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CONCISE REPORT

Exacerbated inflammatory arthritis in response to hyperactive gp130 signalling is independent of IL-17A

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ABSTRACT

Objective Interleukin (IL)-17A producing CD4 T-cells (T_H-17 cells) are implicated in rheumatoid arthritis (RA). IL-6/STAT3 signalling drives T_H-17 cell differentiation, and hyperactive gp130/STAT3 signalling in the *gp130*$^{F/F}$ mouse promotes exacerbated pathology. Conversely, STAT1-activating cytokines (eg, IL-27, IFN-γ) inhibit T_H-17 commitment. Here, we evaluate the impact of STAT1 ablation on T_H-17 cells during experimental arthritis and relate this to IL-17A-associated pathology.

Methods Antigen-induced arthritis (AIA) was established in wild type (WT), *gp130*$^{F/F}$ mice displaying hyperactive gp130-mediated STAT signalling and the compound mutants *gp130*$^{F/F}$*:Stat1*$^{-/-}$ and *gp130*$^{F/F}$*:Il17a*$^{-/-}$ mice. Joint pathology and associated peripheral T_H-17 responses were compared.

Results Augmented gp130/STAT3 signalling enhanced T_H-17 commitment in vitro and exacerbated joint pathology. Ablation of STAT1 in *gp130*$^{F/F}$ mice (*gp130*$^{F/F}$*:Stat1*$^{-/-}$) promoted the hyperexpansion of T_H-17 cells in vitro and in vivo during AIA. Despite this heightened peripheral T_H-17 cell response, disease severity and the number of joint-infiltrating T-cells were comparable with that of WT mice. Thus, gp130-mediated STAT1 activity within the inflamed synovium controls T-cell trafficking and retention. To determine the contribution of IL-17A, we generated *gp130*$^{F/F}$:IL-17a$^{-/-}$ mice. Here, loss of IL-17A had no impact on arthritis severity.

Conclusions Exacerbated gp130/STAT-driven disease in AIA is associated with an increase in joint infiltrating T-cells but synovial pathology is IL-17A independent.

INTRODUCTION

Interleukin (IL)-17A is increasingly linked with chronic disease progression, and several targeted therapies against IL-17A are in clinical development.[1–5] IL-17A-producing CD4 T-cells (T_H-17 cells) are widely acknowledged as pathogenic in many diseases, including rheumatoid arthritis (RA).[1 6] Here, IL-17A production by T-cells contributes to synovial inflammation through regulation of proinflammatory cytokines and chemokines (IL-1β, tumour necrosis factor (TNF)-α, IL-6, granulocyte/macrophage-colony stimulating factor (GM-CSF), receptor activator of nuclear factor-kappa-B ligand (RANKL), CC-chemokine ligand 20 (CCL20)), and the control of matrix metalloproteinases and osteoclastogenic processes.[6–9] Consequently, in experimental arthritis, IL-17A deficiency or blockade of IL-17A signalling reduces inflammation-associated joint pathology.[10]

While cytokines including transforming growth factor-β (TGF-β), IL-6, IL-21 and IL-23[1] promote T_H-17 effector functions murine T_H-17 differentiation is dependent on TGF-β and IL-6.[11] IL-6 stimulates cells through a non-signalling IL-6R α-chain and gp130, which activates signal transducer and activator of transcription 1 (STAT1) and STAT3, and represents the signalling β-receptor for IL-6-related cytokines.[12] Mice displaying enhanced gp130-mediated STAT1 and STAT3 signalling, as a consequence of a phenylalanine (F) knock-in substitution of the cytoplasmic tyrosine (Y)$_{757}$ residue in gp130 (*gp130*$^{F/F}$ mice) show exacerbated joint pathology in experimental arthritis.[13] Here, disease was linked to gp130-driven STAT3 and was associated with increased synovial T-cell production of IL-17A.[13] However, the role of gp130-mediated STAT1 signalling during inflammatory arthritis is ill defined. STAT1 activity often counteracts STAT3 transactivation, and recent data highlight an inhibitory role in T_H-17 differentiation.[14] Here, we investigate STAT1 control of T_H-17 responses during experimental arthritis and determine the role of gp130-regulated IL-17A in arthritis pathology.

METHODS

Mice

The generation of *gp130*$^{F/F}$ and *gp130*$^{F/F}$ compound mutant mice homozygous null for *Stat1* (*gp130*$^{F/F}$*:Stat1*$^{-/-}$) or *Il17a* (*gp130*$^{F/F}$*:Il17a*$^{-/-}$) and heterozygous for the *Stat3* (*gp130*$^{F/F}$*:Stat3*$^{+/-}$) genes have been described previously.[15 16] Mice were bred and maintained under specified pathogen-free conditions.

T-cell cultures

Splenic T-cells were cultured in RPMI-1640 supplemented with 10% (v/v) foetal calf serum (FCS), 2 mM L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, 1 mM sodium pyruvate and 50 μM 2-mercaptoethanol (all from Life Technologies). A total of 1×10^5 cells/well were cultured in 96-well plates, and T-cells activated by plate-bound anti-CD3 (1 μg/mL; 45-2C11; R&D Systems) and soluble anti-CD28 (5 μg/mL; 37.51; BD Biosciences). Cultures were supplemented with TGF-β (1 ng/mL; R&D Systems) and IL-6 (10 ng/mL; R&D Systems) and incubated at 37°C for 4 days before evaluation

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of T_H-1 and T_H-17 polarisation by flow cytometry (see online supplementary methods).

Antigen-induced arthritis

Experiments were performed on 8–12-week-old mice in accordance with UK Home Office Project License PPL-30/2361. Antigen-induced arthritis (AIA) was induced as previously described and disease severity determined by histological assessment of knee-joint sections.[13] See online supplementary methods for further details.

Statistics

Disease activity was statistically evaluated using the non-parametric Mann–Whitney U test. Otherwise, differences were determined using an unpaired Student t test. In all cases, $p<0.05$ was considered significant.

RESULTS

T-cells from *gp130*$^{F/F}$ mice lacking STAT1 exhibit hyperexpansion of T_H-17 cells

We have previously shown that *gp130*$^{F/F}$ mice display exacerbated histopathology in experimental arthritis, as a consequence of elevated STAT3 signalling.[13] In this respect, the severity of joint pathology was associated with increased infiltration of synovial IL-17A-producing T-cells.[13] Enhanced gp130-mediated STAT3 activity promotes T_H-17 differentiation in vitro.[16] However, STAT1 activating cytokines (eg, IFN-γ and IL-27) inhibit T_H-17 differentiation, and are protective in experimental arthritis.[14 17 18] Thus, a balance between gp130-mediated STAT1 and STAT3 signalling would be predicted to influence the course of disease. To test this, we first considered the impact of STAT1 deletion on T_H-17 development in T-cell cultures from *gp130*$^{F/F}$:*Stat1*$^{-/-}$ compound mice (figure 1). Compared with wild type (WT) controls, T-cells from *gp130*$^{F/F}$ mice showed more than a twofold increase in the proportion of CD4 IL-17A$^+$ T-cells when cultured under T_H-17 polarising conditions (figure 1A,B). This response was STAT3 dependent as the proportion of CD4 IL-17A$^+$ T-cells from *gp130*$^{F/F}$:*Stat3*$^{+/-}$ mice were significantly reduced and T_H-17 expansion was comparable with that seen in WT mice (figure 1A,B). Conversely, a loss of STAT1 signalling in *gp130*$^{F/F}$:*Stat1*$^{-/-}$ T-cell cultures caused a 'hyperexpansion' of T_H-17 cells (figure 1A,B), which was further reflected by the quantification of IL-17A in culture supernatants (figure 1C). While no differences were observed in the frequency of IFN-γ-producing T_H-1 cells between the genetic strains, the proportion of IFN-γ$^+$IL-17A$^+$ double producers was elevated in *gp130*$^{F/F}$:*Stat1*$^{-/-}$ T-cell cultures (see online supplementary figure S1). Thus, altered bioavailability of gp130-mediated STAT1 and STAT3 signalling dramatically skews T_H-17 commitment in vitro.

Increased T_H-17 responses in *gp130*$^{F/F}$:*Stat1*$^{-/-}$ mice do not enhance arthritis severity

To determine the in vivo consequence of STAT1 deletion in experimental arthritis, AIA was established in *gp130*$^{F/F}$:*Stat1*$^{-/-}$ mice (figure 2). On day 10 of arthritis induction, inguinal lymph

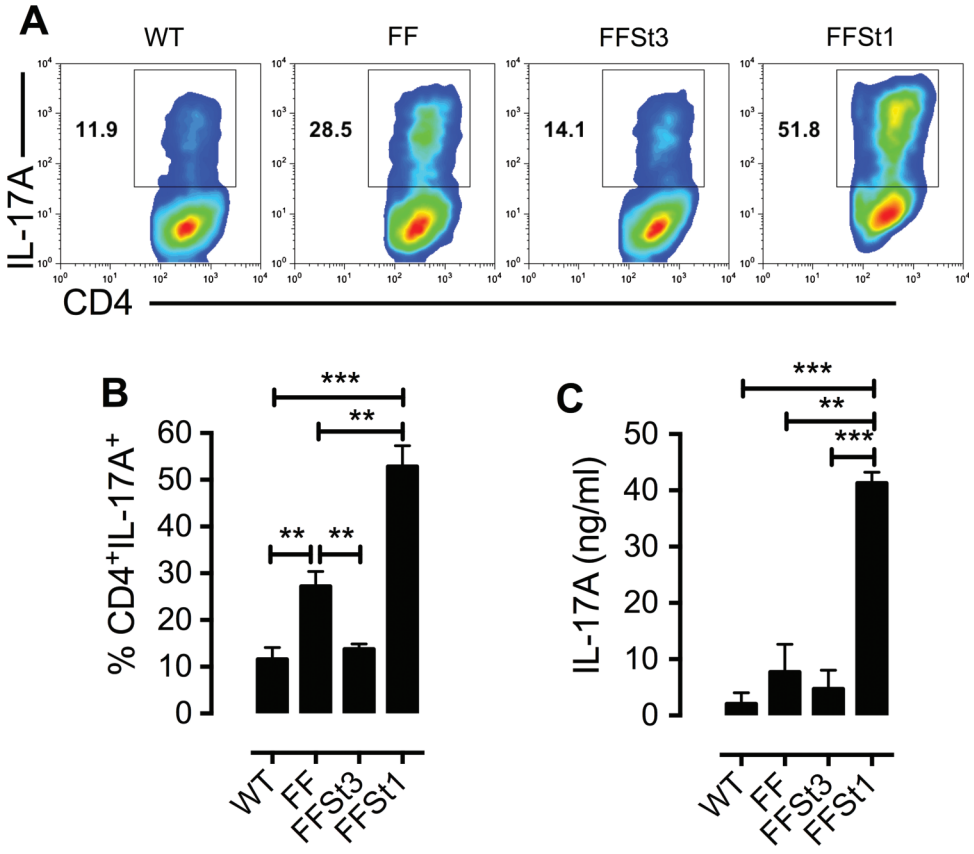

Figure 1 T-cells from *gp130*$^{F/F}$:*Stat1*$^{-/-}$ mice are hyper-responsive to T_H-17 polarisation. (A) Representative flow cytometric analysis of the development of IL-17A producing CD4 T-cells from (WT), *gp130*$^{F/F}$ (FF), *gp130*$^{F/F}$:*Stat3*$^{+/-}$ (FFSt3) and *gp130*$^{F/F}$:*Stat1*$^{-/-}$ (FFSt1) mice cultured under T_H-17 polarising conditions (TGF-β and IL-6). (B) Percentage of CD4 IL-17A$^+$-producing cells generated under T_H-17 polarising conditions for each mouse genotype (C) ELISA measurements of IL-17A protein concentrations in splenic T-cell culture supernatants. Data are presented as mean±SD of three independent experiments (**p<0.01, ***p<0.001). WT, wild type.

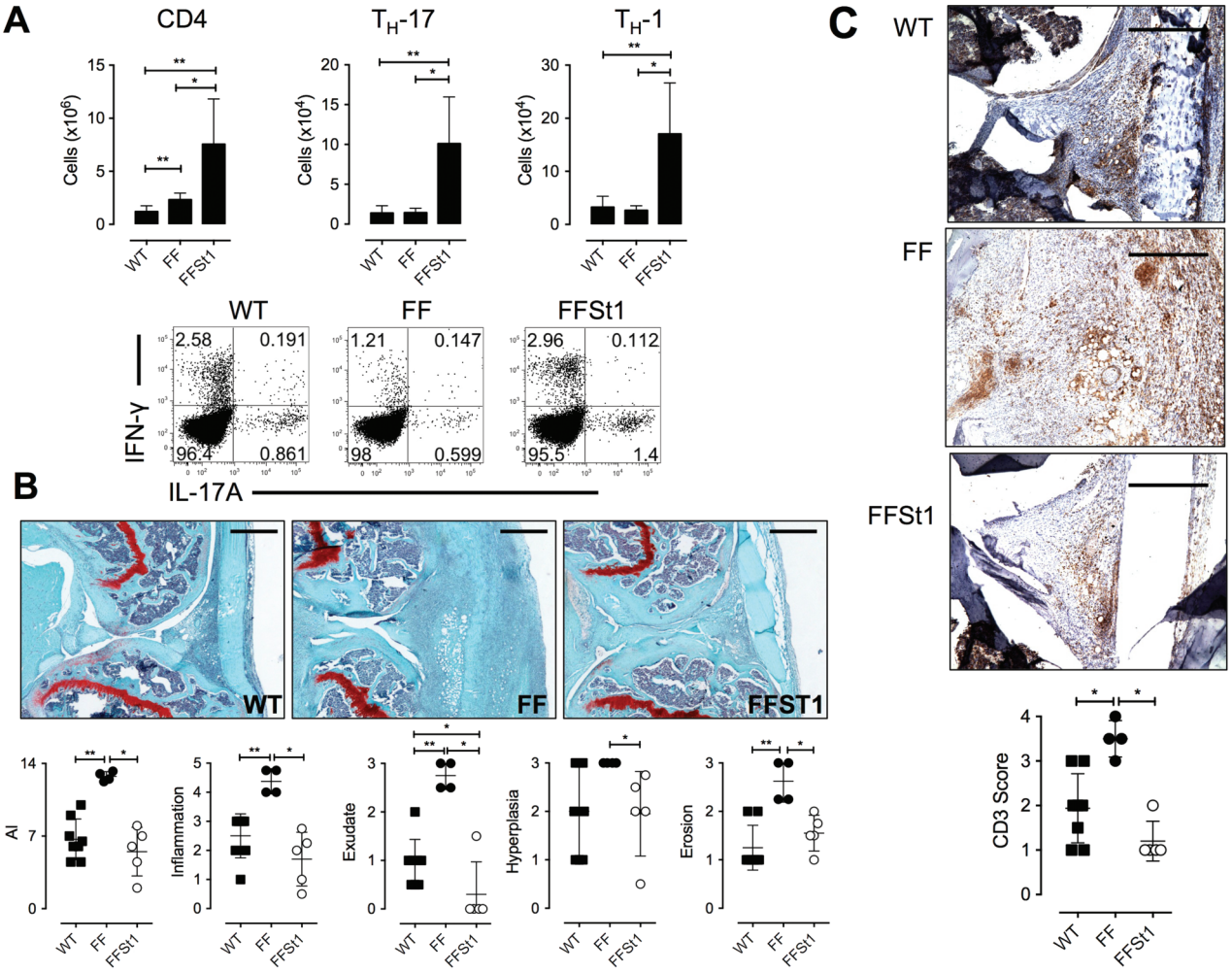

Figure 2 Heightened peripheral T_H-17 cell responses in *gp130*$^{F/F}$:*Stat1*$^{-/-}$ mice during antigen-induced arthritis (AIA) is not associated with exacerbated joint pathology. (A) AIA was established in wild type (WT), *gp130*$^{F/F}$ (FF) and *gp130*$^{F/F}$:*Stat1*$^{-/-}$ (FFSt1) mice and the number of peripheral CD4, T_H-17 and T_H-1 cells assessed by flow cytometry of inguinal lymph nodes at 10 days postarthritis induction. Representative dot plots indicating the percentage of IFN-γ (T_H-1) and IL-17A (T_H-17) producing T-helper cells are also shown. (B) Histological evaluation of joint pathology. Values are presented for individual joints taken at day 10 post AIA (*p<0.05, **p<0.01). Representative parasagittal knee-joint sections stained with haematoxylin, Safranin-O and Fast Green are shown for WT, FF and FFSt1 mice (scale bars, 500 μm). (C) Evaluation of synovial CD3 T-cell infiltration by immunohistochemistry in WT, FF and FFSt1 synovial tissue (scale bars 200 μm). Quantification of staining is also presented. Values represent mean±SD (n=8/4/5 for WT/FF/FFSt1 mice, respectively).

nodes were isolated and the number of T_H-17 cells compared with those observed in *gp130*$^{F/F}$ and WT mice (figure 2A). Here, *gp130*$^{F/F}$:*Stat1*$^{-/-}$ mice displayed a heightened peripheral T_H-17 response, reflecting our in vitro observations and supporting a role for STAT1 as a negative regulator of T_H-17 expansion in vivo. The increased peripheral response was not, however, limited to T_H-17 cells as *gp130*$^{F/F}$:*Stat1*$^{-/-}$ mice also displayed elevated total CD4 and T_H-1 cell numbers (figure 2A). While *gp130*$^{F/F}$:*Stat1*$^{-/-}$ mice displayed an increased expansion in absolute T_H-1 and T_H-17 cell numbers compared with WT and *gp130*$^{F/F}$ mice, the proportion of CD4 T-cells secreting IFN-γ and IL-17A was comparable between genotypes (figure 2A and see online supplementary table S1). This increase in peripheral T-cell commitment did not, however, equate to worse joint pathology during the T-cell prominent phase of the model (day-10). While *gp130*$^{F/F}$ mice displayed exacerbated disease, *gp130*$^{F/F}$:*Stat1*$^{-/-}$ mice showed attenuated histopathology and scores were comparable with WT mice (figure 2B). Also, immunohistochemistry (IHC) for synovial CD3 T-cells demonstrated a dramatic reduction of infiltrates in *gp130*$^{F/F}$:*Stat1*$^{-/-}$ mice compared with *gp130*$^{F/F}$ joints (IHC CD3 score of 1.2±0.4 compared with 3.5 ±0.4 respectively; figure 2C). Synovial STAT1 signalling therefore contributes to gp130-driven joint inflammation. These findings illustrate two contrasting STAT1 activities for the control of T-cell responses, where STAT1 negatively regulates peripheral T-cell expansion, but supports local effector cell recruitment.

IL-17A does not drive arthritis pathology in *gp130*$^{F/F}$ mice

We previously observed an association between joint infiltrating IL-17A producing T-cells and exacerbated AIA in *gp130*$^{F/F}$ mice.[13] While *gp130*$^{F/F}$:*Stat1*$^{-/-}$ mice displayed exaggerated peripheral T-cell responses, the failure to recruit these cells to the inflamed joint during AIA prevented us from determining the contribution of T_H-17 cells to local joint pathology. We therefore generated *gp130*$^{F/F}$:*Il17a*$^{-/-}$ compound mice to investigate the importance of the T_H-17 signature cytokine, IL-17A, in local joint pathology. Consistent with our previous data,[13] end-stage histopathology (day-28 & 35) was exacerbated in AIA challenged *gp130*$^{F/F}$ mice (see online supplementary table S2). However, comparison of *gp130*$^{F/F}$ and *gp130*$^{F/F}$:*Il17a*$^{-/-}$ mice

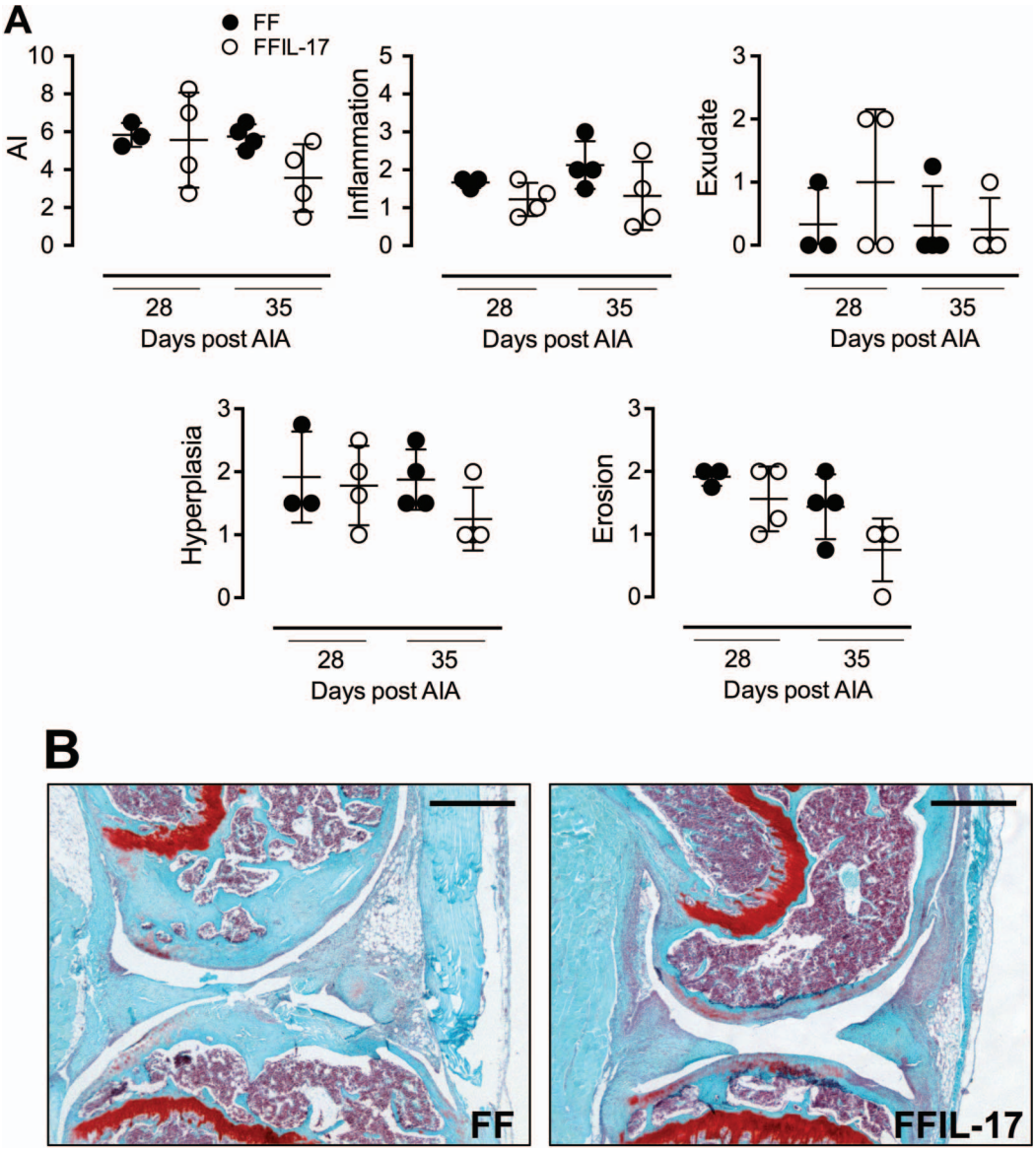

Figure 3 Antigen-induced arthritis (AIA) pathology in *gp130*$^{F/F}$ mice is independent of IL-17A. (A) Evaluation of arthritic index (AI), inflammation, exudate, hyperplasia and erosion scores in histological joint sections from *gp130*$^{F/F}$ (FF, closed circles) and *gp130*$^{F/F}$:*IL-17*$^{-/-}$ (FFIL-17, open circles) joint sections. Values are presented for individual joints taken at day 28 and day 35 post AIA. (C) Representative haematoxylin, Safranin-O and Fast Green stained parasagittal joint sections taken on day-28 for FF and FFIL-17 mice (scale bars, 200 μM). Graphs represent mean±SD.

showed no significant differences in arthritic index, inflammation, exudate, hyperplasia or erosion (figure 3A,B). Therefore, IL-17A has minimal impact in local joint pathology during inflammatory arthritis in *gp130*$^{F/F}$ mice.

DISCUSSION

While IL-17A and T_H-17 cells are associated with the progression of autoimmune diseases, IL-17A targeted therapies have delivered contrasting clinical outcomes. Inhibition of IL-17A in psoriasis is extremely promising,[4 5] but less favourable results have come from trials in rheumatoid and psoriatic arthritis.[19 20] Such varied clinical outcomes may reflect the nature of the underlining pathology and infer mechanistic differences in disease progression. To appreciate T_H-17/IL-17A involvement in inflammatory arthritis we used the gain-of-function *gp130*$^{F/F}$ knock-in mouse model, which display enhanced IL-6/gp130-mediated T_H-17 commitment, increased IL-17A expression and severe AIA pathology.[13] These responses are attributed to enhanced and prolonged gp130-driven STAT1 and STAT3 activation. Importantly, deregulated gp130/STAT3 signalling is associated with experimental models of autoimmunity and cancer. Here, polymorphisms in several IL-6/STAT3 target genes are considered risk factors for RA.[21] Critically, STAT1 often opposes the action of STAT3 (termed cross-regulation). Our results reinforce this, with STAT1 negatively regulating the STAT3 control of T_H-17 cells in vitro. Prior AIA experiments comparing *gp130*$^{F/F}$ with *gp130*$^{F/F}$:*Stat3*$^{+/-}$ mice show that a partial STAT3 deficiency ameliorates disease.[13] We therefore postulated that *gp130*$^{F/F}$:*Stat1*$^{-/-}$ mice would display severe joint pathology. Although *gp130*$^{F/F}$:*Stat1*$^{-/-}$ mice showed heightened peripheral effector T-cell characteristics, joint inflammation in *gp130*$^{F/F}$:*Stat1*$^{-/-}$ mice closely resembles that seen in *gp130*$^{F/F}$:*Stat3*$^{+/-}$ mice. Thus, STAT1/STAT3 cross-regulation appears to more prominently impact peripheral adaptive immunity.

Both STAT1 and STAT3 control chemokine-directed T-cell trafficking to inflamed tissue. STAT1 induces CXCR3 expression

on CD4 T-cells[22] and local expression of CXCR3 ligands CXC-chemokine ligand (CXCL)9, CXCL10 and CXCL11.[23 24] Similarly, gp130/STAT3 activity controls inflammatory chemokine expression and *IL-6*$^{-/-}$ mice show impaired T-cell infiltration and reduced T-cell CC-chemokine receptor (CCR)3, CCR5 and CXCR3 expression.[25] Here, STAT1 and STAT3 did not drive a selective trafficking of defined T-cell subsets, but instead regulated all T-cell recruitment.[25] We therefore generated *gp130*$^{F/F}$:*Il17a*$^{-/-}$ mice to investigate T_H-17-driven joint pathology in *gp130*$^{F/F}$ mice. Critically, IL-17A did not majorly contribute to the pathology seen in *gp130*$^{F/F}$ mice, and data were consistent with results from inflammation-associated gastric tumourigenesis in *gp130*$^{F/F}$ mice, where tumour progression was also independent of IL-17A.[16] While alternative effector T-cell subsets may contribute to gp130-mediated joint pathology in *gp130*$^{F/F}$:*Il17a*$^{-/-}$ mice, it is also possible that other T_H-17 effector cytokines (eg, IL-17F, GM-CSF) substitute for IL-17A.[1 26 27] Such findings may reflect recent trials in RA where secukinumab (anti-IL-17A mAb) failed to meet its clinical endpoint.[19 20] The clinical efficacy of a dual targeting strategy for IL-17A/IL-17F (eg, brodalumab - the anti-IL-17 receptor A mAb) remains to be determined. Loss of STAT1 or STAT3 activity had a profound effect on gp130-driven AIA, whereas loss of IL-17A had minimal impact on disease. Therefore, gp130/STAT signalling regulates T-cell responses through control of T-cell effector functions and may determine the severity of local synovial inflammation by driving T-cell trafficking.

In summary, our results illustrate that peripheral markers of inflammatory disease may not correlate with local pathology and can be an inadequate predictor of disease severity or local joint pathology. When reflecting on clinical blockade of IL-17A,[4 5 19 20] our findings may be relevant in determining the contrasting efficacy of drugs like secukinumab in psoriasis and RA.

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Competing interests None.

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