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Poly-ADP-ribosyl polymerase-14 promotes T helper 17 and follicular T helper development

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Summary

Transcription factors are critical determinants of T helper cell fate and require a variety of co-factors to activate gene expression. We previously identified the ADP ribosyl-transferase poly-ADP-ribosyl polymerase 14 (PARP-14) as a co-factor of signal transducer and activator of transcription (STAT) 6 that is important in B-cell and T-cell responses to interleukin-4, particularly in the differentiation of T helper type 2 (Th2) cells. However, whether PARP-14 functions during the development of other T helper subsets is not known. In this report we demonstrate that PARP-14 is highly expressed in Th17 cells, and that PARP-14 deficiency and pharmacological blockade of PARP activity result in diminished Th17 differentiation *in vitro* and in a model of allergic airway inflammation. We further show that PARP-14 is expressed in T follicular helper (Tfh) cells and Tfh cell development is impaired in PARP-14-deficient mice following immunization with sheep red blood cells or inactivated influenza virus. Decreases in Th17 and Tfh development are correlated with diminished phospho-STAT3 and decreased expression of the interleukin-6 receptor α-chain in T cells. Together, these studies demonstrate that PARP-14 regulates multiple cytokine responses during inflammatory immunity.

Keywords: differentiation; knockout; T helper cell; transcription factor.

Introduction

Naive CD4 T cells upon an antigen encounter differentiate into various effector T-cell lineages.[1] This process depends upon the cytokines in the environment and transcription factors activated by that environment. Cytokines upon binding to specific receptors activate Janus kinase-dependent phosphorylation of signal transducer and activator of transcription (STAT) proteins.[2] Phosphorylated STATs dimerize, translocate to the nucleus, and bind DNA to activate gene transcription.

Activation of STAT3 is required for the development of T helper type 17 (Th17) cells that secrete interleukin-17A (IL-17A), IL-17F and IL-21, and for the development of T follicular helper (Tfh) cells that promote germinal centre (GC) formation and antibody maturation.[3–5] The development of Th17 cells is induced by cytokines including IL-6, IL-23 and IL-21 that activate STAT3. STAT3 activates overlapping sets of genes during Th17 and Tfh differentiation including *Maf*, *Irf4* and *Batf*.[6] STAT3 also regulates *Rorc* and *Bcl6*, primary transcription factors required for the development of Th17 and Tfh cells, respectively. Whether STAT3 requires co-factors in promoting Th17 and Tfh development is not yet known.[4,7–9]

Poly (ADP) ribose polymerase-14, a member of the poly-ADP-ribosyl polymerase (PARP) superfamily, is defined by the PARP catalytic domain.[10] The enzymatic activity of PARPs catalyses the transfer of ADP ribose from NAD^+ onto target proteins thereby regulating various cellular processes including inflammation and immunity. PARP-14 was identified as a transcriptional co-factor for STAT6, following IL-4 stimulation in B cells.[11,12] PARP-14 (ADP)-ribosylates histone deacetylases 2 and 3 bound to a gene promoter, displacing the histone

Abbreviations: BAL, bronchoalveolar lavage; GC, germinal centre; IL-17, interleukin-17; PARP, poly (ADP) ribose polymerase; RORγt, RAR-related orphan receptors; SRBC, sheep red blood cell; STAT, signal transducer and activator of transcription; Tfh, T follicular helper; TGF-β, transforming growth factor-β; Th 17, T helper 17; WT, wild-type

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deacetylases and facilitating STAT6 binding and gene activation.[13] PARP-14 also promotes STAT6 phosphorylation.[13] In T cells, PARP-14 promotes Th2 differentiation via STAT6-dependent *Gata3* expression.[14,15] PARP-14 is also required for STAT6-dependent Th9 development.[16] PARP-14-deficient mice as well as mice treated with a pharmacological inhibitor of PARP activity are protected from airway allergic diseases.[15]

Although a requirement for PARP-14 in STAT6-dependent T helper cell development and function is clearly established, it is not clear that PARP-14 function is strictly limited to the IL-4/STAT6 pathway. To explore this question, we tested the expression and function of PARP-14 in other T helper cell lineages.

Materials and methods

Mice

C57BL/6 mice were purchased from Harlan (Indianapolis, IN). Six- to 8-week-old *Parp14*$^{-/-}$ mice on a C57BL/6 background and wild-type (WT) littermates were used for allergic airway disease studies, or BALB/c mice were used in the PARP inhibitor allergic airway disease experiments. Mice were maintained in pathogen-free conditions, and studies were approved by the Indiana University Institutional Animal Care and Use Committee.

Differentiation of T cells

Naive CD4$^+$ CD62L$^+$ T cells were isolated from spleen using magnetic selection (Miltenyi Biotec, San Diego, CA). CD4$^+$ T cells were activated with plate-bound anti-CD3 (2 μg/ml; BD Pharmingen, San Jose, CA) and soluble anti-CD28 (1 μg/ml; BD Pharmingen) and cultured under the following conditions: Th17 with transforming growth factor-β (TGF-β) (100 ng/ml IL-6; 10 ng/ml IL-23; 10 ng/ml IL-1β; 2 ng/ml TGF-β; 10 μg/ml anti-IL-4, and 10 μg/ml anti-interferon-γ) and Th17 without TGF-β (100 ng/ml IL-6; 10 ng/ml IL-23; 10 ng/ml IL-1β; 10 μg/ml anti-IL-4, and 10 μg/ml anti-interferon-γ). Cells were expanded after 3 days with half-concentration of the original cytokines in fresh medium. Cells were harvested on day 5 for analysis.

Surface and intracellular flow cytometry

Th17 primed cells were stimulated with PMA and ionomycin for a total of 4 hr. Monesin was added in between for 3 hr, the cells were then permeabilzed using 0·1% saponin and stained for antibodies against IL-17a (Biolegend, San Diego, CA) and IL-17 (Biolegend). Antibodies to phosphorylated STAT3 (Biolegend) were used for staining methanol-fixed Th17 cells. To detect Tfh cells, splenic cells were stained for CD4-Alexa Fluor 700 (Biolegend), PD-1-Peridinin chlorophyll protein-Cy5.5 (Biolegend), and biotinylated CXCR5 (eBioscience, San Diego, CA). B220-Peridinin chlorophyll protein (Biolegend), GL7 FITC (Biolegend) and Fas A647 (Biolegend) were used to stain for GC B cells.

Sheep red blood cell immunization and antibody titre measurement

Sheep red blood cells (SRBC; VWR International, Radnor, PA) were washed three times with PBS. Wild-type and *Parp14*-deficient mice were injected with 1×10^9 cells (intraperitoneal). Mice were killed after 10 days for the analysis. Serum was collected by cardiac puncture, and SRBC-specific antibodies were measured using ELISA as described previously.[17]

Induction of airway inflammation and PJ34 administration. Mice were sensitized by using intraperitoneal injections on days 0 and 7 of ovalbumin/alum (20 μg of ovalbumin in BALB/c mice and 50 μg of ovalbumin in C57BL/6 mice per 2 mg of alum). On day 14, mice were challenged with ovalbumin (100 μg) intranasally for 7 days. Mice were killed 24 hr after the last challenge. Mice were injected intraperitoneally with PJ34 (10 mg/kg) on day 0 and 7, and intranasally from day 14 to day 21.

Heat-inactivated influenza immunization. Influenza A/PR/8/34 (H1N1) was heat inactivated at 55° for 20 min. Wild-type and *Parp14*$^{-/-}$ mice were injected with 1×10^7 heat-inactivated influenza virus (intraperitoneal). Mice were killed after 10 days for the analysis.

Results

PARP-14 promotes cytokine production in Th17 cells

Regulation of Th2 cell development by PARP-14 has been described, but the role of PARP-14 on other T helper subsets has not been extensively examined. To explore the role of PARP-14 in the generation of T-cell lineages, we examined *Parp14* expression from *in vitro* differentiated T helper cells. Interestingly, *Parp14* mRNA levels were increased (twofold) in Th17 differentiated cells compared with non-polarized Th0 cells (Fig. 1a). *Parp14* mRNA in other T-cell lineages was similar to those in Th0 cells (data not shown).

To define the role of PARP-14 in Th17 differentiation, we assessed cytokine secretion and gene expression from Th17 cultures derived from *Parp14*$^{-/-}$ and WT naive CD4$^+$ T cells. TGF-β promotes Th17 polarization, but recent reports suggest that it also represses the pathogenicity of T cells.[18] Hence, we compared Th17 differentiation in the presence or absence of TGF-β. The

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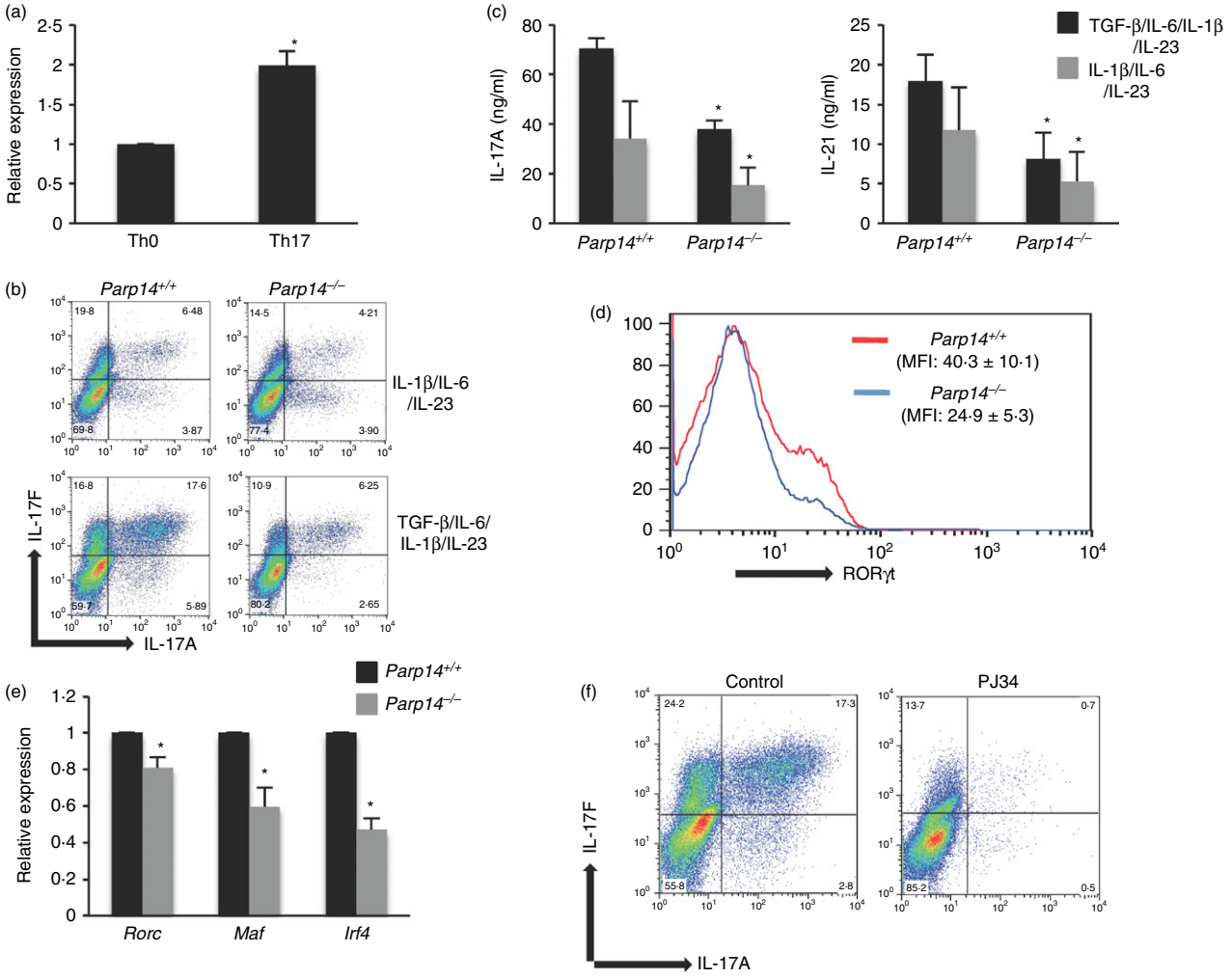

Figure 1. Poly (ADP) ribose polymerase 14 (PARP-14) regulates T helper type 17 (Th17) differentiation: (a) Naive T cells isolated from C57BL/6 mice were cultured under Th0 or Th17 conditions. *Parp14* expression was measured in helper T-cell subsets using quantitative RT-PCR. (b, c) Naive wild-type and *Parp14*−/− $CD4^+$ T cells were cultured under Th17 polarizing conditions with or without transforming growth factor-β (TGF-β) and harvested on day 5. Differentiated cells were stimulated with PMA and ionomycin for 4 hr before intracellular cytokine staining for interleukin-17A (IL-17A) and IL-17F production (b) or with anti-CD3 stimulation for 24 hr before supernatants were harvested for analysis by ELISA (c). (d–e) TGF-β-induced Th17 cells from wild-type and $Parp14^{-/-}$ mice were stained for intracellular RORγt (d) and gene expression for transcription factors by quantitative PCR (e). (f) Naive $CD4^+$ T cells were cultured under Th17 polarizing conditions in the presence or absence of PJ34 and stained for intracellular cytokines. Data are mean ± SEM of four independent experiments $^*P < 0{\cdot}05$.

TGF-β induced higher amounts of IL-17A, IL-17F and IL-21 (Fig. 1b and c). PARP-14-deficient Th17 cells had a significantly reduced frequency of IL-17A and IL-17F single- and double-positive cells (Fig. 1b), compared with WT Th17 cells irrespective of the presence or absence of TGF-β. Protein and message levels of RORγt/*Rorc*, the master regulator of Th17 cells, were lower in PARP-14-deficient cells (Fig. 1d,e). Message RNA of additional Th17-inducing transcription factors, *Maf* and *Irf4*, was also decreased in $Parp14^{-/-}$ Th17 cells (Fig. 1e). To determine whether impaired PARP catalytic activity would lead to diminished Th17 differentiation, we cultured naive T cells under Th17 skewing conditions in the presence and absence of the PARP inhibitor PJ34.[19] The frequencies of IL-17A- and IL-17F-positive cells were reduced by 50% in TGF-β-primed Th17 cells (Fig. 1f). These observations suggested that PARP-14 and its activity are required for optimal Th17 differentiation.

Impaired Th17 development in the absence of PARP14 during the development of allergic inflammation

To determine if PARP-14 also impacts Th17 development *in vivo*, we used the ovalbumin/alum-induced airway inflammation model that is Th2 and Th17 cell depen-

dent.[20–22] Previous work in our laboratory has demonstrated that ablation of PARP-14 expression or administration of a pharmacological inhibitor of PARP activity attenuated airway inflammation as measured by reduced cellular lung infiltration, serum IgE concentration and Th2 cytokines.[15] To test *in vivo* Th17 development in this model, we assessed the role of Th17 secretion in lung tissue and bronchoalveolar lavage (BAL) fluid. There was a significant decrease in IL-17 production from $CD4^+$ T cells in both lung tissue and BAL (Fig. 2a and c) isolated from immunized $Parp14^{-/-}$ mice compared with WT mice. We also examined other IL-17-secreting populations in the lung and observed decreased percentages of IL-17-secreting $\gamma\delta$ T cells in both lung tissue and BAL

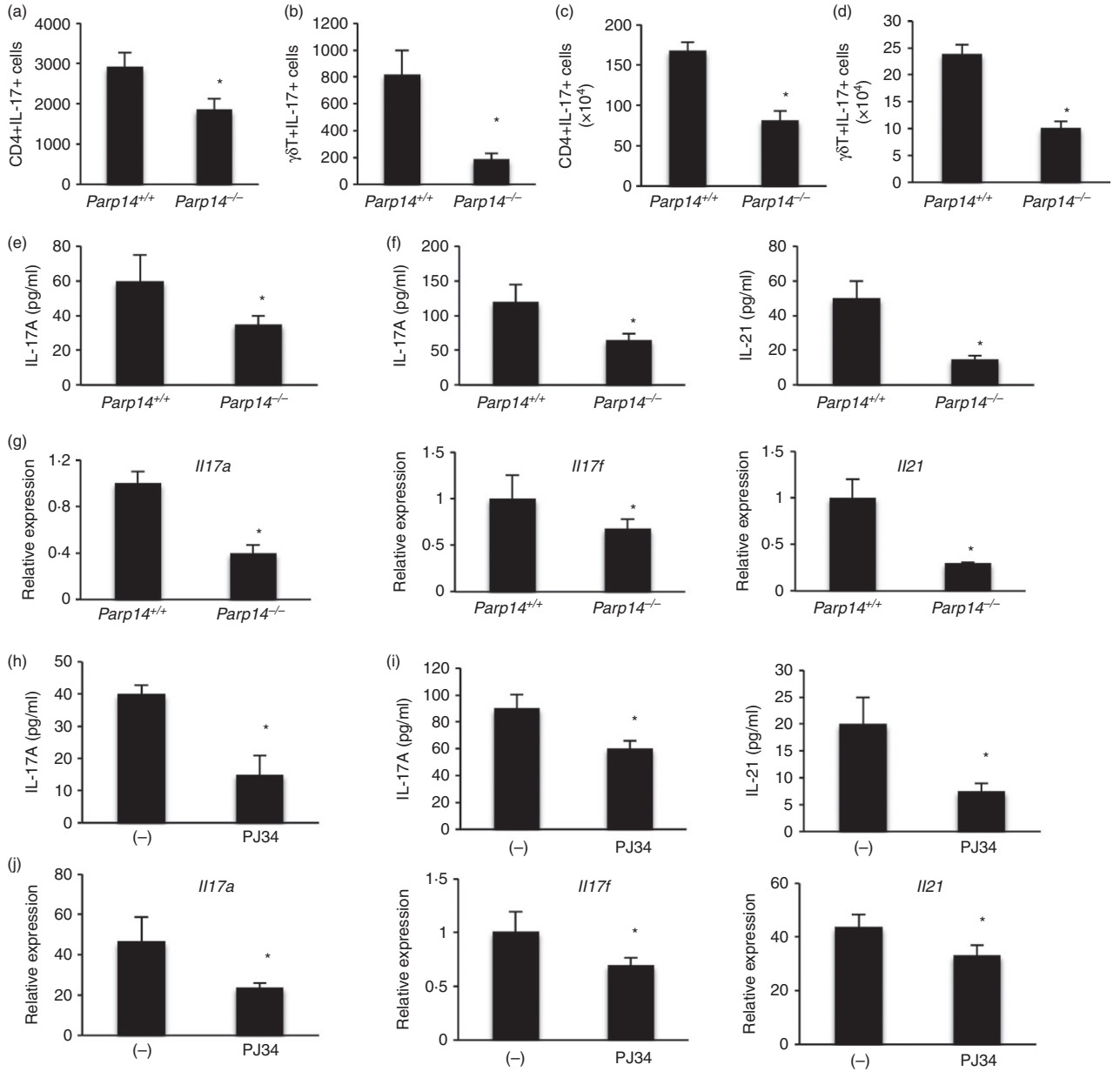

Figure 2. Poly (ADP) ribose polymerase 14 (PARP-14) regulates T helper type 17 (Th17) cell cytokine production in allergic inflammation. (a–j) $Parp14^{+/+}$ and $Parp14^{-/-}$ mice were sensitized with ovalbumin and alum and challenged with ovalbumin to induce allergic inflammation. The number of $CD4^+$ T cells and $\gamma\delta$ T cells secreting interleukin-17 (IL-17) in the lungs (a, b) and bronchoalveolar lavage (BAL) cells (c, d) are depicted. Cytokines were measured in the BAL fluid (e) or in the supernatant of antigen-stimulated splenocytes (f) by ELISA. Expression of cytokines in the lung tissue of mice was assessed by quantitative PCR (g). (d–f) Allergic inflammation was induced in C57BL/6 mice and treated with or without PJ34. Cytokines were measured in the BAL fluid (h) and in antigen-stimulated splenocytes (i) by ELISA. (j) Cytokine expression in the lung tissue was measured by quantitative PCR. Data are means ± SEMs of three independent experiments, $^*P < 0{\cdot}05$.

(Fig. 2b and d) isolated from immunized *Parp14*$^{-/-}$ mice, compared with WT mice. We did not observe significant IL-17 production from other cell types in the lung. Consistent with our flow cytometric data, reduced IL-17A levels were detected in the BAL fluid of *Parp14*$^{-/-}$ mice compared with control mice (Fig. 2e). Concentrations of IL-17A, IL-17F and IL-21 were significantly reduced from supernatants of antigen-stimulated cells (Fig. 2f and data not shown). Message levels of *Il17a*, *Il17f* and *Il21* were also significantly reduced in lungs of PARP-14-deficient mice (Fig. 2g). To evaluate the role of PARP catalytic activity in Th17-dependent airway inflammation, we treated WT mice with PJ34 during the sensitization and challenge phases. Levels of IL-17A in the BAL fluid were reduced in mice treated with PJ34 (Fig. 2h). Decreased amounts of IL-17A and IL-21 were measured in the antigen-stimulated splenocytes isolated from treated versus untreated mice, suggesting impaired peripheral T-cell responses (Fig. 2i). Similarly, mRNA levels of *Il17a*, *Il17f* and *Il21* were also reduced in the lung tissue of the treated mice compared with untreated control (Fig. 2j). Collectively, these results suggest that PARP-14 and its activity promote Th17 differentiation *in vivo* during the development of allergic airway inflammation.

PARP-14 limits Tfh development

Because PARP-14 was required for STAT3-dependent Th17 development, and PARP-14 also regulated genes important for Tfh cell development including *Maf* and *Irf4*, we wanted to determine whether PARP-14 deficiency results in impaired Tfh development. To test this, PARP-14-deficient mice and control littermates were immunized with SRBC and analysed on day 10. As expected, SRBC immunization increased Tfh cell percentages ($CD4^+$ $CXCR5^+$ $PD\text{-}1^{high}$) in WT mice (Fig. 3a). The number and frequency of Tfh cells was significantly reduced in PARP-14-deficient mice (Fig. 3a). This was not correlated with preferential expression of PARP-14 because the expression of PARP-14 was similar between Tfh cells and non-Tfh cells ($CD4^+$ $CXCR5^-$ $PD\text{-}1^{lo}$) (Fig. 3b). Although the expression of *Bcl6* in the Tfh cells derived from *Parp14*$^{-/-}$ mice was similar to Tfh cells from WT mice there was a significant reduction in *Maf* and *Irf4* message levels in Tfh cells sorted from *Parp14*$^{-/-}$ mice, compared with WT mice (Fig. 3c).

The Tfh cells promote GC B-cell development. To test the functional consequences of reduced Tfh in *Parp14*$^{-/-}$ mice in GC B cells, splenocytes from SRBC immunized mice were stained with antibodies against B220, GL7 and Fas ligand. We observed a 45% reduction in the number and frequency of GC B cells in *Parp14*$^{-/-}$ mice compared with WT mice (Fig. 3d). To further document the functional outcome of decreased GC B cells, we analysed SRBC-specific serum antibody titres. Although SRBC-specific IgM serum concentrations were similar in WT and PARP-14-deficient mice, SRBC-specific IgG1 and IgG2a/c titres were reduced in mice that lack PARP-14 (Fig. 3e–g).

To further demonstrate the importance of PARP-14 in the regulation of Tfh development, we immunized *Parp14*$^{-/-}$ mice and WT mice with heat-inactivated influenza virus. As observed during SRBC immunization, numbers and percentages of Tfh cells were significantly reduced in influenza-immunized *Parp14*$^{-/-}$ mice compared with WT mice (Fig. 4a). PARP-14 deficiency also leads to 38% reduction in GC B cells (Fig. 4b). Collectively, the data demonstrate that PARP-14 limits Tfh development and function *in vivo*.

Decreased STAT3 activation in the absence of PARP-14

The IL-6-induced STAT3 signalling cascade is crucial for both Th17 and Tfh differentiation.[23–26] To test whether PARP-14 regulates STAT3 activation, we investigated the kinetics of STAT3 activation during Th17 development. The frequency of phospho-STAT3 (pSTAT3) was decreased on days 1 and 2 of Th17 differentiation during the peak of activation, though there were comparable levels of STAT3 activation measured thereafter (Fig. 5a). We next wanted to determine whether reduced pSTAT3 correlates with reduced expression of *Il6ra*. RNA expression of *Il6ra* was reduced in *Parp14*$^{-/-}$ Th17 cultures, compared with wild-type cells, in the same time-frame as diminished pSTAT3 (Fig. 5b). The effect on Il6ra was specific, as there was no difference in the expression of *Il6st* (encoding gp130) or *Il21r* between WT and *Parp14*$^{-/-}$ Th17 cells (Fig. 5c, d). A similar reduction in *Il6ra* expression and pSTAT3 was observed when Th17 cells were treated with PJ34, compared with control treated cells (Fig. 5e, f). To determine if these observations were consistent with a role of PARP-14 in regulating pSTAT3 in Tfh development, we stained Tfh cells from SRBC-immunized mice with antibodies against pSTAT3. In the $CD4^+$ $CXCR5^+$ $PD\text{-}1^{high}$ population, there was a higher percentage of pSTAT3-positive cells in WT mice than in *Parp14*$^{-/-}$ mice (Fig. 5g). Together, these data suggest that PARP-14 regulates STAT3 activation, potentially by regulating *Il6ra*, thereby influencing Th17 and Tfh development.

Discussion

Differentiation of naive T cells into specialized T helper subsets is dependent upon the cytokines in the milieu and the array of transcription factors induced in response to stimulation. In activating target genes necessary for T helper cell differentiation, transcription factors use a variety of co-factors. We previously identified PARP-14 as a co-factor for STAT6 and demonstrated that in the

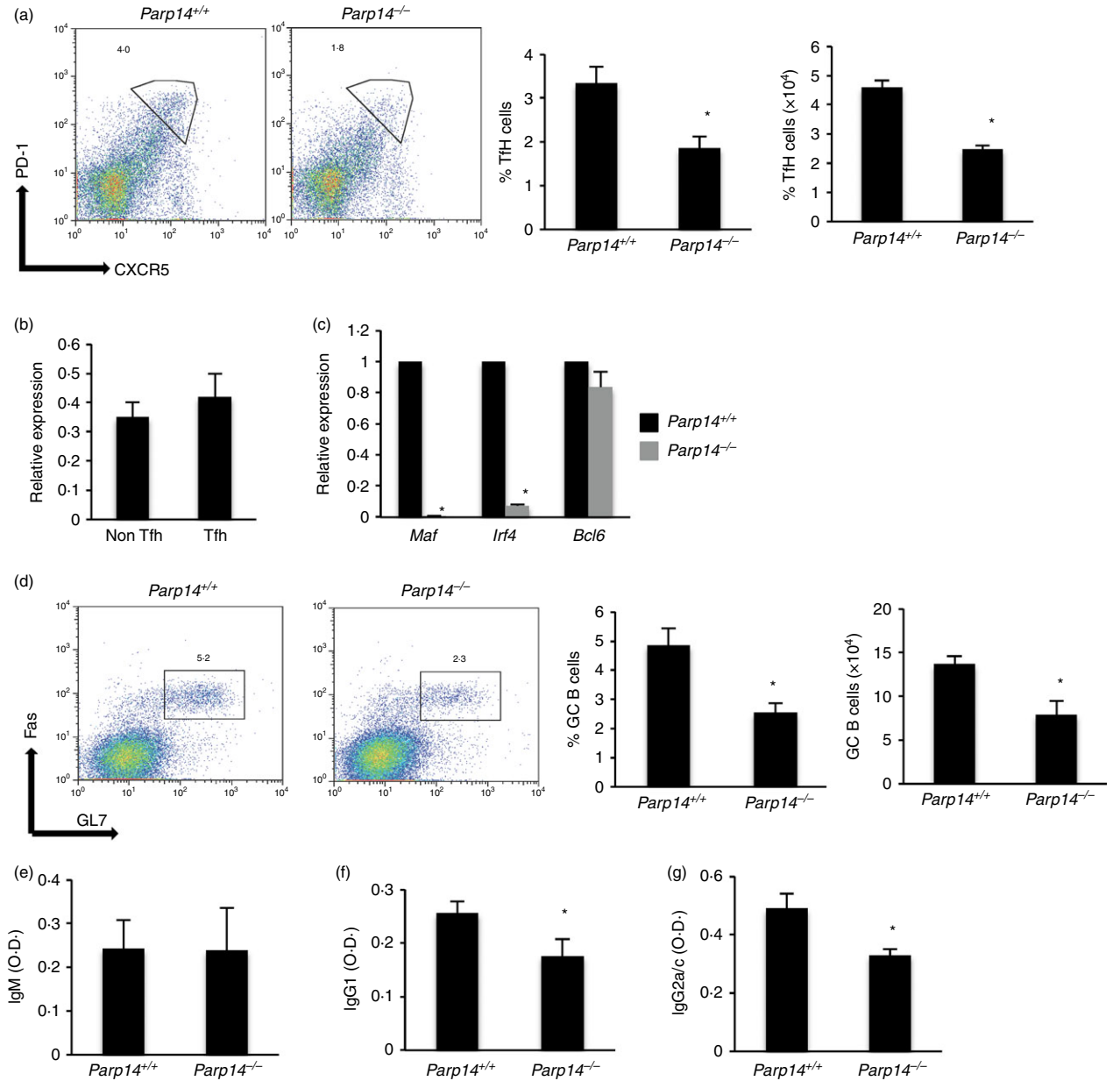

Figure 3. Poly (ADP) ribose polymerase 14 (PARP-14) promotes T follicular helper (Tfh) and germinal centre (GC) B-cell development in response to sheep red blood cell (SRBC) immunization. Wild-type (WT) and *Parp14*$^{-/-}$ mice were immunized with SRBC. On day 9, splenocytes were analysed for Tfh cell (a) and GC B-cell (d) populations with quantitative analysis shown next to the representative gating. Data are gated on CD4^{+} and B220^{+} for Tfh and GC B cells, respectively. (b) Absolute mRNA levels of Parp14 from sorted Tfh cells and non-Tfh cells were measured by quantitative PCR. (c) Expression of transcription factors in Tfh (CD4^{+} CXCR5^{+}PD-1^{high}) cells sorted from SRBC immunized WT and *Parp14*$^{-/-}$ mice. (e) Serum from WT and *Parp14*$^{-/-}$ mice was used to measure SRBC-specific antibody titres by ELISA. Data are mean ± SE of four or five mice per group and representative of three independent experiments. *$P < 0{\cdot}05$.

absence of PARP-14, activity of the IL-4/STAT6 signalling pathway was impaired. In this report, we examined the effects of PARP-14 deficiency on other T helper subsets and observed a role in Th17 and Tfh cell development. As development of these subsets does not require STAT6,[3,25] these studies identify new roles for PARP-14 in the immune system.

The role of PARPs in adaptive immunity has been investigated using pharmacological inhibitors and gene-deficient mice. PARP family members and PARP activity are involved in T-cell development as well as in peripheral T-cell activation and differentiation.[27] *Parp2*$^{-/-}$ mice have reduced thymic T-cell numbers and decreased double-positive CD4^{+} CD8^{+} T cells.[28] PARP-1, the prototypical representa-

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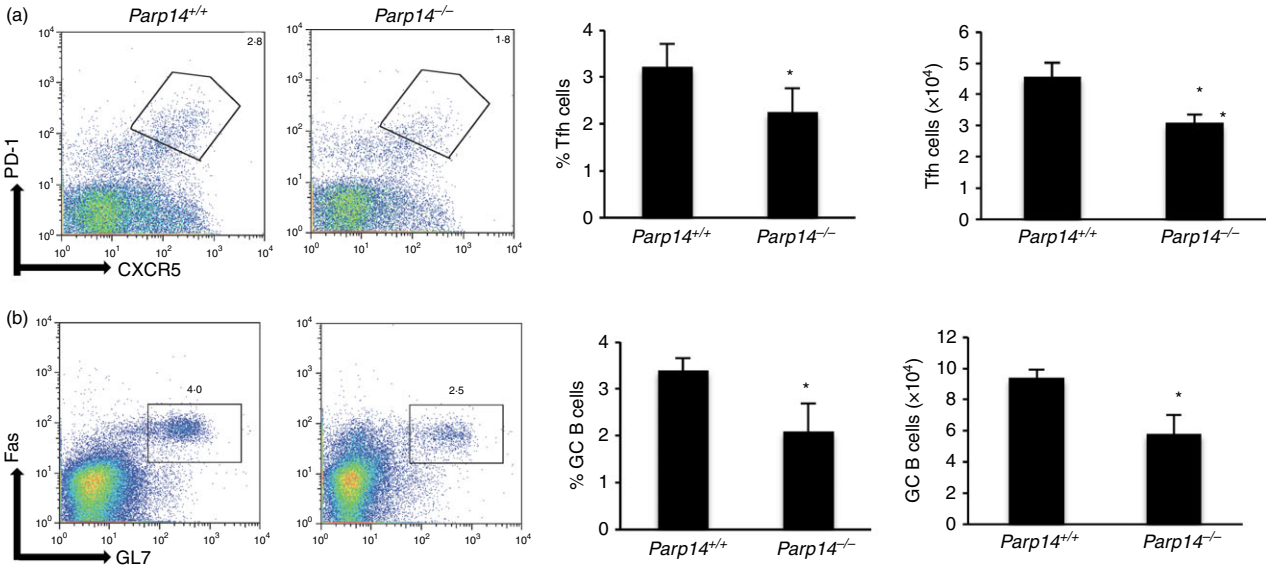

Figure 4. Poly (ADP) ribose polymerase 14 (PARP-14) promotes T follicular helper (Tfh) and germinal centre (GC) B-cell development in response to heat-inactivated influenza immunization. Wild-type (WT) and *Parp14*$^{-/-}$ mice were immunized with heat-inactivated influenza virus. On day 9, splenocytes were analysed for Tfh cell (a) and GC B-cell (b) populations with quantitative analysis shown next to the representative gating. Data are gated on CD4^{+} and B220^{+} for Tfh and GC, respectively. Data are mean ± SE of four or five mice per group and representative of three independent experiments. *$P < 0{\cdot}05$.

tive of the PARP family, is expressed in all cells and plays a central role in inflammation. PARP-1 regulated peripheral T-cell activation at multiple stages.[29] It interacts with and poly-ADP-ribosylates components of nuclear factor-κB, thereby regulating genes including tumour necrosis factor-α, inducible nitric oxide synthase and NFATc.[30,31] Like PARP-14, PARP-1 influences Th2 differentiation by affecting STAT6 activation. Lack of protein or enzymatic inhibition of PARP-1 exposes STAT6, which is required for IL-4 signalling, to calpain-mediated degradation, with consequent reduction in GATA-3 and IL-5 mRNA expression.[32,33] In contrast, PARP-14 interacts with STAT6 at gene promoters, and subsequently mediates STAT6-dependent *Gata3* induction.[15] Hence, two PARP family members impact T helper differentiation through entirely different mechanisms.

PARP inhibition has beneficial effects in a wide spectrum of inflammatory conditions like asthma,[32,33] experimental allergic encephalomyelitis[34] and contact hypersensitivity,[35,36] though the precise mechanisms are unclear. Although work from our laboratory and others suggested that PARPs function in allergic inflammation by perturbing the Th1–Th2 balance, it is not clear that this is an important effect in all models. In the collagen-induced arthritis[37] and experimental allergic encephalomyelitis,[34] animal models of the human diseases rheumatoid arthritis and multiple sclerosis that have dominant Th1 and Th17 responses, administration of PARP inhibitors also reduces disease severity. In the absence of PARP-1 there was decreased production of tumour necrosis factor and inducible nitric oxide synthase, but no effects on IL-17 production.[38] PARP-1 deficiency also results in increased frequency of Treg cells in the spleen, thymus and lymph nodes, and impaired cell proliferation coincident with reduced IL-2 secretion.[39] It is not yet clear if PARP-14 deficiency results in altered development of these autoimmune diseases.

We observed that PARP-14 and its activity influence IL-17 secretion during Th17 differentiation. In a recent report, Cho *et al.*[40] observed similar results. In that study, CD4^{+} T cells were reconstituted with a truncated form of PARP-14 and cultured under Th17 skewing conditions. Smaller amounts of IL-17 were measured in cells with impaired PARP activity. We extended those studies by showing that IL-21, which re-enforces the Th17 phenotype, is also diminished in the absence of PARP-14. We have further established that PARP-14 regulates *Il6ra* and STAT3 phosphorylation coincident with reduced expression of STAT3-induced transcription factors including *Rorc*, *Maf* and *Batf* that contribute to Th17 and Tfh development. The role of IL-17 in airway inflammation has been described in mouse models and human populations. Increased mRNA levels of *IL17A*, *IL17F* and *IL21* are found in asthmatics.[41,42] Further, elegant studies in mice observed that IL-17 promotes neutrophil and eosinophil trafficking into the lungs.[43] We have shown previously that PARP-14-deficient mice have reduced cellular infiltration in the lungs with the greatest reduction of neutrophils and eosinophils.[15] The decreased levels of Th17 cytokines

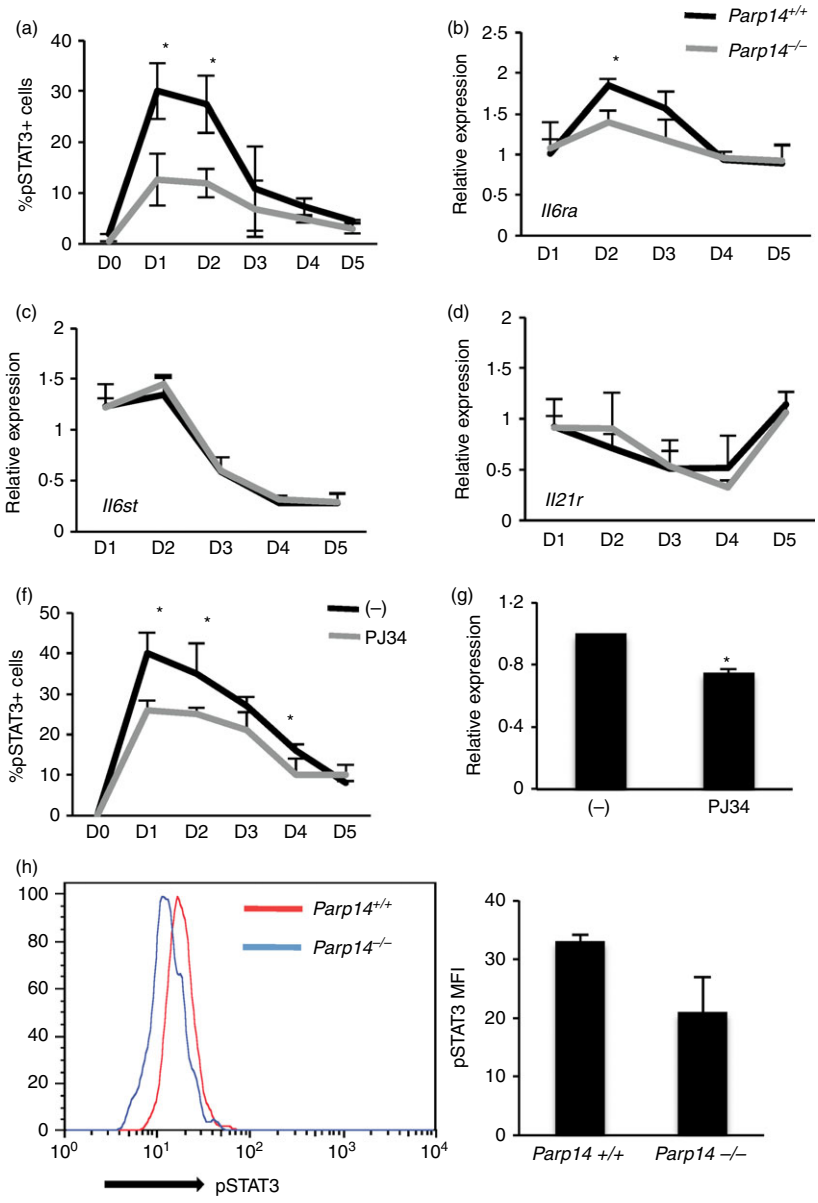

Figure 5. Decreased signal transducer and activator of transcription (STAT3) activation in the absence of poly (ADP) ribose polymerase 14 (PARP-14) (a, b) Naive $CD4^+$ T cells were isolated from wild-type (WT) and *Parp14*$^{-/-}$ mice and differentiated under T helper type 17 (Th17) polarizing conditions. (a) The levels of phospho-STAT3 (pSTAT3) were measured by intracellular cytokine staining daily during differentiation. (b–d) Expression of the indicated genes was measured daily by quantitative PCR during Th17 differentiation. (f, g) Naive T cells from C57BL/6 mice were differentiated under Th17 polarizing condition in the presence or absence of PJ34. (f) The pSTAT3 was measured daily by intracellular cytokine staining. (g) Gene expression in the differentiated Th17 cells was measured by quantitative PCR. (h) *Parp14*$^{-/-}$ and wild-type mice were immunized with sheep red blood cells (SRBC). On day 9, splenocytes were analysed by flow cytometry with histograms of pSTAT3 staining on gated $CD4^+$ $CXCR5^+$ $PD\text{-}1^+$ cells. Percentages are mean ± SE of four or five mice per group and representative of three independent experiments. $^*P < 0{\cdot}05$.

observed in BAL fluid and the supernatants of antigen-stimulated splenocytes could contribute to this observation. Although the airway inflammation in the model we used is not strictly dependent on IL-17, these observations support the further study of PARP-14-deficient mice in infection models where inflammation is entirely IL-17-dependent.

Parp14$^{-/-}$ mice have impaired antibody responses and PARP-14 might act mechanistically at several points. We and another group demonstrated that primary responses were diminished and recall responses were further affected by PARP-14 deficiency.[15,40] Cho *et al.*[40] observed that PARP-14 deficiency had no effect on the primary IgA response but the IgA recall response was

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reduced compared with that in controls. Basal serum IgA levels were also reduced in *Parp14*$^{-/-}$ mice. Similarly, we observed defects in IgE antibody production in response to OVA sensitization and challenge.[15] We extended these studies, by specifically examining the development of Tfh cells. We observed diminished Tfh and GCB cell frequency and numbers in response to SRBC and influenza virus immunizations, and decreased titres of SRBC-specific IgG. These studies define a novel role for PARP-14 in Tfh cell development, and suggest that impaired antibody levels in PARP-14-deficient mice are not restricted to specific antigens. In Cho *et al.*[40] elegant adoptive transfer studies showed that impaired IgA levels were the result of a B-cell extrinsic pathway, whereas the IgE phenotype was B-cell intrinsic. Hence, whereas PARP-14 might be acting in a B-cell intrinsic manner with STAT6 in the induction of IgE class switching, the PARP-14-dependent B-cell extrinsic mechanism might rely upon PARP-14-dependent Tfh and GC B-cell development.

Studies have indicated that PARP-14 influenced STAT6-mediated gene expression. However, RNA pol II ChIP-seq experiments identified genes that were PARP-14-dependent but STAT6-independent.[14] It was not clear what other pathways PARP-14 might impact. The current study indicates that PARP-14 regulates STAT3 activation, thereby regulating Th17 and Tfh differentiation. STAT3 phosphorylation is observed early on during Th17 differentiation and PARP-14 deficiency reduced pSTAT3 on days 1 and 2 of culture. The effect of PARP-14 on STAT3 activation is not limited to Th17 differentiation; Tfh cells derived from SRBC-immunized mice also had reduced pSTAT3. It is still unclear whether the effects of PARP-14 on STAT3 activation are entirely by decreasing *Il6ra* expression, or whether there is a direct physical interaction between STAT3 and PARP-14 as there is between STAT6 and PARP-14. Regardless, as the IL-6–STAT3 pathway is an important signalling cascade in various cancers including gastric cancer, and is also important in autoimmune disease, PARP-14 may be a potential therapeutic target for the modulation of this pathway.

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Author contributions

PM and PK performed the experiments. JS provided influenza virus. PM, SG and MHK designed the experiments. PM, JS and MHK wrote the paper.

Disclosures

The authors have no financial conflicts of interest.

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