CONCISE REPORT

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

G W Jones,1,2 C J Greenhill,1 J O Williams,1 M A Nowell,1 A S Williams,1 B J Jenkins,2 S A Jones1

ABSTRACT

Objective Interleukin (IL)-17A producing CD4 T-cells (Th17 cells) are implicated in rheumatoid arthritis (RA). IL-6/STAT3 signalling drives Th17 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 Th17 commitment. Here, we evaluate the impact of STAT1 ablation on Th17 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 Th17 responses were compared.

Results Augmented gp130/STAT3 signalling enhanced Th17 commitment in vitro and exacerbated joint pathology. Ablation of STAT1 in gp130F/F mice promoted the hyperexpansion of Th17 cells in vitro and in vivo during AIA. Despite this heightened peripheral Th17 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.1–5 IL-17A-producing CD4 T-cells (Th17 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.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-231 promote T17 effector functions murine T17 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 differentiation.14 Here, we investigate STAT1 control of T17 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.

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 1×105 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.
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-\(\gamma\) 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-\(\gamma\)-producing T\(_{H}1\) cells between the genetic strains, the proportion of IFN-\(\gamma\) 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 nodes were removed, and the proportion of CD4 IL-17A\(^+\) T-cells was determined by flow cytometry.
nodes were isolated and the number of TH-17 cells compared with those observed in gp130F/F and WT mice (figure 2A). Here, gp130F/F: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 gp130F/F:Stat1−/− mice also displayed elevated total CD4 and TH-1 cell numbers (figure 2A). While gp130F/F:Stat1−/− mice displayed an increased expansion in absolute TH-1 and TH-17 cell numbers compared with WT and gp130F/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 gp130F/F mice displayed exacerbated disease, gp130F/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 gp130F/F:Stat1−/− mice compared with gp130F/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 gp130F/F mice**

We previously observed an association between joint infiltrating IL-17A producing T-cells and exacerbated AIA in gp130F/F mice.13 While gp130F/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 TH-17 cells to local joint pathology. We therefore generated gp130F/F:Il17a−/− compound mice to investigate the importance of the T17 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 gp130F/F mice (see online supplementary table S2). However, comparison of gp130F/F and gp130F/F:Il17a−/− mice

---

**Figure 2**  
Heightened peripheral TH-17 cell responses in gp130F/F:Stat1−/− mice during antigen-induced arthritis (AIA) is not associated with exacerbated joint pathology. (A) AIA was established in wild type (WT), gp130F/F (FF) and gp130F/F:Stat1−/− (FFSt1) mice and the number of peripheral CD4, TH-17 and TH-1 cells assessed by flow cytometry of inguinal lymph nodes at 10 days postarthritis induction. Representative dot plots indicating the percentage of IFN-γ (TH-1) and IL-17A (TH-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,^{45} 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 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 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 TH-17 cells in vitro. Prior AIA experiments comparing gp130F/F with gp130F/F:Stat3^{+/−} mice show that a partial STAT3 deficiency ameliorates disease.^{13} 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

**Figure 3** Antigen-induced arthritis (AIA) pathology in gp130F/F mice is independent of IL-17A. (A) Evaluation of arthritic index (AI), inflammation, exudate, hyperplasia and erosion scores in histological joint sections from gp130F/F (FF, closed circles) and gp130F/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.

on CD4 T-cells and local expression of CXCR3 ligands CXC-chemokine ligand (CXCL)9, CXCL10 and CXCL11. Similarly, gp130/STAT3 activity controls inflammatory chemokine expression and IL-6/IL17A 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/STAT3 activity controls inflammatory chemokine expression and IL-6/IL17A mice show impaired T-cell infiltration and reduced T-cell CC-chemokine receptor (CCR3), CCR5 and CXCR3 expression.

References


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

G W Jones, C J Greenhill, J O Williams, M A Nowell, A S Williams, B J Jenkins and S A Jones

Ann Rheum Dis published online July 26, 2013

Updated information and services can be found at:
http://ard.bmj.com/content/early/2013/07/26/annrheumdis-2013-203771

These include:

Supplementary Material
Supplementary material can be found at:
http://ard.bmj.com/content/suppl/2013/07/26/annrheumdis-2013-203771.DC1

References
This article cites 25 articles, 8 of which you can access for free at:
http://ard.bmj.com/content/early/2013/07/26/annrheumdis-2013-203771#BIBL

Open Access
This is an Open Access article distributed in accordance with the terms of the Creative Commons Attribution (CC BY 3.0) license, which permits others to distribute, remix, adapt and build upon this work, for commercial use, provided the original work is properly cited. See:
http://creativecommons.org/licenses/by/3.0/

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections
Articles on similar topics can be found in the following collections
- Open access (602)
- Degenerative joint disease (4641)
- Musculoskeletal syndromes (4951)
- Immunology (including allergy) (5144)
- Connective tissue disease (4253)
- Rheumatoid arthritis (3258)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/