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 (T17-17 cells) are implicated in rheumatoid arthritis (RA). IL-6/STAT3 signalling drives T17-17 cell differentiation, and hyperactive gp130/STAT3 signalling in the gp130F/F mouse promotes exacerbated pathology. Conversely, STAT1-activating cytokines (eg, IL-27, IFN-γ) inhibit T17-17 commitment. Here, we evaluate the impact of STAT1 ablation on T17-17 cells during experimental arthritis and relate this to IL-17A-associated pathology.

Methods Antigen-induced arthritis (AIA) was established in wild type (WT), gp130F/F mice displaying hyperactive gp130-mediated STAT signalling and the compound mutants gp130F/F:Stat1−/− and gp130F/F:Il17a−/− mice. Joint pathology and associated peripheral T17-17 responses were compared.

Results Augmented gp130/STAT3 signalling enhanced T17-17 commitment in vitro and exacerbated joint pathology. Ablation of STAT1 in gp130F/F mice (gp130F/F:Stat1−/−) promoted the hyperexpansion of T17-17 cells in vitro and in vivo during AIA. Despite this heightened peripheral T17-17 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 gp130F/F:Il17a−/− 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.1–3 IL-17A-producing CD4 T-cells (T17-17 cells) are widely acknowledged as pathogenic in many diseases, including rheumatoid arthritis (RA).4–6 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 osteoelastic processes.7–9 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-2311 promote T17-17 effector functions murine T17-17 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 (gp130F/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 T17-17 differentiation.14 Here, we investigate STAT1 control of T17-17 responses during experimental arthritis and determine the role of gp130-regulated IL-17A in arthritis pathology.

METHODS

Mice

The generation of gp130F/F and gp130F/F compound mutant mice homozygous null for Stat1 (gp130F/F:Stat1−/−) or Il17a (gp130F/F:Il17a−/−) and heterozygous for the Stat3 (gp130F/F:Stat3+/−) genes have been described previously.15 16 Mice were bred and maintained under specified pathogen-free conditions.
of T<sub>H</sub>-1 and T<sub>H</sub>-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. 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<sup>F/F</sup> mice lacking STAT1 exhibit hyperexpansion of T<sub>H</sub>-17 cells**

We have previously shown that gp130<sup>F/F</sup> mice display exacerbated histopathology in experimental arthritis, as a consequence of elevated STAT3 signalling. In this respect, the severity of joint pathology was associated with increased infiltration of synovial IL-17A-producing T-cells. Enhanced gp130-mediated STAT3 activity promotes T<sub>H</sub>-17 differentiation in vitro. However, STAT1 activating cytokines (eg, IFN-γ and IL-27) inhibit TH-17 differentiation, and are protective in experimental arthritis. 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<sub>H</sub>-17 development in T-cell cultures from gp130<sup>F/F</sup>:Stat1<sup>−/−</sup> compound mice (figure 1). Compared with wild type (WT) controls, T-cells from gp130<sup>F/F</sup> mice showed more than a twofold increase in the proportion of CD4 IL-17A<sup>+</sup> T-cells when cultured under T<sub>H</sub>-17 polarising conditions (figure 1A,B). This response was STAT3 dependent as the proportion of CD4 IL-17A<sup>+</sup> T-cells from gp130<sup>F/F</sup>:Stat3<sup>+/−</sup> mice were significantly reduced and T<sub>H</sub>-17 expansion was comparable with that seen in WT mice (figure 1A,B). Conversely, a loss of STAT1 signalling in gp130<sup>F/F</sup>:Stat1<sup>−/−</sup> T-cell cultures caused a ‘hyperexpansion’ of T<sub>H</sub>-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<sub>H</sub>-1 cells between the genetic strains, the proportion of IFN-γ<sup>+</sup>IL-17A<sup>+</sup> double producers was elevated in gp130<sup>F/F</sup>:Stat1<sup>−/−</sup> T-cell cultures (see online supplementary figure S1). Thus, altered bioavailability of gp130-mediated STAT1 and STAT3 signalling dramatically skews T<sub>H</sub>-17 commitment in vitro.

**Increased T<sub>H</sub>-17 responses in gp130<sup>F/F</sup>:Stat1<sup>−/−</sup> mice do not enhance arthritis severity**

To determine the in vivo consequence of STAT1 deletion in experimental arthritis, AIA was established in gp130<sup>F/F</sup>:Stat1<sup>−/−</sup> mice (figure 2). On day 10 of arthritis induction, inguinal lymph
nodes were isolated and the number of TH-17 cells compared with those observed in gp130\(^{−/−}\) and WT mice (figure 2A). Here, gp130\(^{−/−}\):Stat1\(^{−/−}\) mice displayed a heightened peripheral TH-17 response, reflecting our in vitro observations and supporting a role for STAT1 as a negative regulator of TH-17 expansion in vivo. The increased peripheral response was not, however, limited to TH-17 cells as gp130\(^{−/−}\):Stat1\(^{−/−}\) mice also displayed elevated total CD4 and TH-1 cell numbers (figure 2A). While gp130\(^{−/−}\):Stat1\(^{−/−}\) mice displayed an increased expansion in absolute TH-17 and TH-1 cells compared with WT and gp130\(^{−/−}\) mice, the proportion of CD4 T-cells secreting IFN-\(\gamma\) 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\(^{−/−}\):Stat1\(^{−/−}\) mice displayed exacerbated disease, gp130\(^{−/−}\):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\(^{−/−}\):Stat1\(^{−/−}\) mice compared with gp130\(^{−/−}\) 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\(^{−/−}\) mice

We previously observed an association between joint infiltrating IL-17A producing T-cells and exacerbated AIA in gp130\(^{−/−}\) mice.\(^{13}\) While gp130\(^{−/−}\):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 TH-17 cells to local joint pathology. We therefore generated gp130\(^{−/−}\):Il17a\(^{−/−}\) compound mice to investigate the importance of the TH-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\(^{−/−}\) mice (see online supplementary table S2). However, comparison of gp130\(^{−/−}\) and gp130\(^{−/−}\):Il17a\(^{−/−}\) mice...
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\textsuperscript{F/F} mice.

**DISCUSSION**

While IL-17A and T\textsubscript{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\textsuperscript{4, 5} but less favourable results have come from trials in rheumatoid and psoriatic arthritis.\textsuperscript{19, 20} Such varied clinical outcomes may reflect the nature of the underlining pathology and infer mechanistic differences in disease progression. To appreciate T\textsubscript{H}17/IL-17A involvement in inflammatory arthritis we used the gain-of-function gp130\textsuperscript{F/F} knock-in mouse model, which display enhanced IL-6/gp130-mediated T\textsubscript{H}17 commitment, increased IL-17A expression and severe AIA pathology.\textsuperscript{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.\textsuperscript{21} Critically, STAT1 often opposes the action of STAT3 (termed cross-regulation). Our results reinforce this, with STAT1 negatively regulating the STAT3 control of TH-17 cells in vitro. Prior AIA experiments comparing gp130\textsuperscript{F/F} with gp130\textsuperscript{F/F}:Stat3\textsuperscript{+/−} mice show that a partial STAT3 deficiency ameliorates disease.\textsuperscript{13} We therefore postulated that gp130\textsuperscript{F/F}:Stat1\textsuperscript{−/−} mice would display severe joint pathology. Although gp130\textsuperscript{F/F}:Stat1\textsuperscript{−/−} mice showed heightened peripheral effector T-cell characteristics, joint inflammation in gp130\textsuperscript{F/F}:Stat1\textsuperscript{−/−} mice closely resembles that seen in gp130\textsuperscript{F/F}:Stat3\textsuperscript{−/−} 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 and local expression of CXCR3 ligands CXCL9, CXCL10 and CXCL11. Similarly, gp130/STAT3 activity controls inflammatory chemokine expression and IL-6−/− mice show impaired T-cell infiltration and reduced T-cell CC-chemokine receptor (CCR3, CCR5 and CXCR3) expression. Here, STAT1 and STAT3 did not drive a selective trafficking of defined T-cell subsets, but instead regulated all T-cell recruitment. We therefore generated gp130−/−Il17a−/− mice to investigate T+17-driven joint pathology in gp130−/− mice. Critically, IL-17A did not majorly contribute to the pathology seen in gp130−/− mice, and data were consistent with results from inflammation-associated gastric tumourigenesis in gp130−/− mice, where tumour progression was also independent of IL-17A. While alternative effector T-cell subsets may contribute to gp130-mediated joint pathology in gp130−/−Il17a−/− mice, it is also possible that other T+17 effector cytokines (e.g., IL-17F, GM-CSF) substitute for IL-17A.26 Such findings may reflect recent trials in RA where secukinumab (anti-IL-17A mAb) failed to meet its clinical end-point.29 The clinical efficacy of a dual targeting strategy for IL-17A/IL-17F (e.g., 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 indicate 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, 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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