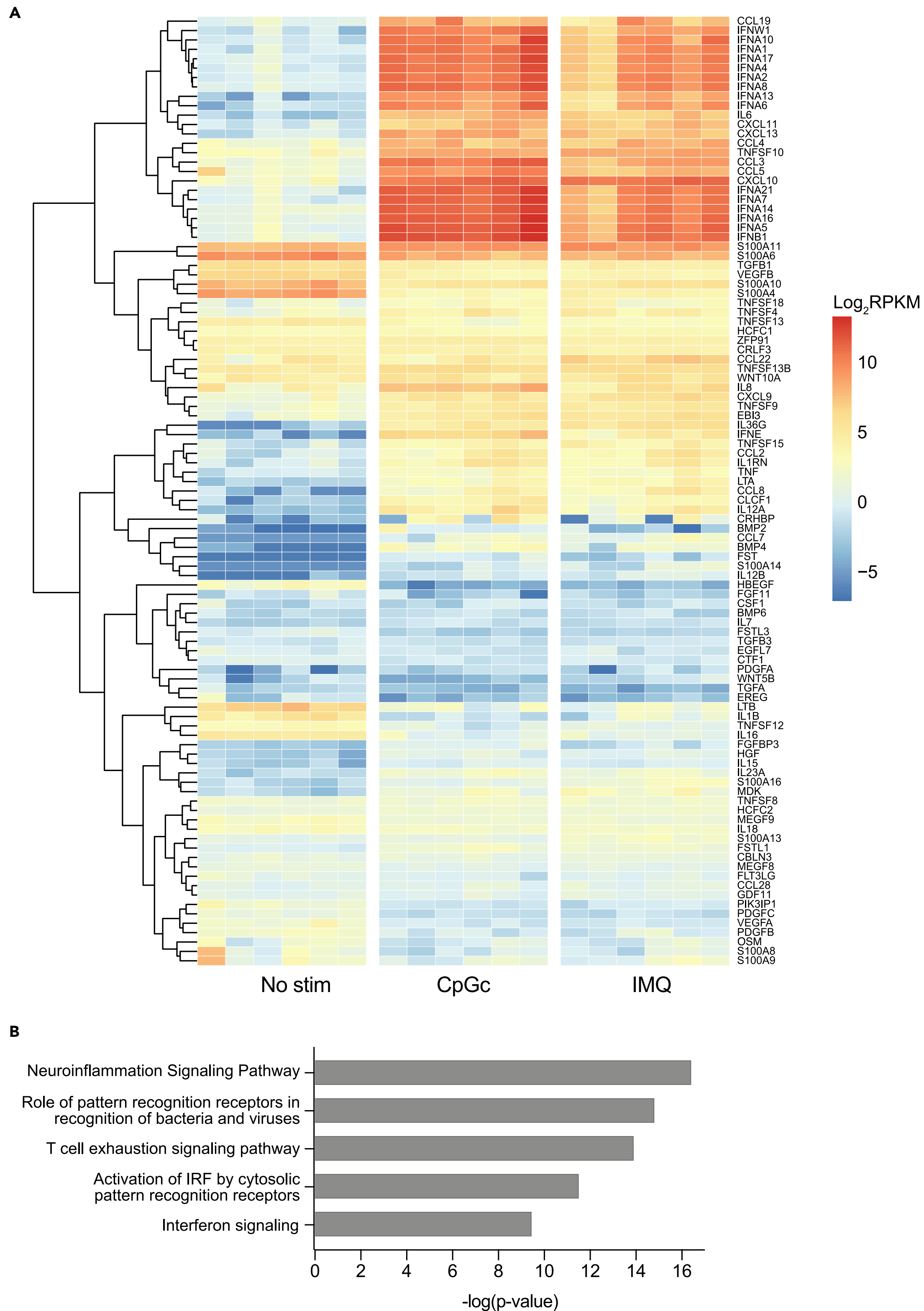


Figure 3. Soluble factors transcribed by pDCs when stimulated with TLR7 and TLR9 agonists

PBMC from healthy donors (n = 6) were cultured for 18 h with 0.5 μM CpGc (TLR9 agonist) or 0.5 μg/mL Imiquimod (IMQ; TLR7 agonist) before pDCs were isolated by FACS sorting and analyzed by RNA-seq.

Figure 3. *Continued*

(A) Transcripts of secreted proteins (NABA secreted protein gene list[44]) are shown.

(B) The genes differentially expressed (absolute log2FC >2, FDR <0.05) between sorted pDCs stimulated with CpGc or unstimulated were subjected to pathway analysis with Ingenuity (IPA). The top 5 altered processes, selected based on p value of overlap, are shown.

PBMC with CSL362 resulted in complete inhibition of the CpGc-induced gene expression profile with no effect on LPS or poly I:C-induced genes, confirming previous data.[35] CSL362 dramatically reduced the expression of all genes induced by SLE Ig + NCL, with most reduced to background expression. Although more variable, CSL362 also greatly reduced gene expression induced by SLE sera + NCL.

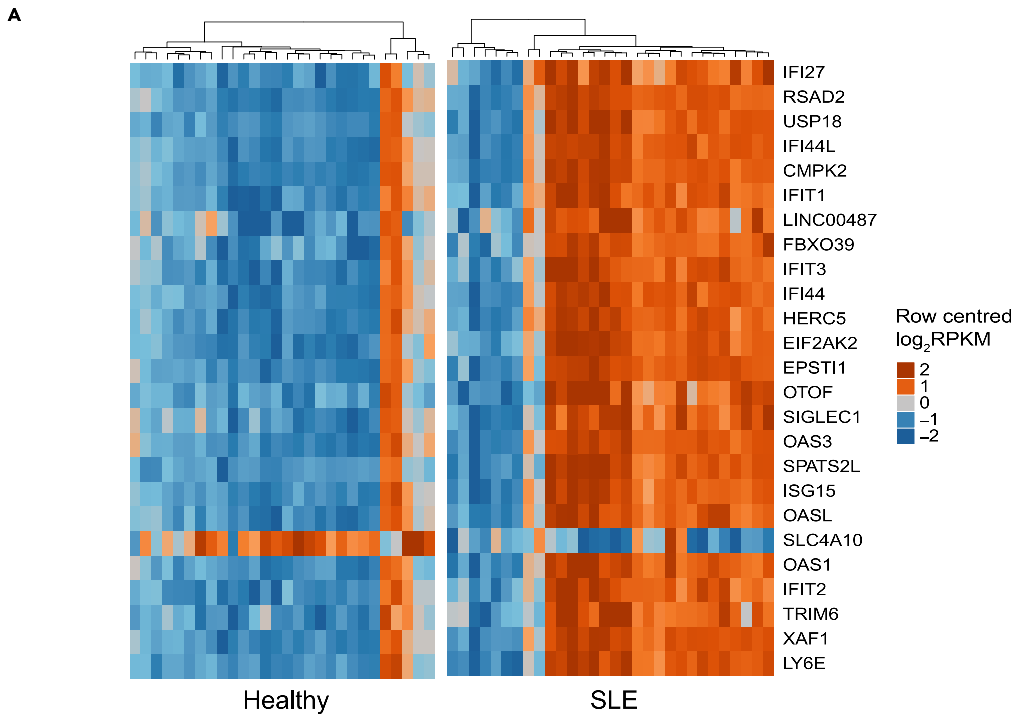
pDC derived cytokines and chemokines are increased in SLE

Our data show that pDCs are the only cell type completely depleted by CSL362 in SLE and HD blood *in vitro*, suggesting that loss of pDCs is primarily responsible for the CSL362-abrogated IFN response to SLE Ig + NCL. Given that CSL362 likely reduces IFN production through depletion of pDCs, we sought to understand additional soluble factors pDCs produce that may contribute to SLE pathology. As pDCs respond to DNA- and RNA-containing IC via TLR9 and TLR7[17,42] we stimulated PBMC from HD with TLR9 (CpGc) or TLR7 (imiquimod) agonists before isolating pDCs and performing RNA-seq. We identified transcripts of secreted proteins from the NABA secreted factors gene list[43,44] and determined their expression (Figure 3A). pDCs express transcripts for most type I IFNs, including all IFNα subtypes, IFNβ, IFNω, and IFNε, as well as IFNγ and IFN-inducible factors such as IP-10/CXCL10, ITAC/CXCL11 and MCP-2/CCL8. The molecules of the type III IFN group, which signal through a different receptor to type I and II IFNs, were only recently classified and thus are not included in the NABA list. However, all the type III IFNs were also significantly upregulated in activated pDCs (fold change >2 and p value <0.05). Stimulated pDCs also express high levels of transcripts for factors that regulate B-cell survival and recruitment, such as BAFF/TNFSF13B, APRIL/TNFSF13 and BCA1/CXCL13, and other pro-inflammatory molecules including IL-6 and TNFα. Pathway analysis of the differentially expressed genes from the NABA list predicted increases in processes like leukocyte migration (p value = 7.09×10^{-37}), activation (p value = 7.27×10^{-49}) and proliferation (p value = 1.53×10^{-41}), suggesting that pDCs contribute to the inflammatory environment characteristic of SLE. Altogether, these data suggest that pDCs drive SLE pathogenesis by producing a number of pro-inflammatory cytokines in addition to type I IFN.

To further investigate the role of activated pDCs in SLE pathogenesis, we performed pathway analysis on all genes identified as differentially expressed in pDCs following stimulation with TLR9 (CpGc). As expected, activation of pDCs induced transcriptional changes consistent with increased IFN signaling as well as enrichment of pathways supporting activation of Pattern Recognition Receptor signaling (Figure 3B). Of interest, the top identified pathway was one related to neuroinflammatory signaling, further suggesting that pDCs contribute to SLE pathogenesis by secreting a range of pro-inflammatory cytokines.

We next analyzed our SLE and HD cohorts to determine the gene expression profiles that are found in SLE patients and how these relate to the genes that are driven by pDC stimulation and modulated by CSL362 treatment discussed above. RNA-seq analysis of our dataset[35] showed 605 significantly upregulated and 498 significantly downregulated genes (absolute FC >2, FDR <0.05) in SLE patients compared to HD; the top 25 differentially expressed genes contained many IFN regulated genes (Figure 4A). Pathway analysis of the differentially expressed genes in SLE patients compared to HD identified increased IFN and Pattern Recognition Receptor signaling as the top ranked pathways (Figure 4B), consistent with deregulation of IFN signaling as a key event in SLE pathogenesis. Of note is that the pathway analysis of the gene expression profile of SLE patients identified alteration of similar processes to those we have previously shown to be driven by stimulated pDCs (Figure 3B) and those that can be targeted by CSL362 treatment (Figure 2C), further supporting the potential therapeutic benefit of CSL362 in this disease cohort.

The majority of the SLE patient cohort had a significantly elevated IFN-gene score (Figure 4C) based on our previously described 11-gene signature (p <0.0001). We investigated whether SLEDAI-2K and IFN-gene score correlated with clinical parameters or pDC numbers in the peripheral blood (Figure 4D). SLEDAI-2K had a positive correlation with IFN-gene score, anti-dsDNA, erythrocyte sedimentation rate (ESR) BAFF, ITAC/CXCL11 and M-CSF and was negatively correlated with C3 and C4 levels as well as pDC and NK cell number. The IFN-gene score also positively correlated with anti-dsDNA level, BAFF and ITAC/CXCL11 but was not correlated with pDC number.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OTOF
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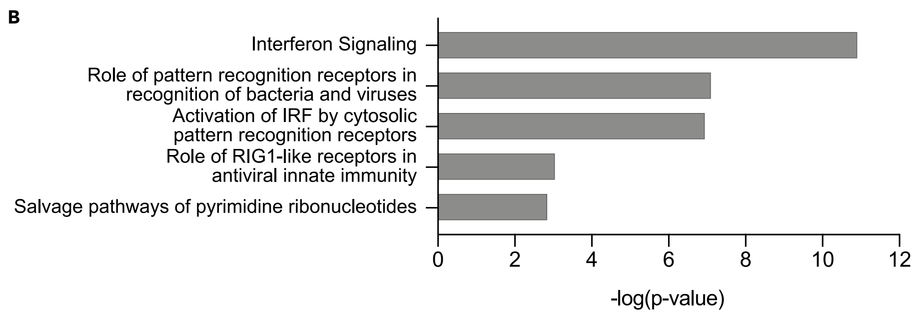
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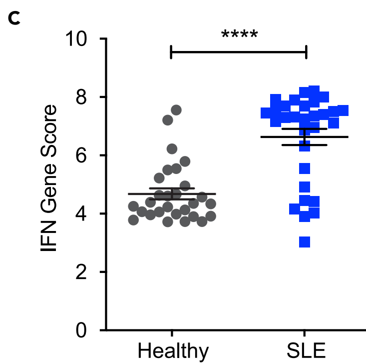
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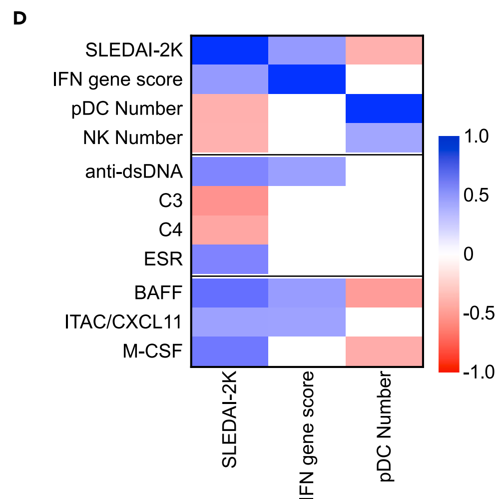
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NK Number
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ESR
BAFF
ITAC/CXCL11
M-CSF
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0.5
0
-0.5
-1.0
SLEDAI-2K
IFN gene score
pDC Number

Figure 4. SLE patients show upregulated expression of IFN genes and this can be correlated with other indicators of disease

RNA from SLE samples (n = 30) and HD Samples (n = 29) was analyzed by RNA-seq.

(A) A large proportion of the top 25 differentially expressed genes with the highest fold-change differences that were significantly different between SLE donors and matched HD were IFN inducible genes.

(B) The genes differentially expressed (absolute log2FC >0.8, FDR <0.05) between whole blood from SLE patients and HD were subjected to pathway analysis with Ingenuity (IPA). The top 5 altered processes, selected based on p value of overlap, are shown.

(C) An IFN gene score was determined based on the average log2 fold change of 11 gene transcripts (*IFI44L, IFIT1, IFIT3, IRF7, ISG15, MX1, MX2, OAS1, OAS2, SERPING1 and XAF1*) with SLE samples showing a significantly increased IFN gene score compared to HD. Bars depict mean $\pm$ SEM (Mann-Whitney test **** p value <0.0001).

(D) SLEDAI-2K, IFN gene score, NK cell number and pDC number correlate with various other parameters (experimental data - IFN gene score, absolute NK cell number per microliter of whole blood, absolute pDC number per microliter of whole blood; clinical parameters - adjusted mean SLEDAI-2K, ratio of anti-dsDNA titer to the upper limit of normal, C3 protein, C4 protein, Erythrocyte sedimentation rate (ESR); and serum protein levels of - BAFF, ITAC/CXCL11 and M-CSF) only correlation coefficients that were significant p = <0.05 are shown, with values closest to 1 having strongly positive correlations and values closest to −1 having strongly negative correlations.

We next evaluated the expression of 119 soluble analytes in the serum from both the SLE and HD cohorts. Of these, 94 were detectable in at least one sample and 27 were significantly upregulated (fold change >1.5 and adj p value <0.05) in the SLE patient group compared to HD (Table S3). Of the 27 proteins upregulated in SLE serum, transcripts for 11 were inducible in pDCs by TLR7 and TLR9 agonists (as seen in Figure 3A). IFNα was detected in only nine SLE patients but also in eight HD, with levels close to the lower limit of quantitation of the assay (range 7.6–18 pg/mL, data not shown).

Subsequently, we determined which soluble factors were affected by depletion of pDCs with CSL362. The expression of 87 different soluble analytes were evaluated in supernatants from SLE or HD PBMC stimulated with CpGc with or without prior pDC depletion. Twenty analytes were significantly upregulated (fold change >2 and FDR <0.05) by CpGc in both SLE and HD (Figure 5A); 14/20 CpGc-inducible proteins also had increased transcript levels in CpGc-stimulated pDCs. Notably there were increased levels of IFNα and IFN-inducible proteins such as MCP-2/CCL8, IP-10/CXCL10, ITAC/CXCL11 and MIP-3β/CCL19. The production of these and most other induced proteins was greatly reduced when pDCs were depleted by treatment with CSL362 before stimulation (Figure 5A). The levels of four of these IFN inducible and CSL362 modulated proteins in the sera of SLE patients is shown in detail (Figure 5B) confirming their dysregulation in SLE.

DISCUSSION

There has been a significant lag in the development of therapeutics for SLE patients, despite improved understanding of disease pathogenesis, and poor patient outcomes including markedly reduced life expectancy persist.[45] Type I IFNs have emerged as key cytokines that contribute to the perpetuation of autoimmunity and inflammation in SLE. Therapeutic targeting of the IFN pathway has shown great potential, from earlier strategies of using neutralizing antibodies against IFN, to the more recent approval of anifrolumab, an anti IFN-receptor monoclonal antibody. pDCs are known to be the most potent producers of type I IFN[15,16] and strategies that directly target pDC may yield an effective strategy to modulate type I IFN activity in a more precise and disease-tailored manner.

CSL362 is a humanized, affinity matured therapeutic monoclonal antibody against CD123 that has been Fc engineered for enhanced ADCC activity.[36] Herein we provide further evidence that pDCs express a high level of CD123[29,35] and can be potently depleted by CSL362 *in vitro*. This is supported by previously published data demonstrating *in vivo* depletion of pDCs.[36,40] We found SLE patients had significantly fewer pDCs in blood than HD, consistent with previous studies.[27,28,46,47] We also found significantly fewer basophils,[48,49] and many other cell types, including mDCs, monocytes, NK cells and various T and B-cells, consistent with the cytopenia commonly observed in SLE.[50] Although these cell types were reduced in SLE blood, they retained normal levels of CD123. Here, we show that the depletion of cell subsets by CSL362 is proportional to the cell surface expression of CD123, in both HD and SLE patients, with cells expressing less than ∼1000 receptors per cell not depleted.

pDCs are the primary producers of IFNα in response to viral infection.[51] They also produce significant amounts of IFNα in response to DNA- and RNA-containing IC[41,52] found in autoimmune conditions such as SLE, through internalization via CD32a and subsequent stimulation of TLR7 and TLR9.[19] The amounts

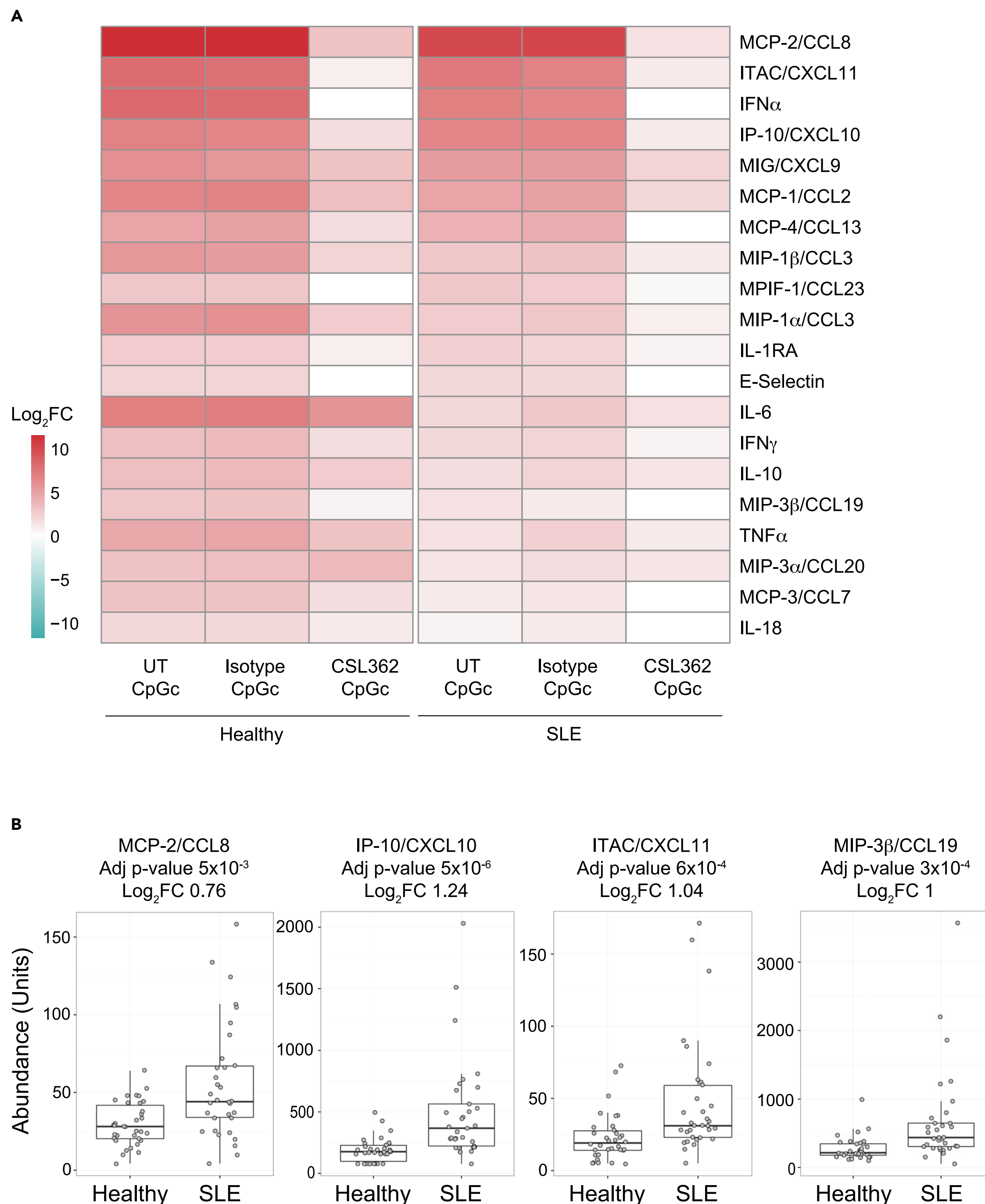

Figure 5. CSL362 reduces production of TLR9-induced proteins including proteins that are significantly elevated in SLE patients sera

(A) PBMC from 9 healthy controls and 8 SLE patients were incubated in media alone (untreated; UT) or treated with 1 μg/mL CSL362 or isotype control for 20 h before wells were stimulated with 0.5 μM CpGc for 24 h. Supernatant was collected and assayed for various soluble proteins. Heatmap shows proteins that were significantly upregulated by CpGc stimulation when normalized to unstimulated control (fold change >2 and FDR <0.05).

(B) Sera was collected from 33 SLE patients and 34 HD and analyzed for levels of 119 soluble proteins, 27 of which were found to be upregulated in SLE patient sera (see also Table S3). The IFN inducible proteins MCP-2/CCL8, IP-10/CXCL10, ITAC/CXCL11 and MIP-3β/CCL19 were among those significantly upregulated in the sera of SLE patients (Benjamini and Hockberg method, fold change >1.5 and adj p value <0.05).

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of IFNα induced by SLE Ig + NCL from different donors varied considerably between different Ig and PBMC donors, consistent with published reports.[41] The source of the variation is unknown but may reflect different amounts of autoantibody, specificity of the autoantibodies, number of pDCs or genetic factors. TLR9-, SLE Ig- and SLE serum + NCL-stimulation was also able to induce multiple genes, many of which were IFN program genes and included a specific IFN signature which was also significantly increased in the peripheral blood of the SLE patients we studied. The induction of these genes was completely prevented by pDC depletion by CSL362. It was interesting, however, that when donor PBMC were stimulated with SLE patient sera mixed with NCL, depletion of pDCs was not sufficient to completely block the IFN signature. Other cells may contribute to the IFN signature under some circumstances or, alternatively, this could also be the effect of IFN itself present at low levels in the SLE sera. CSL362 pre-treatment specifically prevented the upregulation of TLR9-induced genes and did not affect those upregulated by TLR4 and TLR3 stimulation. As pDCs are most potently and completely depleted by CSL362, we propose pDC depletion is the chief mechanism for the reduction of IFN-inducible genes as a result of CSL362 treatment. Given the positive correlation between SLEDAI and IFN-gene score, and the impact of CSL362 on IFN-related genes, these findings suggest that depletion of pDCs by CSL362 and subsequent reduction in IFN could result in decreased disease activity. Furthermore the specific ability of CSL362 targeting of pDCs to selectively modulate type I IFN production and activity in response to lupus specific triggers such as ICs whereas preserving responses to other non-lupus related triggers like LPS (TLR4) and poly I:C (TLR3) could offer significant clinical benefit when compared to approaches that involve complete type I IFN blockade. This is supported by data from others showing SLE patient PBMC depleted of CD123+ pDCs were still able to produce IFNα in response to viral stimuli.[47]

The primary mechanism of action of CSL362 is NK cell mediated ADCC, as previously determined.[36] SLE patients have aberrant immune systems, including decreased NK cells.[5,53,54] As a prelude to clinical trials it was important to establish whether SLE NK cells were effective mediators of ADCC. NK cells from SLE patients can have impaired cytotoxic function *in vitro* and decreased perforin and granzyme.[54] However, it has also been reported that NK cells can have enhanced cytotoxic function in the presence of IFNα[55,56] which can be upregulated in SLE patients. CD107a expression is a measure of NK cell degranulation capacity and activity and can correlate with ADCC,[57] whereas CD16 downregulation is associated with production of various cytokines.[58] Incubation of PBMC with CSL362 increased the expression of CD107a on NK cells and downregulated CD16. We also found increased CD107a and decreased CD16 expression on SLE patient NK cells in untreated cultures when compared to HDs, suggesting increased basal activation of NK cells in SLE patients. Activation-induced cell death could contribute to the decreased NK cell numbers in SLE patients.[3] Regardless of the decreased NK cell numbers and CD16 expression in SLE PBMC, we saw comparable dose dependent depletion *in vitro* of pDCs by CSL362 in SLE patients and HD. These data suggest that SLE patient NK cells are capable of ADCC-mediated depletion of pDCs *in vitro* despite the majority of SLE patients in our cohort receiving treatment with immunosuppressive medications.

Given that SLE NK cells were able to potently mediate CSL362-induced depletion of pDCs, we also measured depletion of other blood cell subsets. Basophils, which also express high levels of CD123, were partially depleted by CSL362 *in vitro*, however both pDCs and basophils are potently and completely depleted by CSL362 *in vivo*, in both non-human primates and humans without SLE.[36,40] The depletion of basophils in SLE patients could provide additional therapeutic benefits in contrast to molecules that only target pDCs, as basophils have been implicated in SLE pathogenesis and the support of autoreactive B cells.[48,59] A small clinical study of the anti-IgE mAb omalizumab showed a significant reduction in SLEDAI-2K which could be related to the reduction of IgE autoantibodies and thus decreased basophil activation.[60] Basophils express receptors for soluble factors that are elevated in SLE including MCP-2/CCL8, MIP-3β/CCL19, IL-18 and MCP-1/CCL2, which were significantly upregulated in our study. The blockade of IL-3 signaling by CSL362 could also affect basophil survival, as IL-3 is a survival factor for basophils.[61,62] There was some depletion of mDCs in both the SLE and HD samples, but this was of a lesser magnitude than pDC and basophil depletion at the doses used. Importantly, other immune cells were not depleted including monocytes and T, B and NK cells, which express much lower levels of CD123, suggesting CSL362 is unlikely to be generally immunosuppressive.

The pathways that were overrepresented in samples stimulated with SLE IC showed a strong IFN related theme and did not include any basophil related pathways suggesting the changes are primarily driven by the removal of pDCs. Basophils do express TLRs including TLR4 and TLR9,[63] and can respond to the

stimuli such as poly I:C, LPS and CPG used in our experiments.[64,65] However, in our experiments we only saw changes after CSL362 treatment in the responses to TLR9 and SLE IC and sera, so if basophils are responding to TLR3 or TLR4 agonism these responses are being masked by the responses of other cell types present. Of interest, basophils also express CD32a[63] which potentially opens the possibility that basophils may also be able to respond to IC found in autoimmune conditions such as SLE. The binding of Ig-allergen complexes to mouse basophils mediated by CD32a and subsequent cellular responses has been demonstrated[66] which supports the need for further investigation into the effects of IC on basophils in autoimmune diseases.

Although there is an established role for IFNα in SLE, studies to measure the protein in peripheral blood have yielded mixed results.[2–5,67] Herein we found low levels of IFNα detectable only in some SLE patients and similar results in HD. Importantly pDCs produce many other soluble factors in addition to IFNα. We found that TLR9 or TLR7 stimulation of pDC *in vitro* induced the transcription of many soluble factors, including all IFN subtypes and multiple IFN-inducible proteins including cytokines and chemokines such as MCP-2/CCL8, IP-10/CXCL10, ITAC/CXCL11 and MIP-3β/CCL19. Like others[22] we found that gene expression of many of these were also induced in pDCs stimulated with SLE Ig + NCL. We were also able to detect these IFN-inducible factors as soluble proteins after TLR9 stimulation of SLE or HD PBMC, and they were significantly decreased by CSL362-mediated depletion of pDCs before stimulation. Moreover, these proteins were among the cytokines found to be significantly upregulated in the sera of SLE patients. Type III IFNs are also produced by pDCs and have been implicated in SLE pathogenesis.[68] These findings imply that pathologically important molecules in addition to type I IFN would be reduced by pDC depletion with CSL362, suggesting a set of pharmacodynamic effects broader than those achievable with type I IFN or IFN receptor 1 blockade. Some of these factors might also represent useful biomarkers of CSL362 activity. Other clinical studies have shown blocking IFN can reduce the serum levels of IFN inducible proteins, including MCP-2/CCL8, in patients with SLE and other diseases that have IFN signatures.[69,70] The majority of the pDC-derived factors are chemokines involved in the recruitment of immune cells, suggesting that pDCs may be involved in immune cell recruitment to sites of pathology and the reduction of these soluble factors could also provide some clinical benefit in SLE.

We have shown that depletion of pDCs by CSL362 can potently inhibit IFN pathway activation in PBMC from patients with SLE. This study confirms pDC as the dominant source of IFN program activation found in SLE, and provides strong supportive evidence for the potential therapeutic effects of pDC depletion with CSL362 in SLE. Further studies on the pharmacokinetic and pharmacodynamic effects of CSL362 in humans, as well as its safety, are required to confirm the feasibility of using CSL362 to deplete pDC in the treatment of human IFN-driven diseases such as SLE.

Limitations of the study

There are several limitations of the current study. Although we demonstrated depletion of pDC and commensurate silencing of the IFN program and other inflammatory pathways by CSL362 *ex vivo*, it remains to be shown that CSL362 would exert these effects if administered to SLE patients. Most patients from whom blood was obtained were taking at least one SLE medicine and medication use was variable between patients, with unknown effects on the results. We note, however, that the profile of medication use is similar to that of patients who might be treated with CSL362 in a clinical trial. In addition, samples were restricted to a single time point per donor and there is limited sample size and ethnic diversity of patients within the study, and although it may be representative of Caucasian and Asian SLE patients, African American SLE patients were not available for our study. The majority of samples collected were from female SLE patients which is representative of the sex bias of the condition. CSL362 targets CD123, which we show is expressed on cells other than pDC; different effects might be observed through pDC targeting via another mechanism.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability

- ○ Data and code availability
- EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS
  - ○ Human Subjects and clinical protocol
  - ○ Primary cultures and cell lines
- METHOD DETAILS
  - ○ Absolute cell counts and CD123 expression
  - ○ *In vitro* depletion cultures
  - ○ PBMC stimulation cultures
  - ○ pDC stimulation and isolation
  - ○ Protein analysis
  - ○ RNA extractions and transcriptome analysis
  - ○ Bioinformatics analysis
- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2023.107173.

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AUTHOR CONTRIBUTIONS

Conceptualization, K.A.M., N.W., J.J., J.B., and I.W.

Methodology, K.A.M. and S.O.

Investigation, K.A.M. and T.Y.T.

Formal Analysis, K.A.M., B.L., T.S., M.C., M.N., and C.G.

Resources, A.H. and E.M.

Writing – Original Draft, K.A.M., N.W., and C.G.

Writing – Review and Editing, K.A.M., N.W., E.M., and A.H.

Visualization, K.A.M., M.C., M.N., C.G.

Supervision, N.W. and E.M.

DECLARATION OF INTERESTS

This work was done as part of study by CSL Limited and Janssen/Johnson and Johnson.

K.A.M., N.W., M.N., and C.G. are employees and shareholders of CSL Limited. During the study T.Y.T. was an employee of CSL.

During the study J.B., T.S., B.L., and J.J. were employees and/or shareholders of Johnson and Johnson.

I.W. has acted as a scientific advisor to CSL and his laboratory has received funding for pre-clinical evaluation of CSL362.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Lin1 FITC (anti-CD3, -CD14, -CD16, -CD19, -CD20, -CD56)	Becton Dickinson	Cat# 340546; RRID:AB_398456
anti-human CD123 PE	Becton Dickinson	Cat# 555644; RRID:AB_396001
anti-human BDCA2 APC	Miltenyi Biotec	Cat# 130-090-905; RRID:AB_244165
anti-human HLA-DR APC-H7	Becton Dickinson	Cat# 561358; RRID:AB_10611876
anti-human CD11c BV421	Becton Dickinson	Cat# 562561; RRID:AB_2737656
anti-human CD4 FITC	Becton Dickinson	Cat# 340133; RRID:AB_400007
anti-human CD3 PECY7	Becton Dickinson	Cat# 557851; RRID:AB_396896
anti-human CD19 APC	Becton Dickinson	Cat# 555415; RRID:AB_398597
anti-human CD8 APC-H7	Becton Dickinson	Cat# 561423; RRID:AB_10682894
anti-human CD56 BV421	Becton Dickinson	Cat# 562751; RRID:AB_2732054
anti-human CD27 BV510	Biolegend	Cat# 356420; RRID:AB_2562603
anti-human CCR3 FITC	Miltenyi Biotec	Cat# 130-097-064; RID:AB_2655897
anti-human CD49d PECY7	Biolegend	Cat# 304314; RRID:AB_10643278
anti-human CD11b APC	Becton Dickinson	Cat# 550019; RRID:AB_398456
anti-human CD14 APC-H7	Becton Dickinson	Cat# 561384; RRID:AB_10611720
anti-human CD16 BV510	Biolegend	Cat# 302048; RRID:AB_2562085
anti-human CD3 FITC	Becton Dickinson	Cat# 340542; RRID:AB_400051
anti-human CD16 PE	Becton Dickinson	Cat# 555407; RRID:AB_395807
anti-human NKp46 APC	Becton Dickinson	Cat# 558051; RRID:AB_398653
anti-human BDCA1 PE	Miltenyi Biotec	Cat# 130-090-508; RRID:AB_244315
anti-human CD123 BV510	Becton Dickinson	Cat# 563072; RRID:AB_2728102
anti-human IgE FITC	KPL	Cat# 01-10-04
anti-human CCR3 BV421	Becton Dickinson	Cat# 562570; RRID:AB_2737659
anti-human CD11b BV510	Biolegend	Cat# 301334; RRID:AB_2562112
anti-human CD107a PECY7	Becton Dickinson	Cat# 561348; RRID:AB_10644018
CSL362 (anti-human CD123)	CSL Limited, Reference: Busfield et al.[36]	CSL362
Isotype control (BM4-XV90)	CSL Limited, Reference: Oon et al.[35]	BM4-XV90
Biological samples		
SLE patient sera	Astarte Bio	N/A
SLE patient blood	Monash Health	N/A
Healthy donor blood	Skin Health Institute	N/A
Healthy donor buffy packs	Australian Red Cross	N/A
Fetal Calf Serum	Thermo	Cat# SV30176.03
Chemicals, peptides, and recombinant proteins		
Lysis Buffer	Becton Dickinson	Cat# 349202
RNA Protect	Qiagen	Cat# 76104
Propidium Iodide	Thermo Fisher	Cat# P1304MP
CpGc	Invivogen	Cat# tlrl-2395

(*Continued on next page*)

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Continued		
LPS	Invivogen	Cat# eklps
Poly I:C	Invivogen	Cat# k-picw
Imiquimod	Invivogen	Cat# tlrl-imqs
Ficoll Paque Plus	GE	Cat# GE17-1440-03
Critical commercial assays		
Verikine Multiple subtype IFNα ELISA kit	PBL Assay Science	Cat# 41110
PAXgene blood RNA kit	Qiagen	Cat# 762164
RNAeasy plus mini	Qiagen	Cat# 74134
RNAeasy plus micro	Qiagen	Cat# 74034
Deposited data		
SLE patient WB RNAseq dataset	Our Lab, Reference: Oon et al.[35]	GEO: GSE112087
Stimulated pDC RNAseq dataset	Our Lab, Reference: Oon et al.[35]	GEO: GSE79272
Stimulated SLE PBMCs treated with CSL362	Our Lab	GEO: GSE231686
Experimental models: Cell lines		
Human Embryonic Kidney: HEK293T cells	ATCC	Cat# CRL-3216
Software and algorithms		
Ingenuity PathwayAnalysis	Qiagen	Version 01-21-03
Prism Version 7 and 8	Graphpad	Version 7 and 8
Flowjo	Becton Dickinson	Version 9
Illumina HiSeq control Software	Illumina	Versions 2.2.58 and 2.2.68
Illumina Real time analysis	Illumina	Versions 1.18.64, 1.18.66.3, 1.18.64
Illumina bcl2fastq	Illumina	Versions 1.8.4 and 2.17.1.14
TopHat2	Reference: Trapnell et al.[71]	Versions 2.0.12 and 2.1.0
Bowtie	Reference: Trapnell et al.[71]	Version 2.2.5
FeatureCounts	Reference: Liao et al.[72]	Version 1.4.6
Limma	Bioconductor	Versions 3.34.3 and 3.26.8
Other		
Live/Dead Yellow	Life Technologies	Cat# L-34959
Quantibrite tubes	Becton Dickinson	Cat# 340495

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact Katherine Monaghan (Katherine.Monaghan@csl.com.au).

Materials availability

The study did not generate any unique reagents.

Data and code availability

RNAseq data has been deposited at GEO and are publically available (GSE112087, GSE79272, GSE231686).

This paper does not report original code.

Any additional information required to reanalyse the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Human Subjects and clinical protocol

2 cohorts of SLE patients were recruited, the primary cohort (Table S1) between August 2014 and March 2015 (n =33) as consecutive patient samples from a tertiary, specialist SLE clinic (Monash Lupus Clinic, Monash Medical Centre, Victoria, Australia) for the cell numbers, receptor quantification, depletion, serum and supernatant protein quantification and whole blood (WB) RNAseq and a secondary smaller cohort (Table S2) for immune complex stimulation experiments from November 2015 to December 2015 (n = 8). All met the American College of Rheumatology SLE Classification Criteria prior to enrolment. All patients provided informed consent and the study was approved by the Monash Health human research ethics committee. WB samples were collected into lithium heparin, PAXgene and serum separator vacutainers (BD). Patients were evaluated for disease activity using SLE disease activity index (SLEDAI-2K)[73] and had a median SLEDAI-2K of 4 (range 0 – 21) at the time of sample collection. Clinical and demographic details of the patients are described in Tables S1 and S2. The primary cohort of SLE samples were matched with 34 HD based on sex and age ($\pm$10%), and the secondary cohort compared to an additional 3 HD from the skin health institute (Victoria) after informed consent with ethics approval from Bellberry Human Research Ethics Committe. The majority of matched HD for the primary cohort (29/34) were also matched for self-reported ethnicity. Age and sex for each participant (see Tables S1 and S2) was self-reported and sex was not included as a parameter in the analysis as there were too few male participants in the study due to the sex bias associated with SLE. All experiments conform to the relevant regulatory standards.

Primary cultures and cell lines

Peripheral blood mononuclear cells (PBMC) for all culture experiments were isolated by overlaying fresh blood (depletion and stimulation cultures) or healthy donor human buffy pack (for pDC isolation) onto Ficoll Paque Plus (GE), centrifuging at 1000 $\times$ g for 20 min with no brake and collected the cell layer. Cells were thoroughly washed with PBS. Primary cell cultures of PBMC were cultured in RPMI-1640 supplemented with glutamax and 5% fetal calf serum (FCS) in a humidified incubator at 37°C with 5% CO2. HEK293T cells which are human embryonic kidney cells were sourced from the ATCC and used as a source of necrotic cell lysates (NCL) as described below.

METHOD DETAILS

Absolute cell counts and CD123 expression

50 or 100 μL aliquots of WB from 32/33 SLE and 32/34 HD were stained with antibody for 15 min at room temperature before being lysed and fixed with a 10 x volume of BD lysis buffer using a lyse no-wash procedure and acquired on the Miltenyi MACSquant 10. The absolute count of each cell type per microliter of WB was determined. The expression level of CD123 was determined on cell subsets using anti-CD123 PE antibody (9F5 clone, BD) and the number of antibodies bound per cell (ABC) determined by comparing the geometric mean fluorescence intensity (MFI) to the fluorescence minus one control, then converting the MFI to ABC using quantibrite beads (BD) as per the manufacturers instructions.

Cell populations were identified by flow cytometry as follows: all populations were first gated for single cells and FSC and SSC gates were used to gate the major cell populations and remove any additional debris/artefact. pDCs were $SSC^{low/intermediate}$ Lin1- HLA-DR+ CD11c- BDCA2+, mDCs were $SSC^{low/intermediate}$ Lin1- HLA-DR+ BDCA2- CD11c+, basophils were $SSC^{low/intermediate}$ CD14- CCR3+, Monocytes were $SSC^{low/intermediate}$ CCR3- CD49d+ CD11b+, CD4 T cells were SSC^{low} $CD3^+$ $CD56^-$ $CD8^-$ $CD4^+$, CD8 T cells were SSC^{low} $CD3^+$ $CD56^-$ $CD8^+$ $CD4^-$, $CD3^+CD56^+$ cells were SSC^{low} $CD3^+CD56^+$, Naïve B cells were SSC^{low} $CD3^-$ $CD56^-$ $CD19^+$ $CD27^-$, memory B cells were SSC^{low} $CD3^-$ $CD56^-$ $CD19^+$ $CD27^{intermediate}$, plasmablasts were SSC^{low} $CD3^-$ $CD56^-$ $CD19^+$ $CD27^{high}$, neutrophils SSC^{high} CD11b+ CD49d-, eosinophils SSC^{high} CD11b+ CD49d+ $CD16^-$ $CD14^-$. Details of specific antibodies used are includes in the key resources table.

In vitro depletion cultures

Depletion experiments were done with 1×10^6 PBMC in 200 μL media cultured for 20 h at 37°C with or without CSL362 at various doses (0.0005–3 μg/mL) in RPMI-1640 media supplemented with 5% heat-inactivated FCS.

Cells were then stained with live/dead yellow (Life Technologies) and various commercial antibodies (as listed in the key resources table) and gated as listed above (excluding eosinophils and neutrophils) to identify different cell subsets before being fixed in formaldehyde and acquired on a BD fortessa. CD16 and CD107a were also measured on NK cells.

PBMC stimulation cultures

SLE IC were produced by combining purified SLE immunoglobulin G (IgG) or SLE sera with necrotic cell lysates (NCL) as per Kassim et al.[41] Briefly, IgG from the sera of SLE patients (Astarte Bio) was isolated on a protein A/G column (Thermo) according to the manufacturers instructions. This was combined with NCL produced from HEK293T cells (ATCC) that were repeatedly freeze-thawed (4 cycles of freezing at −80°C and thawing at 37°C) before being centrifuged to remove cellular debris. Stimulation experiments were done with PBMC from SLE or HD cultured with 1 μg/mL CSL362 or isotype control for 20 h before the supernatant was removed and cells were resuspended in various stimuli (0.25 μM CpGc, 10 μg/mL LPS, 10 μg/mL poly I:C, 0.1 mg/mL HD Ig + 0.1 mg/mL NCL, 0.05–0.1 mg/mL SLE Ig + 0.1 mg/mL NCL, 20% SLE sera + 0.1 mg/mL NCL, 20% HD sera + 0.1 mg/mL NCL) for an additional 24 h of culture. The stimuli used included CPGc, a TLR9 agonist known to elicit a potent pDC type I IFN response, SLE specific stimuli (SLE-Ig + NCL and SLE sera + NCL) and control stimuli (HD Ig + NCL and HD sera + NCL) and non-SLE specific stimuli (LPS (endotoxin), a TLR4 agonist derived from bacteria and poly I:C a TLR3 agonist with a molecular pattern associated with viral exposure) to understand the specific effects of pDC depletion on different inducible gene transcripts. After 24 h the supernatants were collected and analysed for IFNα concentration by ELISA (as detailed below), and cell pellets were collected and stored in RNAprotect at −80°C, before the RNA was extracted at a later date.

pDC stimulation and isolation

As previously described[35] PBMC were isolated from HD buffy coats (Australian Red Cross Blood Service) and stimulated with the TLR9 agonist 0.5 μM CpGc or TLR7 agonist 0.5 μg/mL imiquimod for 18 h at 37°C before pDCs were isolated by magnetic Lin1 depletion and FACS sorted (BDCA1- BDCA2+ HLA-DR+ CD11c- CD123+ Propidium Iodide-). Purified pDCs were stored in RNAprotect at −80°C before RNA was extracted at a later date.

Protein analysis

Serum samples from the SLE (n=33) and HDs (n=34) were assayed for 119 different soluble analytes (6Ckine, Agouti-Related Protein, Alpha-1-Antitrypsin, Amphiregulin, Angiopoietin-2, Angiotensin- Converting Enzyme, AXL Receptor Tyrosine Kinase, B cell-activating factor, B Lymphocyte Chemoattractant, Betacellulin, Brain-Derived Neurotrophic Factor, Cancer Antigen 15-3, CD27 antigen, CD 40 antigen, CD40 Ligand, Chemokine CC-4, Ciliary Neurotrophic Factor, Dickkopf-related protein 1, E-Selectin, Eotaxin-1, Eotaxin-2, Eotaxin-3, Epidermal Growth Factor, Epidermal Growth Factor Receptor, Epiregulin, Erythropoietin, Factor VII, Fas Ligand, FASLG Receptor, Fibrinogen, Fibroblast Growth Factor 4, Fibroblast Growth Factor 21, Fibroblast growth factor 23, Fibroblast Growth Factor basic, Follicle-Stimulating Hormone, Glucagon-like Peptide 1, total, Granulocyte Colony-Stimulating Factor, Granulocyte-Macrophage Colony-Stimulating Factor, Growth-Regulated alpha protein, Haptoglobin, Heparin-Binding EGF-Like Growth Factor, Hepatocyte Growth Factor, Immunoglobulin A, Immunoglobulin M, Insulin-like Growth Factor-Binding Protein 2, Insulin-like Growth Factor-Binding Protein 7, Intercellular Adhesion Molecule 1, Interferon alpha, Interferon gamma, Interferon gamma Induced Protein 10, Interferon-inducible T-cell alpha chemoattractant, Interleukin-1 alpha, Interleukin-1 beta, Interleukin-1 receptor antagonist, Interleukin-2 receptor alpha, Interleukin-3, Interleukin-4, Interleukin-5, Interleukin-6, Interleukin-6 receptor, Interleukin-7, Interleukin-8, Interleukin-10, Interleukin-12 Subunit p40, Interleukin-12 Subunit p70, Interleukin-15, Interleukin-17, Interleukin-18, Interleukin-22, Interleukin-23, Interleukin-31, Latency- Associated Peptide of Transforming Growth Factor beta 1, Luteinizing Hormone, Macrophage Colony-Stimulating Factor 1, Macrophage Inflammatory Protein-1 alpha, Macrophage Inflammatory Protein-1 beta, Macrophage Inflammatory Protein-3 alpha, Macrophage inflammatory protein 3 beta, Macrophage Migration Inhibitory Factor, Matrix Metalloproteinase-1, Matrix Metalloproteinase-3, Matrix Metalloproteinase-7, Matrix Metalloproteinase-9, Matrix Metalloproteinase-9, total, Matrix Metalloproteinase-10, Monocyte Chemotactic Protein 1, Monocyte Chemotactic Protein 2, Monocyte Chemotactic Protein 3, Monocyte Chemotactic Protein 4, Monokine Induced by Gamma Interferon, Myeloid Progenitor Inhibitory Factor 1, Myeloperoxidase, Osteoprotegerin, Pancreatic Polypeptide, Placenta Growth Factor, Platelet-Derived Growth Factor BB, Progranulin, Prolactin, Resistin, Stem Cell Factor, Stromal cell-derived factor-1,

T Lymphocyte-Secreted Protein I-309, Tenascin-C, Thymus and activation-regulated chemokine, Thymus-Expressed Chemokine, Tissue Inhibitor of Metalloproteinases 3, TNF- Related Apoptosis-Inducing Ligand Receptor 3, Transforming Growth Factor alpha, Transforming Growth Factor beta-3, Tumor Necrosis Factor alpha, Tumor Necrosis Factor beta, Tumor necrosis factor ligand superfamily member 12, Tumor necrosis factor ligand superfamily member 13, Tumor Necrosis Factor Receptor I, Vascular Endothelial Growth Factor, Vitamin D- Binding Protein) by bead-based multiplex assay (Myriad RBM, U.S.A). SLE patient PBMC and HD PBMC from some donors were cultured for 20 h with 1 μg/mL CSL362 or isotype control before addition of CpGc and culture for an additional 24 h, supernatants were collected and analysed for 87 different soluble proteins from the above list by bead based multiplex assay (Myriad RBM). IFNα was quantitated in culture supernatants using Verikine multiple subtype IFNα ELISA kit as per the manufacturer's instructions which detects IFN-αA -α2, -αA/D, -αB2, -αC, -αD, -αG, -αH, -αI, -αJ1, -αK, -α1, -α4a, and - αWA.

RNA extractions and transcriptome analysis

As previously described[35] SLE (n=30) and HD (n=29) peripheral blood (PB) samples were collected into PAXgene RNA tubes and stored at −80°C. RNA was extracted using the PAXgene blood RNA kit (Preanalytix) as per the manufacturer's instructions and RNAseq was performed. RNA was extracted from stimulated PBMCs and stimulated and sorted pDCs using Qiagen RNeasy mini plus and micro plus kits respectively. RNA libraries were prepared for sequencing using standard Illumina protocols including whole transcriptome library preparation with rRNA and Globin depletion for whole blood samples, and polyA extraction for samples from stimulated cells. Image analysis was performed in real time by the HiSeq Control Software and Real Time Analysis running on the instrument computer. The Illumina bcl2fastq pipeline was used to generate the sequence data. At least 6 million 100bp single reads were kept per sample from the total generated on a HiSeq2500 (Illumina, California, USA) at the Australian Genome Research Facility (Melbourne, Australia).

Bioinformatics analysis

Sequenced reads were mapped to human reference genome GRCh37.p10 using TopHat2 (TopHat, bowtie[71]). Mapped reads were summaried on a gene level using Ensembl ID gene annotation (Ensembl Release 70 based on GRCh37.p10 genome build) and featureCounts[72] software. Read counts were summarised using Bioconductor edgeR package functions: i) calcNormFactors (TMM method) to normalise between samples based on library sizes, and ii) rpkm function to normalise between genes within a sample based on gene lengths. Differential expression was determined using limma from the Bioconductor package. The lists of differentially expressed (DE) genes were used to perform pathway analysis using the Ingenuity Pathway Analysis (IPA) software (QIAGEN Inc.). The core analysis was carried out on each gene list separately using the default settings. Cut-off values of fold change and FDR were applied as specified in the text. Finally, the top-ranked canonical pathways, diseases and functions, and upstream regulators were selected from the analysis to derive biological interpretations of the transcriptome analysis. An 11-gene IFN signature was determined by the expression of the IFN-inducible genes *EPSTI1, ISG15, HERC5, IFI44, OAS3, OAS1, LY6E, CMPK2, RSAD2, IFI44L, IFIT1, IFIT3, USP18, SIGLEC1, IFI27* and *OTOF*.[35] For each isotype control and CSL362 treated samples the score was calculated by the average log2 fold change compared to control (HD PBMC unstimulated and treated with isotype control).

QUANTIFICATION AND STATISTICAL ANALYSIS

Details of individual statisticals tests, sample sizes, precision measures and levels of significance used can be found in the figure legends (**** p $\leq$0.0001, *** p $\leq$0.001, ** p $\leq$0.01, * p $\leq$0.05 and n.s p >0.05). Statistical analyses were performed as specified using GraphPad Prism Versions 7 and 8. All flow cytometric data was analysed using FlowJo 9. The details of the specific software versions used for the transcriptomic and bioinformatic analysis is captured in the key resources table. Graphical abstract created with biorender.com.