CONCISE REPORT

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

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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 (T17 cells) are widely acknowledged as pathogenic in many diseases, including rheumatoid arthritis (RA).1,5 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. 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 promote T17 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 cell commitment. Here, we evaluate the impact of STAT1 ablation on T17 cell differentiation during experimental arthritis and relate this to IL-17A-associated 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,Ill17a−/−) and heterozygous for the Stat3 (gp130F/F,Stat3+/−) genes have been described previously.14-16 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 1×106 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 TGFB (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.
of T\textsubscript{H}1 and T\textsubscript{H}17 polarisation by flow cytometry (see online supplemental 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.\textsuperscript{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\textsuperscript{F/F} mice lacking STAT1 exhibit hyperexpansion of T\textsubscript{H}17 cells**

We have previously shown that gp130\textsuperscript{F/F} mice display exacerbated histopathology in experimental arthritis, as a consequence of elevated STAT3 signalling.\textsuperscript{13} In this respect, the severity of joint pathology was associated with increased infiltration of synovial IL-17A-producing T-cells.\textsuperscript{13} Enhanced gp130-mediated STAT3 activity promotes T\textsubscript{H}17 differentiation in vitro.\textsuperscript{16} However, STAT1 activating cytokines (eg, IFN-\textgamma and IL-27) inhibit T\textsubscript{H}17 differentiation, and are protective in experimental arthritis.\textsuperscript{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\textsubscript{H}17 development in T-cell cultures from gp130\textsuperscript{F/F}:Stat1\textsuperscript{−/−} compound mice (figure 1). Compared with wild type (WT) controls, T-cells from gp130\textsuperscript{F/F} mice showed more than a twofold increase in the proportion of CD4 IL-17A\textsuperscript{+} T-cells when cultured under T\textsubscript{H}17 polarising conditions (figure 1A,B). This response was STAT3 dependent as the proportion of CD4 IL-17A\textsuperscript{+} T-cells from gp130\textsuperscript{F/F}:Stat3\textsuperscript{+/−} mice were significantly reduced and T\textsubscript{H}17 expansion was comparable with that seen in WT mice (figure 1A,B). Conversely, a loss of STAT1 signalling in gp130\textsuperscript{F/F}:Stat1\textsuperscript{−/−} T-cell cultures caused a ‘hyperexpansion’ of T\textsubscript{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-\textgamma-producing T\textsubscript{H}1 cells between the genetic strains, the proportion of IFN-\textgamma-IL-17A\textsuperscript{+} double producers was elevated in gp130\textsuperscript{F/F}:Stat1\textsuperscript{−/−} T-cell cultures (see online supplementary figure S1). Thus, altered bioavailability of gp130-mediated STAT1 and STAT3 signalling dramatically skew T\textsubscript{H}17 commitment in vitro.

**Increased T\textsubscript{H}17 responses in gp130\textsuperscript{F/F}:Stat1\textsuperscript{−/−} mice do not enhance arthritis severity**

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

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**Figure 1**  T-cells from gp130\textsuperscript{F/F}:Stat1\textsuperscript{−/−} mice are hyper-responsive to T\textsubscript{H}17 polarisation. (A) Representative flow cytometric analysis of the development of IL-17A producing CD4 T-cells from (WT), gp130\textsuperscript{F/F} (FF), gp130\textsuperscript{F/F}:Stat3\textsuperscript{−/−} (FFS\textsubscript{3}) and gp130\textsuperscript{F/F}:Stat1\textsuperscript{−/−} (FFS\textsubscript{1}) mice cultured under T\textsubscript{H}17 polarising conditions (TGF-\beta and IL-6). (B) Percentage of CD4 IL-17A\textsuperscript{+} T-cells generated under T\textsubscript{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.
nodes were isolated and the number of T\textsubscript{H}-17 cells compared with those observed in gp130\textsuperscript{F/F} and WT mice (figure 2A). Here, gp130\textsuperscript{F/F}:Stat1\textsuperscript{−/−} mice displayed a heightened peripheral T\textsubscript{H}-17 response, reflecting our in vitro observations and supporting a role for STAT1 as a negative regulator of T\textsubscript{H}-17 expansion in vivo. The increased peripheral response was not, however, limited to T\textsubscript{H}-17 cells as gp130\textsuperscript{F/F}:Stat1\textsuperscript{−/−} mice also displayed elevated total CD4 and T\textsubscript{H}-1 cell numbers (figure 2A). While gp130\textsuperscript{F/F}:Stat1\textsuperscript{−/−} mice displayed an increased expansion in absolute T\textsubscript{H}-1 and T\textsubscript{H}-17 cell numbers compared with WT and gp130\textsuperscript{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\textsuperscript{F/F} mice displayed exacerbated disease, gp130\textsuperscript{F/F}:Stat1\textsuperscript{−/−} 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\textsuperscript{F/F}:Stat1\textsuperscript{−/−} mice compared with gp130\textsuperscript{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\textsuperscript{F/F} mice

We previously observed an association between joint infiltrating IL-17A producing T-cells and exacerbated AIA in gp130\textsuperscript{F/F} mice.\textsuperscript{13} While gp130\textsuperscript{F/F}:Stat1\textsuperscript{−/−} 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\textsubscript{H}-17 cells to local joint pathology. We therefore generated gp130\textsuperscript{F/F}:Il17a\textsuperscript{−/−} compound mice to investigate the importance of the T\textsubscript{H}-17 signature cytokine, IL-17A, in local joint pathology. Consistent with our previous data,\textsuperscript{13} end-stage histopathology (day-28 & 35) was exacerbated in AIA challenged gp130\textsuperscript{F/F} mice (see online supplementary table S2). However, comparison of gp130\textsuperscript{F/F} and gp130\textsuperscript{F/F}:Il17a\textsuperscript{−/−} mice

Figure 2  
Heightened peripheral T\textsubscript{H}-17 cell responses in gp130\textsuperscript{F/F}:Stat1\textsuperscript{−/−} mice during antigen-induced arthritis (AIA) is not associated with exacerbated joint pathology. (A) AIA was established in wild type (WT), gp130\textsuperscript{F/F} (FF) and gp130\textsuperscript{F/F}:Stat1\textsuperscript{−/−} (FFSt1) mice and the number of peripheral CD4, T\textsubscript{H}-17 and T\textsubscript{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\textsubscript{H}-1) and IL-17A (T\textsubscript{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).
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 gp130F/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, but less favourable results have come from trials in rheumatoid and psoriatic arthritis. Such varied clinical outcomes may reflect the nature of the underlying 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 gp130F/F knock-in mouse model, which displayed increased IL-6/IL-23-mediated T_{H}17 commitment, enhanced IL-17A expression and severe AIA pathology. 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. 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 gp130F/F with gp130F/F:Stat3^{−/−} mice show that a partial STAT3 deficiency ameliorates disease. We therefore postulated that gp130F/F:Stat1^{−/−} mice would display severe joint pathology. Although gp130F/F:Stat1^{−/−} mice showed heightened peripheral effector T-cell characteristics, joint inflammation in gp130F/F:Stat1^{−/−} mice closely resembles that seen in gp130F/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 and local expression of CXCR3 ligands CXC-chemokine ligand (CXCL9, CXCL10 and CXCL11). Similarly, gp130/STAT3 activity controls inflammatory chemokine expression and IL-6/IL-17A mice show impaired T-cell infiltration and reduced T-cell CC-chemokine receptor (CCR)3, 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/STAT3/IL-17A−/− mice to investigate T-cell-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/−/−/IL-17A−/− mice, it is also possible that other T-effector cytokines (eg, IL-17F, GM-CSF) substitute for IL-17A. Such findings may reflect recent trials in RA where secukinumab (anti-IL-17A mAb) failed to meet its clinical endpoint. 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 gp130 signaling 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, 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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