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IL-13Rα1-Mediated Signaling Regulates Age-Associated/ Autoimmune B-Cell Expansion and Lupus Pathogenesis

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Abstract

Objective: Age-Associated/Autoimmune B cells (ABCs) are an emerging B cell subset that aberrantly expand in SLE. ABC generation and differentiation exhibit marked sexual dimorphism and TLR7 engagement is a key contributor to these sex differences. ABC generation is also controlled by IL-21 and its interplay with IFNγ and IL-4. Here we investigated whether IL-13Rα1, an X-linked receptor that transmits IL-4/IL-13 signals, can regulate ABCs and lupus pathogenesis.

Methods: Mice lacking DEF6 and SWAP-70 (Double-Knock-out=DKO) that develop lupus preferentially in females were crossed with IL-13Rα1KO mice. IL-13Rα1KOs were also crossed

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to Yaa-DKO males, which overexpress TLR7 and develop severe disease. ABCs were assessed by FACS and RNA-seq. Lupus pathogenesis was evaluated by serologic and histological analyses.

Results: ABCs express higher levels of IL-13Rα1 than follicular B cells. Absence of IL-13Rα1 in either DKO females or Yaa-DKO males decreased the accumulation of ABCs, their differentiation into plasmablasts, and autoantibody production. Lack of IL-13Rα1 also prolonged survival and delayed the development of tissue inflammation. IL-13Rα1 deficiency diminished the *in vitro* generation of ABCs, an effect that, surprisingly, could be observed in response to IL-21 alone. RNAseq revealed that ABCs lacking IL-13Rα1 downregulated some B cell characteristics but upregulated myeloid markers and proinflammatory mediators.

Conclusions: These studies uncover a novel role for IL-13Rα1 in the control of ABC generation and differentiation suggesting that IL-13Rα1 contributes to these effects by regulating a subset of IL-21-mediated signaling events. These studies also suggest that X-linked genes besides TLR7 participate in the regulation of ABCs in lupus.

Introduction

Autoantibody production, upregulation of interferon stimulated genes (ISGs), and extensive organ damage are hallmarks of Systemic Lupus Erythematosus (SLE), an autoimmune disease that preferentially affects women [1, 2]. Aberrant expansion and dysregulation of T and B cell subsets is critical to SLE pathophysiology [3–6]. In particular, recent studies have highlighted the importance of a novel B cell subset, ABCs (Age/Autoimmune-associated B cells), in SLE development [7, 8]. ABCs accumulate prematurely in murine models of lupus and produce pathogenic autoAbs [9–11]. Furthermore, expansion of human ABCs (also known as DN2 B cells) has been observed in SLE patients where they rapidly differentiate into PB/PCs and are major producers of autoAbs [12, 13]. ABC accumulation occurs to a greater extent in African-American SLE patients, correlates with disease activity and clinical manifestations, and can be detected in SLE kidney biopsies [12–14]. ABCs have also been detected in other autoimmune disorders including Sjogren's, Rheumatoid Arthritis, and Scleroderma [7, 15].

ABCs exhibit a unique phenotype and, in addition to classical B cell markers, also express the transcription factor T-bet and myeloid markers like CD11c, hence these cells are also known as $CD11c^+$ T-bet^+ B cells [7]. Formation of ABCs is promoted by both innate and adaptive signals including engagement of TLR7, an endosomal TLR, which plays a key role in antiviral responses [7]. In line with their regulation by TLR7, ABCs are an important component of the immune response to viruses like flu and their expansion is inappropriately controlled in severe COVID-19 patients [16, 17]. Given the location of TLR7 on the X-chromosome and its ability to partially escape X-chromosome inactivation in B cells [18–20], TLR7 engagement furthermore is a major contributor to the preferential expansion of ABCs in females [9, 21].

In addition to TLRs, the formation of ABCs is also regulated by T-cell cytokines, with IL-21 and IFNγ promoting their formation and IL-4 normally inhibiting it [22]. Similar to IL-21, IL-4 can signal through a receptor complex composed of the γc chain and a ligand binding subunit, the IL-4Rα located immediately adjacent to the IL-21R with which it shares a

high degree of similarity [23, 24]. IL-4 can also signal through the type II heteroreceptor, which is composed of IL-4Rα and IL-13Rα1 and also mediates signaling by IL-13 [25, 26]. In contrast to the ubiquitous expression of IL-4Rα in immune cells, IL-13Rα1 is primarily expressed by myeloid cells and only low levels of IL-13Rα1 expression have been reported on lymphocytes at baseline [26]. Genetic ablation of IL-13Rα1 in murine systems has revealed surprising and complex contributions of this receptor not only to classic T_H2 diseases like atopic dermatitis but also to autoimmune pathophysiology. Lack of IL-13Rα1 ameliorates diabetes in NOD mice but aggravates CNS inflammation in EAE due to distinct effects on T_H17, T_H1, and Treg subsets [27, 28]. Although IL-13Rα1 is located on the X-chromosome and, similarly to TLR7, can also partially escape XCI [20], it is unknown whether signaling mediated by this receptor plays a role in SLE pathogenesis.

The SWEF proteins, SWAP-70 and DEF6, are two homologous proteins that control cytoskeletal reorganization and IRF activity [29–31]. Both human and murine studies support an important immunoregulatory role for these molecules. *SWAP70* is a susceptibility locus for RA and CVD while *Def6* is a risk factor for human SLE in multiple ethnic groups [32–34]. *Def6* mutations moreover result in early-onset autoimmunity, which includes autoAb production and upregulation of an ISG signature, often associated with viral infections [35, 36]. In line with these findings, the concomitant lack of *Def6* and *SWAP-70* in C57BL/6 mice (Double-knockout mice=DKOs) leads to the spontaneous development of SLE, which, similarly to humans, preferentially affects female mice [37]. In particular, lupus development in DKO mice is accompanied by the marked accumulation and aberrant differentiation of ABCs, which occurs in a sex- and TLR7-dependent manner [11, 21].

Given that we had observed that ABCs can express IL-13Rα1, here we have investigated its role in ABC formation and SLE pathogenesis by generating DKO mice deficient for IL-13Rα1 (*Il13rα1*$^{-/-}$DKO). Lack of IL-13Rα1 resulted in significantly fewer ABCs and modulated their ability to further differentiate and produce autoantibodies. Surprisingly, IL-13Rα1 deficiency decreased the *in vitro* generation of ABCs in response to IL-21 alone. RNAseq revealed that ABCs lacking IL-13Rα1 downregulated some of their B cell characteristics but upregulated myeloid markers and proinflammatory mediators. Taken together these studies suggest that IL-13Rα1 can help mediate a subset of IL-21-driven signals and skew the ability of ABCs to acquire autoimmune or pro-inflammatory features.

Methods

Mice.

DEF6-deficient (*Def6*$^{tr/tr}$) mice were generated by Lexicon Pharmaceuticals by a gene-trapping strategy as described [37]. Swap-70-deficient mice (*Swap-70*$^{-/-}$) were previously described [37]. *Def6*$^{tr/tr}$*Swap-70*$^{-/-}$ (DKO) mice were generated by crossing *Def6*$^{tr/tr}$ mice with *Swap-70*$^{-/-}$ mice and have been backcrossed into C57BL/6 background for over 10 generations [37]. *Yaa*DKO mice were recently reported [21]. *Il13rα1*$^{-/-}$ and *Il13rα1*-GFP mice were previously generated [38] and were crossed with DKOs to generate *Il13rα1*$^{-/-}$DKOs and *Il13rα1*-GFP-DKO, respectively. Mice were genotyped in-house and through Transnetyx. Mice of the incorrect genotype were excluded. All the mice were bred under specific pathogen–free conditions and standard housing conditions. All experiments

were carried out following institutional guidelines and with protocols approved by the Institutional Animal Care and Use Committee of the Hospital for Special Surgery and WCM–MSKCC.

Flow Cytometry, sorting, ELISA, and histology

FACS and sorting were performed as described [21]. For cytokines staining, splenocytes were stimulated with PMA, ionomycin and Brefeldin A for 5 hours before staining. Monoclonal antibodies used: to B220 (RA3–6B2), CD19 (6D5), CD11c (N418), CD11b (M1/70), T-bet (4B10), CD4 (RM4–5), GL7 (GL7), CD21/CD35 (7E9), CD23 (B3B4) from Biolegend; Fas (Jo2) and CXCR5 (2G8) from BD biosciences; to PD1 (J43) and Foxp3 (FJK-16s) from Invitrogen. For apoptotic cell analysis, cells were stained with propidium iodide (PI) and Annexin V (BD biosciences) before acquisition. For dead cells exclusion, cells were stained with Fixable Viability Dye (Invitrogen). Efferocytosis assays were performed as recently described [21]. Data were acquired on FACS Canto (Becton Dickinson) and analyzed with FlowJo (TreeStar) software. Autoantibody ELISAs were performed as published [21, 39]. CCL22 (DY439, R&D Systems) and IL-1β (432601, Biolegend) ELISAs were performed according to the manufacturer's instructions. Histological analysis was performed as described [11].

B cell differentiation *in vitro*

$CD23^+$ B cells were purified as described [11] and stimulated with 5μg/ml $F(ab')_2$ anti–mouse IgM (Jackson ImmunoResearch Laboratories), 5μg/ml purified anti–mouse CD40 (Bio X Cell), ± 50ng/ml IL-21 (Peprotech), 10ng/ml IL-4 (Peprotech) or 20ng/ml IL-13 (Peprotech). For proliferation assays, $CD23^+$ B cells were labeled with 2.5μM CellTrace™ Violet dye (Invitrogen) for 2 min at room temperature prior stimulation.

Immunoblot analyses

Extracts were prepared as described [37] and analyzed by immunoblotting with the following antibodies: anti-STAT6 phosphorylated at Tyr641(56554S, Cell Signaling), anti-Stat3 phosphorylated at Tyr705 (9145S, Cell Signaling), anti-Stat1 phosphorylated at Tyr701 (9167S, Cell Signaling), anti-Stat5 phosphorylated at Tyr694 (4322S, Cell Signaling), anti-IRF4 (15106, Cell Signaling), and anti-HDAC1 (2062, Cell Signaling).

Real-time RT-PCR.

Real-time quantitative PCR was performed using the iTaq Universal SYBR Green Supermix (Biorad; #1725121). Gene expression was calculated using the ΔΔCt method and normalized to Cyclophilin a (*Ppia)*. (cyclophilin A Ppia forward, 5' -TTGCCATTCCTGGACCCAAA-3', Ppia reverse, 5'-ATGGCACTGGCGGCAGGTCC-3'). Primers were as follows: IL-21R (Qiagen: QT00137627), IL-1β (mIl1b forward : 5'-AGCTTCCTTGTGCAAGTGTCT-3'; mIl1b reverse : GACAGCCCAGGTCAAAGGTT).

RNA-Seq analyses

Total RNA was extracted using RNeasy Plus Mini kit (Qiagen). Illumina-compatible sequencing libraries were constructed by the Weill Cornell Epigenomics Core. Quality of all RNA and library preparations were evaluated with BioAnalyser 2100 (Agilent) before sequencing. Pair-end sequencing and data evaluation were performed as previously described [11]. Heatmaps were made in Morpheus (https://software.broadinstitute.org/morpheus/). Gene Set Enrichment Analysis (GSEA; http://www.broad.mit.edu/gsea/index.html) was performed using the difference of log-transformed count per million (cpm) for contrasted conditions as a ranking metric. The Molecular Signatures Database (MSigDB v7.2) was used as source of gene sets. Enriched pathway from DEGs and predicted upstream transcriptional factor were performed by IPA (Qiagen).

Statistics

Quantitative data were expressed as mean ± SEM. Unpaired two-tailed Student's t-test followed by Welch's correction was performed for two-group comparisons. One-way ANOVA followed by Bonferroni's multiple-comparisons test was used for multi-group comparisons. Statistical analyses were performed with Graphpad Prism 8 and 9.

Data Availability

The data that support the findings of this study are available from the corresponding author upon request. The RNA-seq data will be deposited on GEO.

Results

Lack of IL-13Rα1 in DKO females decreases ABC accumulation and autoantibody production.

DKO females exhibit an aberrant accumulation of ABCs in their spleens [11, 21]. Interestingly, CUT&RUN and RNA-seq analyses had revealed that, compared to follicular B cells (FoBs), ABCs exhibit a selective loss of repressive chromatin marks at the *IL13Rα1* locus [21] and can express IL-13Rα1 (Supp. Fig.1a). This finding was confirmed by crossing DKO females with IL13Rα1-GFP reporter mice (Supp. Fig.1b–c). To investigate whether signals transmitted via IL-13Rα1 could contribute to the exaggerated expansion of ABCs observed in DKO females, we generated DKOs lacking IL-13Rα1 (*Il13rα1*$^{-/-}$DKO). Lack of IL-13Rα1 in DKO females ameliorated their splenomegaly and resulted in significantly fewer ABCs as determined by either CD11c and T-bet or CD11c and CD11b co-staining (Fig. 1a and Supp. Fig.1d–e).

The robust expansion of germinal center (GC) B cells normally observed in DKO females was also decreased in *Il13rα1*$^{-/-}$DKO females (Fig. 1b). DKO females also accumulate both B220^{int}CD138^{+} plasmablasts (PB) and B220$^{-/lo}$CD138^{+} plasma cells (PC) [21]. Absence of IL-13Rα1 decreased the number of PBs but exerted less of an effect on the numbers of PCs (Fig. 1c). GC and PB/PC populations in DKO females contain CD11c^{+} populations, which are derived from ABCs and whose transcriptional profiles are

distinct from the corresponding $CD11c^-$ subsets [21]. Lack of IL-13Rα1 exerted greater effects on the $CD11c^+$ than the $CD11c^-$ GC populations (Fig. 1d). Similarly, when the PB/PC compartment was subdivided based on CD11c expression, absence of IL-13Rα1 preferentially decreased the CD11c-expressing population, which mainly represents PBs (Fig. 1e). Lack of IL-13Rα1 also resulted in fewer $B220^-CD11c^+CD11b^+$ cells (Suppl. Fig. 1f). These populations were, however, not expanded in DKOs in agreement with our previous findings that myeloid cells do not appear to be involved in promoting the humoral abnormalities in these mice [40]. Consistent with the decrease in ABCs and their effector progeny, the levels of anti-dsDNA IgG2c were lower in *Il13rα1*$^{-/-}$DKO than DKO female mice (Fig. 1f). Thus, lack of IL-13Rα1 decreases ABC formation and differentiation and ameliorates the aberrant humoral responses that characterize the spontaneous autoimmunity that develops in DKO females.

Given the ability of ABCs to act as potent antigen-presenting cells and promote aberrant T_{FH} differentiation [41, 42], we also investigated the effects of the lack of IL-13Rα1 on this compartment. Fewer T_{FH}, but not T_{FR} cells could be observed in *Il13rα1*$^{-/-}$DKO females resulting in a modest decrease in the T_{FH}/T_{FR} ratio (Supp. Fig. 1g). No alterations in the production of IL-21 or IFNγ could be observed (Supp. Fig. 1h–i). An evaluation of T_H2 cytokines revealed that IL-13 production was largely unaffected by the absence of IL-13Rα1 and that only low levels of IL-4 production could be detected (Supp. Fig. 1j). Thus, absence of IL-13Rα1 in lupus-prone DKO females exerts less marked effects on the T cell compartment than on ABCs and does not significantly affect the production of key cytokines known to regulate ABC differentiation.

IL-13Rα1 deficiency diminishes ABC expansion and humoral responses in *Yaa*-DKO males.

The Y-linked autoimmune accelerating (*Yaa*) locus accelerates autoimmunity in lupus models primarily due to the overexpression of TLR7. While DKO males are normally protected from the development of lupus, DKO males crossed to Yaa mice display increased accumulation, differentiation, and dissemination of ABCs [21]. The profound cellular abnormalities of Yaa-DKO males are associated with the production of a broader array of autoantibodies and worse immunopathogenesis than that observed not only in DKO males but also in DKO females [21]. To investigate whether lack of IL-13Rα1 could impact ABCs and disease in this more severe setting, we generated Yaa-DKO males deficient in IL-13Rα1 (*YaaIl13rα1*$^{-/Y}$DKO). *YaaIl13rα1*$^{-/Y}$DKO males exhibited smaller spleens and decreased numbers of ABCs as compared to age-matched Yaa-DKO males (Fig. 2a, Suppl. Fig. 2a–b). Dissemination of ABCs into the blood was also diminished by the lack of IL-13Rα1 (Supp. Fig. 2c). While lack of IL-13Rα1 in Yaa-DKO males did not alter the GC B cell compartment, it again resulted in a decrease in $CD11c^+$ PBs, which were affected to a greater extent than $CD11c^-$ PCs (Fig. 2b–e). T_{FH} numbers were only modestly affected by the absence of IL-13Rα1 and the T_{FH}/T_{FR} ratio remained unchanged (Supp. Fig. 2d). No differences in IL-21, IFNγ, IL-4, or IL-13 production could, furthermore, be observed (Supp. Fig. 2e–f). Thus, even in settings where severe lupus pathogenesis is driven by TLR7 overexpression, IL-13Rα1-mediated signaling contributes to the aberrancies that characterize the ABC compartment, and, in particular, to their accumulation and further differentiation.

In addition to αdsDNA-IgG2c, Yaa-DKO males produce markedly elevated levels of a broad array of autoantibodies, which include anti-SmRNP and antiphospholipid antibodies. We had previously observed a close correlation between ABC frequencies and the production of both anticardiolipin and anti-phosphatidylserine antibodies [21]. In further support of such a connection, the decrease in ABCs in *YaaIl13rα1*$^{-/Y}$DKOs was associated with decreased levels of αdsDNA-IgG2c as well as with lower levels of anticardiolipin and anti-PS antibodies (Fig. 2f). Production of αds-DNA IgG1 and anti-SmRNP was instead not significantly affected (Fig. 2f). Although Yaa-DKO males lacking IL-13Rα1 exhibited enhanced survival compared to Yaa-DKO males (Supp. Fig. 2g), they did eventually succumb to disease and a histopathologic analysis demonstrated that *YaaIl13Rα1*$^{-/Y}$DKO males develop both renal and pulmonary inflammation although this occured at an older age than in Yaa-DKO males (Supp. Fig. 2h–n). Thus, lack of IL-13Rα1 in this severe lupus model broadly impacts the aberrant autoantibody production that characterizes these mice and delays the development of end-organ damage.

IL-13Rα1-mediated signaling regulates ABC formation *in vitro*.

The decrease in ABC accumulation observed in both *Il13Rα1*$^{-/-}$DKO females and *YaaIl13Rα1*$^{-/Y}$DKO males suggested that IL-13Rα1-mediated signaling directly regulates the generation of ABCs. To further investigate this possibility, we employed an established *in vitro* system whereby CD23^{+} B cells purified from young (6–10 wks old) WT, DKO females, or *Il13rα1*$^{-/-}$DKO females were cultured with αIgM and αCD40 in the presence/absence of different combinations of IL-21, IL-4, and IL-13 (Fig. 3a). As we previously reported [11], IL-21 led to greater ABC formation in cultures of DKO B cells than wt B cells as assessed by either CD11c and T-bet or CD11c and CD11b co-staining (Fig. 3a–b and Supp. Fig 3a–b). Surprisingly, *Il13rα1*$^{-/-}$DKO B cells cultured with IL-21 alone generated significantly fewer ABCs than DKO B cells (Fig. 3a–b and Supp. Fig 3a–b). Lack of IL-13Rα1 also resulted in lower surface expression level of CD11c in response to IL-21 (Fig. 3c). No differences in the inhibitory effects of IL-4 on IL-21-mediated ABC generation were observed while IL-13 did not significantly inhibit the IL-21-driven ABC formation (Fig. 3a–b and Supp. Fig. 3a–b). Exposure to IL-13 alone in the absence of IL-21 did not promote ABC generation (Supp. Fig. 3c). IL-13Rα1 deficiency did not affect the expression of IL-21R or diminish B cell proliferation *in vitro* or *in vivo* suggesting that the inhibitory effects of IL-13Rα1 deficiency on ABC generation did not simply result from alterations in the proliferative capabilities of these B cells (Supp. Fig. 3d–g). Thus, deficiency of IL-13Rα1 lessens the aberrant formation of ABCs by DKO B cells upon stimulation with IL-21, an effect that can be observed even in the absence of IL-4 or IL-13.

Given that expression of IL-13Rα1 affected B cell responsiveness to IL-21, we examined STAT phosphorylation to gain insights into the mechanisms by which IL-13Rα1 could impact IL-21 signaling in B cells (Fig. 3d). Interestingly, low levels of Stat6 phosphorylation could be detected upon IL-21 stimulation of WT and DKO B cells but not of *Il13rα1*$^{-/-}$DKO B cells. In contrast, IL-21-mediated Stat3 phosphorylation occurred to a similar extent in B cells from all three genotypes. IL-4 induced comparable levels of Stat6 phosphorylation in WT, DKO, and *Il13rα1*$^{-/-}$DKO B cells while the phosphorylation of Stat6 triggered by IL-13 was completely dependent on the expression of IL-13Rα1 (Fig.

3d). Absence of IL-13Rα1 did not alter the phosphorylation of Stat1 or Stat5 in response to IL-21 stimulation (Suppl. Fig. 3h). IRF4 was furthermore induced at similar levels under all conditions indicating that the lack of IL-13Rα1 did not affect the activation of the cells (Suppl. Fig. 3h). No production of IL-4 or IL-13 was detected in B cell cultures supplemented with IL-21 alone suggesting that Stat6 phosphorylation in these cultures was unlikely to be due to endogenous production of these cytokines (Supp. Fig. 3i–j).

DKO ABCs lacking IL-13Rα1 exhibit enhanced myeloid and proinflammatory features.

To gain further insight into the mechanisms by which IL-13Rα1 regulates ABCs, we sorted ABCs from the spleen of DKO and *Il13ra1*$^{-/-}$DKO females and performed RNA-based next-generation sequencing (RNA-seq) to compare the transcriptomes of DKO ABCs and *Il13ra1*$^{-/-}$DKO ABCs. A total of 789 genes were differentially expressed by the two populations (a change in expression of two-fold (one-fold log value); false-discovery rate (FDR), < 0.05) (Fig. 4a). Lack of IL-13Rα1 did not affect the expression of γc chain, IL-21R, or Stat6 and only minimally decreased IL-4Rα (Supp. Fig. 4a). On the basis of Ingenuity Pathway Analysis (IPA), gene sets that show the greatest enrichment in *Il13ra1*$^{-/-}$DKO ABCs were related to inflammatory responses, cytokine signaling, and phagocytosis and phagocytic markers such as *Mertk* and *Fcgr1* were upregulated in *Il13ra1*$^{-/-}$DKO ABCs compared with DKO ABCs (Fig. 4b, Supp. Fig. 4a). In contrast, pathways involved in BCR signaling were downregulated in DKOs in the absence of IL-13Rα1 (Fig. 4c). In line with these findings the predicted upstream transcription factors regulating the DEGs upregulated in *Il13ra1*$^{-/-}$DKO ABCs by IPA included FoxM1 and MITF while the top upstream TF downregulated in the absence of IL-13Rα1 included EBF1 and XBP1 (Fig. 4d–e). We further analyzed enriched pathways, which were either upregulated or downregulated in *Il13ra1*$^{-/-}$DKO ABCs by Gene-Set-Enrichment-Analyses (GSEA), (Supp. Fig. 4b–c). Lack of IL-13Rα1 in ABCs again resulted in the enrichment of pathways involved in inflammation, cytokine signaling, and cytoskeletal processes. In particular, these cells upregulated a number of proinflammatory mediators that included IL-1β, IL-18, CCL22, CCL5, and mediators of cell-cell and cell-matrix interactions (Fig. 4f–g). Consistent with the RNA-seq results, lack of IL-13Rα1 increased expression of IL-1β and CCL22 upon *in vitro* stimulation with IL-21 and resulted in higher serum levels of these mediators (Suppl. Fig. 4d–g). ABCs from DKOs lacking IL-13Rα1 furthermore exhibited enhanced efferocytic capabilities as compared to DKO ABCs (Suppl. Fig. 4h). Thus, lack of IL-13Rα1 on ABCs downregulates some of their B cell characteristics but enhances their myeloid and proinflammatory features.

Discussion

ABCs are an emerging B cell subset whose aberrant accumulation has been increasingly linked to SLE pathogenesis [7, 8]. Here, we identify a novel role for the X-linked IL-13Rα1 receptor in promoting ABC expansion and differentiation. In line with a major contribution of ABCs to the production of autoantibodies, diminished expansion of ABCs upon ablation of IL-13rα1 in the DKO lupus model was accompanied by decreases in autoantibody production and improved survival. Consistent with our recent studies showing that ABCs can give rise to a heterogenous pool of effector progeny [21], we also observed decreased

accumulation of $CD11c^+$ effectors that include pre-GC B cells and plasmablasts. While the expansion of $CD11c^-$ GC B and $CD11c^-$ PCs was less affected by the absence of IL-13Rα1, we cannot rule out that expression of IL-13Rα1 may impact the functional capabilities of the $CD11c^-$ progeny. Thus, the decreased autoantibody production observed in the absence of IL-13Rα1 may result from multiple effects on ABC formation and differentiation. Interestingly, the remaining ABCs generated in the absence of IL-13Rα1 upregulated several myeloid features including enhanced expression of phagocytic receptors and inflammatory mediators. The shift of ABCs lacking IL-13Rα1 toward a more proinflammatory phenotype likely limited the beneficial effects of the absence of this receptor on humoral responses and, albeit a delay, the mice eventually developed end-organ inflammation. These studies thus uncover an important role for IL-13Rα1 in modulating the plasticity and functional capabilities of ABCs.

The *in vivo* effects of the absence of IL-13Rα1 were coupled with diminished formation of ABCs *in vitro* supporting the idea that IL-13Rα1 controls ABC generation in a cell-intrinsic manner. Surprisingly, the *in vitro* defects in ABC formation imparted by the lack of IL-13Rα1 were detected upon stimulation of B cells with IL-21 alone and were associated with differences in the activation of Stat6 but not of other Stats suggesting that IL-13Rα1 helps modulate only a subset of IL-21-mediated signaling events. Given that the IL-21R and IL-4Rα are neighboring genes and share a high degree of homology [23, 24], these findings raise the possibility that, similarly to IL-4, IL-21 may signal not only via the IL-21R and γc chain but also via an alternative receptor complex that involves the IL-21R and IL-13Rα1 chain. Alternatively, the effect may be indirect and the pathways may intersect with each other further downstream of each receptor signaling. Since the IL-13Rα1 receptor is normally expressed by myeloid cells but not by B cells [25, 26], upregulation of this receptor chain by ABCs may thus enable them to respond to IL-21 in a manner distinct from other B cells and represents another example of the unique combination of myeloid and B cell features displayed by ABCs.

Signaling mediated by the IL-13Rα1 chain in response to IL-4 or IL-13 is known to be controlled by a complex interplay dictated by ligand levels and affinity, its relative abundance as compared to the IL-4Rα and γc chains, and actin-dependent recruitment dynamics [25, 26]. While the involvement of IL-13Rα1 in IL-21 signaling would add additional complexity to the system, the stepwise production of IL-21 and IL-4 that normally accompanies the differentiation of T_{FH} cells as the GC response evolves [43] could help restrict the contribution of IL-13Rα1 to IL-21 or IL-4 signaling events. Such spatial organization could also help limit the known inhibitory actions of IL-4 on IL-21 driven ABC differentiation. In line with this notion, the greater variability in autoantibody production observed upon deleting the IL-13Rα1 chain in Yaa-DKO males than in DKO females could be linked to the greater degree of splenic disorganization observed in these mice as well as to their increased ability to produce IL-4 [21]. The finding that, in contrast to IL-4, addition of IL-13 did not affect the IL-21-driven formation of ABCs *in vitro* suggests that the inhibitory effects of IL-4 on ABCs are mediated by IL-4Rα/γc chain complexes and that binding of IL-13 to IL13Rα2, the high affinity receptor that may serve as a decoy receptor but also signals, has also no role and implies that production of IL-13, for instance by the newly recognized $T_{FH}13$ cells [44], may not impact the generation of ABCs.

IL-13Rα1 is one of the genes on the X-chromosome, which can partially escape XCI together with TLR7 and TASL/CXorf21, an adaptor controlling the TLR-mediated activation of IRF5 [45–47]. Both TLR7 engagement and IRF5 play crucial roles in promoting ABC generation [9, 11, 21]. raising the intriguing possibility that ABC formation relies on the coordinated employment of multiple X-linked components, which can then promote the preferential expansion of this compartment in females and contribute to the profound sex-bias that accompanies SLE. Given the emerging evidence that proper regulation of TLR7 engagement and ABCs may be important in determining the severity of SARS-CoV2 infections, such pathways may also be relevant to the sex-differences that have emerged in responses to this virus [48].

While the lack of IL13Rα1 exerted pronounced inhibitory effects on the generation and differentiation of ABCs and a subsequent decrease in autoantibody production, the ABCs generated in the absence of IL-13Rα1 expressed a transcriptional profile enriched for inflammatory targets. This phenotype was furthermore coupled with the upregulation of several myeloid markers suggesting that the remaining ABCs despite downregulating their humoral capabilities can acquire more robust myeloid-like proinflammatory functions and potentially mediate a distinct set of pathogenic effects and/or complications. Indeed, the lack of IL-13Rα1 resulted in increased expression of IL-1β and CCL22 *in vitro* as well as *in vivo*. The capability of IL-13Rα1-deficient ABCs to acquire this alternative fate may limit the beneficial effects that the absence of IL-13Rα1 exerts on autoantibody responses and contribute to the renal and pulmonary inflammation that eventually develops in these mice. Whether ABCs that express these proinflammatory capabilities can be enriched in subsets of SLE patients will be an important question to be investigated.

Although our studies primarily focused on the role of IL-13Rα1 in lupus pathogenesis, the expression of IL-13Rα1 has been shown to be upregulated in PBMCs of patients with other autoimmune conditions such as scleroderma where expansion of ABCs (also known as $CD21^{low}$) has recently been recognized [49]. Thus, these studies may be relevant to autoimmune disorders in addition to SLE. The ability of IL-13Rα1 to regulate ABCs and lupus pathogenesis could furthermore have important therapeutic implications given that IL-13Rα1 neutralizing monoclonal antibodies have been recently developed [50]. Although the efficacy of targeting this receptor by itself could be limited by the emergence of proinflammatory effects, combination therapy could potentially obviate such shortcomings.

Supplementary Material

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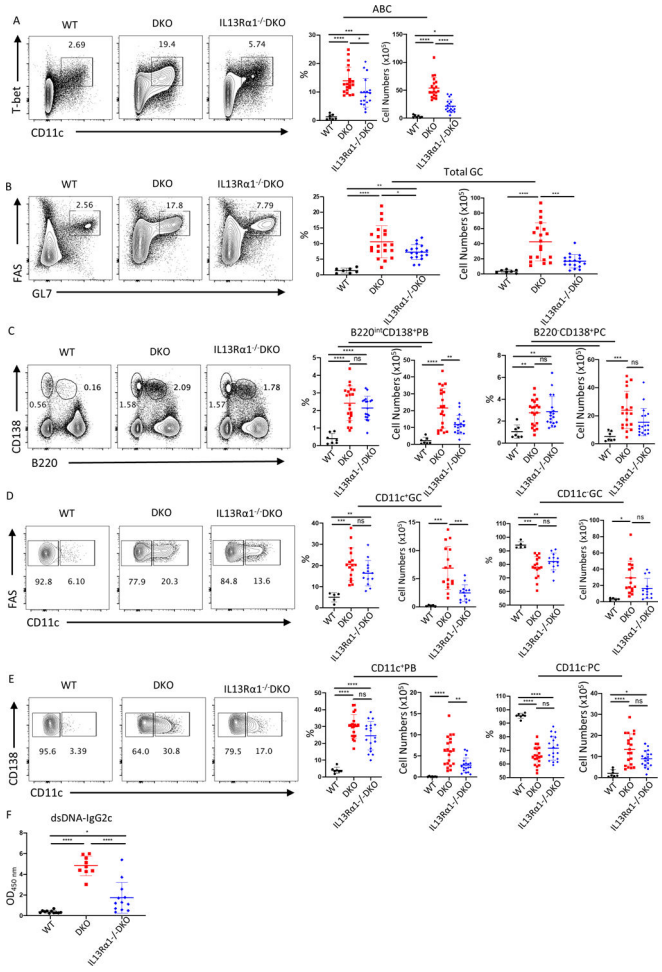

Figure 1. Lack of IL-13Rα1 decreases ABC accumulation and autoantibody production in DKO females.

Representative FACS plots and quantifications of **a**, $CD11c^{+}Tbet^{+}$ ABCs (gated on $B220^{+}$) from the spleens of aged (24 wk) WT, DKO, and *Il13ra1*$^{-/-}$DKO female mice. b, total GC B cells (gated on $B220^{+}$ $GL7^{+}Fas^{+}$ splenocytes). c, total PB/PC ($B220^{mid/lo}CD138^{+}$). d, $CD11c^{+}GL7^{+}Fas^{+}$ cells (gated on $B220^{+}$ splenocytes). e, $CD11c^{+}$ PB/PC ($B220^{mid/lo}CD138^{+}$). f, anti-dsDNA IgG2c levels in the serum as assessed by ELISA. Data show 7–20 mice that represent the total number of mice per group pooled from seven independent experiments (sample size derived from previous studies [21]) (a, b, c, e); 5–16 mice that represent the total number of mice per group pooled from five independent experiments (d); 9–12 mice that represent the total number of mice per group pooled from three independent experiments (f). All data show mean +/− SEM. **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001 (one-way ANOVA followed by Bonferroni's multiple-comparisons test).

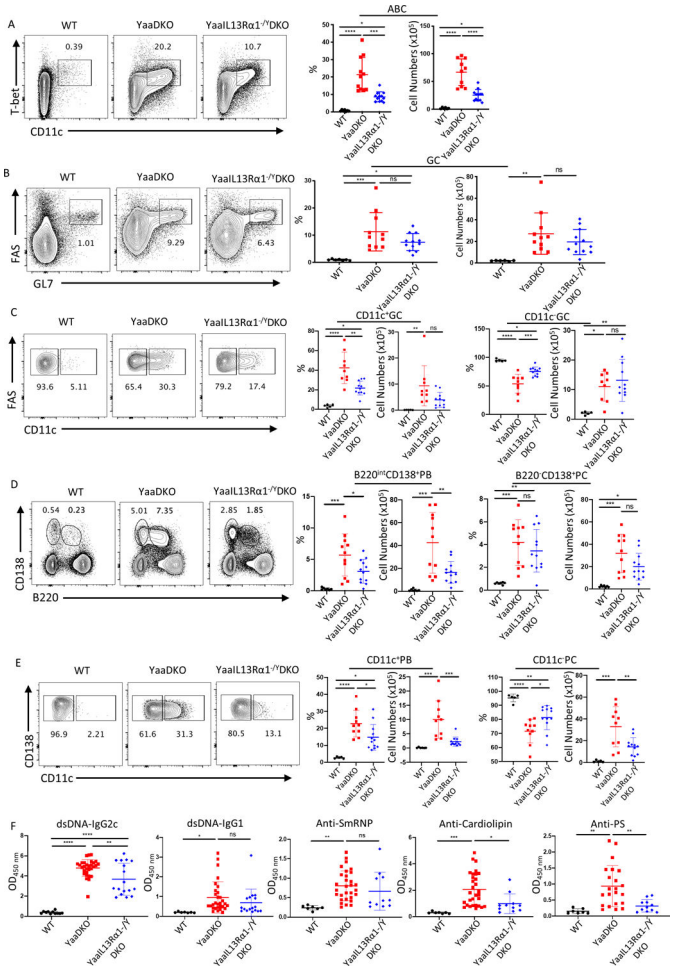

Figure 2. IL-13Rα1 deficiency diminishes TLR7-driven ABC expansion and humoral responses in *Yaa*-DKO male mice.

a, Representative FACS plots and quantifications of splenic CD11c^{+}Tbet^{+} ABCs (gated on B220^{+}) of WT, *Yaa*DKO and *YaaIl13rα1*$^{-/Y}$DKO males (>20 wk). b, Representative FACS plots and quantifications of total GC B cells (gated on B220^{+} GL7^{+}Fas^{+} splenocytes) from WT, *Yaa*DKO and *YaaIl13rα1*$^{-/Y}$DKO males (>20 wk). c, Representative FACS plots and quantifications of CD11c^{+}GL7^{+}Fas^{+} cells (gated on B220^{+} splenocytes). d, Representative FACS plots and quantifications of total splenic PB/PC (B220$^{mid/lo}$CD138^{+}). e, Representative FACS plots and quantifications of CD11c^{+} PB/PC (B220$^{mid/lo}$CD138^{+}). f, Anti-dsDNA IgG2c, anti-dsDNA IgG1, anti-SmRNP, anticardiolipin IgG, and anti-phosphatidylserine (PS) IgG serum levels assessed by ELISA. Data show 7–12 mice that represent the total number of mice per group pooled from seven independent experiments (a, b, d); 5–11 mice that represent the total number of mice per group pooled from five independent experiments (c); 5–12 mice that represent the total number of mice per group pooled from five independent experiments (e); 7–30 mice that represent the total number of mice per group pooled from three independent experiments (f.) All data show mean +/− SEM; **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001 (one-way ANOVA followed by Bonferroni's multiple-comparisons test).

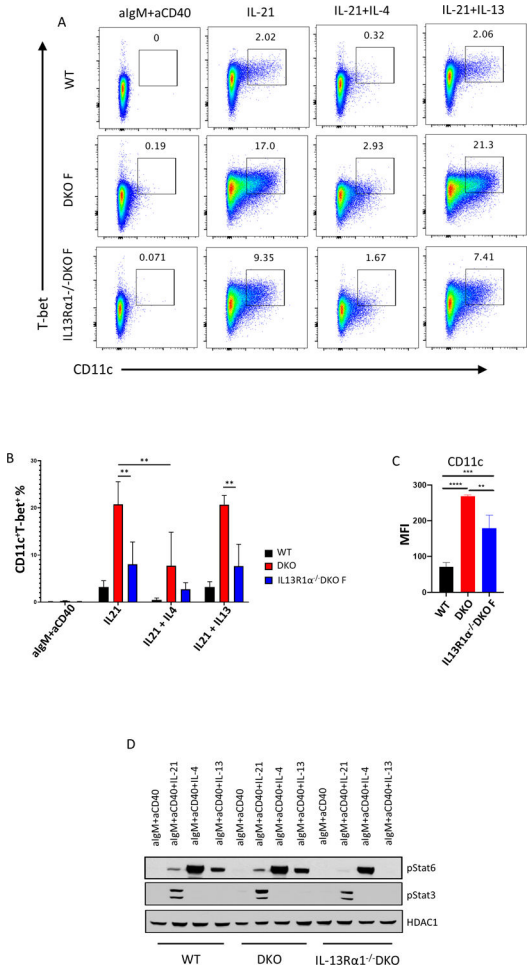

Figure 3. IL-13Rα1-mediated signaling regulates ABC formation *in vitro*.

a, Representative FACS plot of CD11c and T-bet expression by $CD23^+$ B cells purified from WT, DKO and *Il13rα1*$^{-/-}$DKO female mice (6–10 weeks) stimulated for 3 d with anti-IgM (5μg/ml) and anti-CD40 (5μg/ml) alone or together with IL-21 (50ng/ml), IL-4 (10ng/ml), and IL-13 (20ng/ml). Numbers in quadrants indicate percent cells in each. b, Quantification of $CD11c^+T\text{-}bet^+$ ABCs in the cultures described in a. c, Quantification of CD11c expression in $CD23^+$ B cells stimulated with anti-IgM, anti-CD40, and IL-21 described in a. Data show 6–8 mice that represent the total number of mice per group pooled from three independent experiments (a-c). $^{**}P<0.01$, $^{***}P<0.001$, $^{****}P<0.0001$ (one-way ANOVA followed by Bonferroni's multiple-comparisons test). d, Western blot of phosphorylated Stat6 (pStat6) and phosphorylated Stat3 (pStat3) from nuclear extracts of cells stimulated with anti-IgM (5μg/ml) and anti-CD40 (5μg/ml) alone or together with IL-21 (50ng/ml), IL-4 (10ng/ml), and IL-13 (20ng/ml) as described in a. Reprobing with HDAC1 was used as a loading control. Data are representative of two independent experiments.

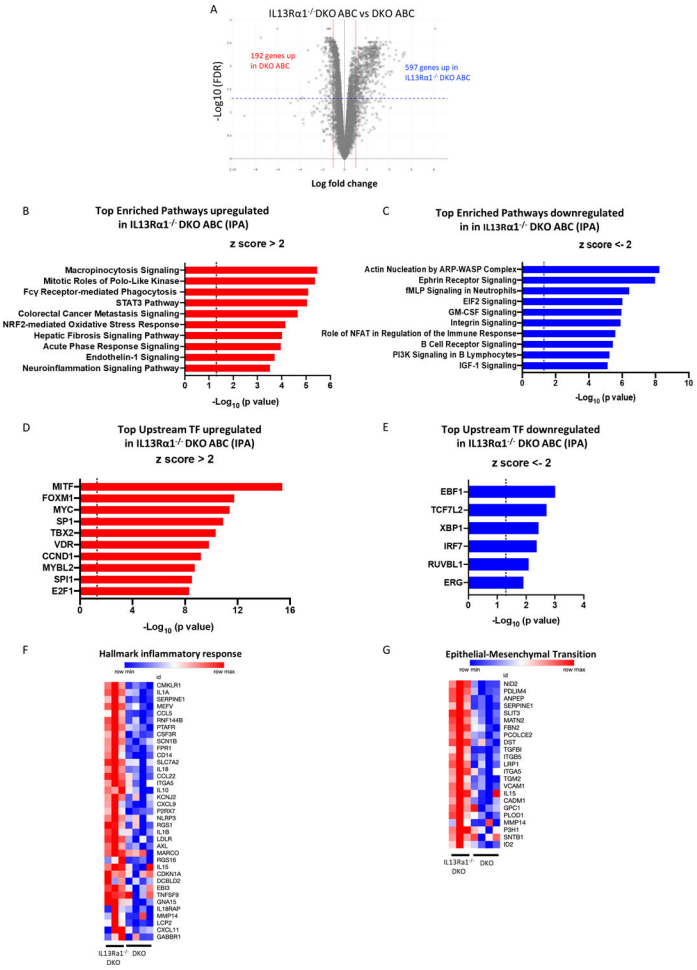

Figure 4. DKO ABCs lacking IL-13Rα1 exhibit enhanced myeloid and proinflammatory features.

a-g, RNA-seq analysis was performed on RNA from ABCs ($B220^+CD19^+CD11c^+CD11b^+$) sorted by flow cytometry from DKO (n=5) and *Il13rα1*$^{-/-}$DKO female mice (n=3) (>24 wk). a, Volcano plot showing differentially expressed genes (change in expression of over one-fold) in cells from DKO female mice relative to their expression in cells from *Il13rα1*$^{-/-}$DKO female mice, plotted against FDR-corrected *P* value ($P < 0.05$; dashed horizontal line indicates cutoff of $P = 0.05$). b-c, Plot showing the top pathways upregulated (b) or downregulated (c) in *Il13rα1*$^{-/-}$DKO ABCs by IPA. Dotted line indicates significance threshold at FDR q <0.25. Data were presented as z scores of expression by k means clustering of log transformed expression (counts per million) of genes expressed differentially by these cells.d-e, Top upstream transcription factors upregulated (d) or downregulated (e) in *Il13rα1*$^{-/-}$DKO ABCs as predicted by IPA. f, Heatmap shoring differentially expressed genes in Hallmark Inflammatory Response pathway. g, Heatmap showing differentially expressed genes in Hallmark Epithelial Mesenchymal Transition.