

Blockade of tumor necrosis factor in collagen-induced arthritis reveals a novel immunoregulatory pathway for Th1 and Th17 cells

Clare A. Notley, Julia J. Inglis, Saba Alzabin, Fiona E. McCann, Kay E. McNamee, and Richard O. Williams

Kennedy Institute of Rheumatology Division, Imperial College London, London, W6 8LH, England, UK

IL-17 is implicated in the pathogenesis of rheumatoid arthritis (RA) and has previously been shown to be induced by tumor necrosis factor (TNF) *in vitro*. The aim of this study was to assess the impact of TNF inhibition on IL-17 production in collagen-induced arthritis, a model of RA. TNF blockade using TNFR-Fc fusion protein or anti-TNF monoclonal antibody reduced arthritis severity but, unexpectedly, expanded populations of Th1 and Th17 cells, which were shown by adoptive transfer to be pathogenic. Th1 and Th17 cell populations were also expanded in collagen-immunized TNFR p55^{-/-} but not p75^{-/-} mice. The expression of IL-12/IL-23 p40 was up-regulated in lymph nodes (LN) from p55^{-/-} mice, and the expansion of Th1/Th17 cells was abrogated by blockade of p40. Treatment of macrophages with rTNF also inhibited p40 production *in vitro*. These findings indicate that at least one of the ways in which TNF regulates Th1/Th17 responses in arthritis is by down-regulating the expression of p40. Finally, although TNF blockade increased numbers of Th1 and Th17 cells in LN, it inhibited their accumulation in the joint, thereby providing an explanation for the paradox that anti-TNF therapy ameliorates arthritis despite increasing numbers of pathogenic T cells.

CORRESPONDENCE
Richard O. Williams:
richard.o.williams@imperial.ac.uk

Rheumatoid arthritis (RA) is a chronic autoimmune disease in which proinflammatory cytokines, such as TNF α , IL-6, and IL-1, play dominant pathological roles. More recently, IL-17 has been suggested to play an important additional role in the induction and maintenance of RA (1, 2). Thus, IL-17 is present in the synovium of RA patients and contributes to the production of IL-6 and MMP-1 in the joint (2, 3), whereas treatment of human macrophages with IL-17 *in vitro* stimulates the production of TNF α and IL-1 β (4). IL-17 can also synergize with TNF α to induce cytokine and chemokine production by synovial fibroblasts and cartilage destruction *in vitro* and can promote osteoclastogenesis (1, 5, 6).

IL-17 is a proinflammatory cytokine produced predominantly by T helper cells (Th17 cells) and, although there is controversy over

the signals required for the differentiation of murine and human Th17 cells, both murine and human CD4⁺ Th17 T cells require IL-23 for their proliferation and maintenance (7). IL-23 is a heterodimeric protein composed of a p19 subunit and a p40 subunit, whereas IL-12, an important cytokine for Th1 cell differentiation, is formed when the p40 subunit dimerizes with p35 (8).

The role of TNF α in RA is well documented, with TNF α -blocking biologics causing amelioration of clinical symptoms (e.g., pain, joint swelling, and stiffness), laboratory parameters of inflammation (e.g., CRP and ESR), and radiological progression of disease (9, 10). Although TNF α plays a direct pathological role in RA, its contribution to disease pathogenesis is amplified by its ability to promote the expression

C.A. Notley's present address is Centre for Rheumatology, University College London, Windeyer Building, London W1T 4JF, England, UK.

The online version of this article contains supplemental material.

© 2008 Notley et al. This article is distributed under the terms of an Attribution-Noncommercial-Share Alike-No Mirror Sites license for the first six months after the publication date (see <http://www.jem.org/misc/terms.shtml>). After six months it is available under a Creative Commons License (Attribution-Noncommercial-Share Alike 3.0 Unported license, as described at <http://creativecommons.org/licenses/by-nc-sa/3.0/>).

of other proinflammatory cytokines. For example, TNF α has been shown *in vitro* to drive the production of IL-17 by equipping DC with the ability to differentiate T cells toward a Th17 phenotype (11). On this basis, it would be predicted that TNF α blockade would result in reduced IL-17 expression, and to test this hypothesis *in vivo*, we investigated the dependence of IL-17 expression on TNF α in collagen-induced arthritis (CIA). Surprisingly, our data show that TNF α is an important negative regulator, not only of IL-17 but also of IFN γ production by T cells. We propose that this forms a part of a negative feedback loop that attempts to limit the intensity and/or duration of Th17 and Th1 responses.

RESULTS AND DISCUSSION

To investigate the effect of blockade of TNF α on the production of IL-17, DBA/1 mice were immunized with bovine type II collagen in CFA. After onset of arthritis, mice were treated with soluble TNFR-Fc for 10 d and the production of IL-17 and IFN γ by LN cells was determined by ELISA. Significantly increased IL-17 and IFN γ production was observed after stimulation of LN cells from TNFR-Fc-treated mice with collagen or anti-CD3 mAb *in vitro*, and a trend toward enhanced production of these cytokines was observed even in unstimulated LN cells (Fig. 1). As expected, arthritis severity was significantly reduced in TNFR-Fc-treated mice despite the increased IL-17 and IFN γ production (Fig. 1).

We next set out to establish whether the increased IFN γ and IL-17 production *in vitro* by LN cells after blockade of TNF α was paralleled by increased numbers of Th1 and Th17 cells *in vivo*. In addition, we compared the effect of TNF α blockade on numbers of Th1/Th17 cells during the T cell expansion phase (days 0–14 after immunization) versus the phase of T cell contraction (days 1–14 after disease onset). For this experiment, we used anti-TNF α mAb (TN3-19.12), which has been characterized extensively in CIA (12). Anti-TNF α mAb treatment lead to a significant expansion of the proportion of CD4⁺IFN γ ⁺ cells in inguinal LN, irrespective of whether it was administered from days 0–14 after immunization or days 1–14 after disease onset (Fig. S1, available at <http://www.jem.org/cgi/content/full/jem.20072707/DC1>). There was a trend toward increased numbers of CD4⁺IL-17⁺ cells during the expansion phase and a significant increase in CD4⁺IL-17⁺ cells during the contraction phase.

It was concluded that early TNF α blockade led to a significant expansion of Th1 cells, whereas late TNF α blockade led to a significant expansion of both Th1 and Th17 cells. The coordinate expansion of both Th17 and Th1 cells *in vivo* after blockade of TNF α is surprising and challenges the assumption that the differentiation of these two subsets is mutually antagonistic (13, 14). Less than 0.2% of CD4⁺ cells from control or anti-TNF α -treated mice were double positive for IFN γ and IL-17 (Fig. S1).

Thus far, the data suggest that signaling via the TNF receptor inhibits Th1/Th17 responses. To confirm this finding, and to establish which of the two TNF receptors (p55 or p75)

is responsible for delivering the inhibitory signal, Th1/Th17 responses were assessed in p55^{-/-}, p75^{-/-}, and WT mice immunized 14 d previously with type II collagen in CFA. This time point was chosen because maximal T cell responses are normally detected around this time.

The results show conclusively that inhibition of Th1/Th17 responses occurs via the p55 and not the p75 TNF receptor. Thus, the production of IL-17 and IFN γ was dramatically higher in collagen or anti-CD3 mAb-stimulated LN cell cultures from p55^{-/-} mice compared with either WT or p75^{-/-} mice (Fig. 2 A). Further analysis by flow cytometry confirmed

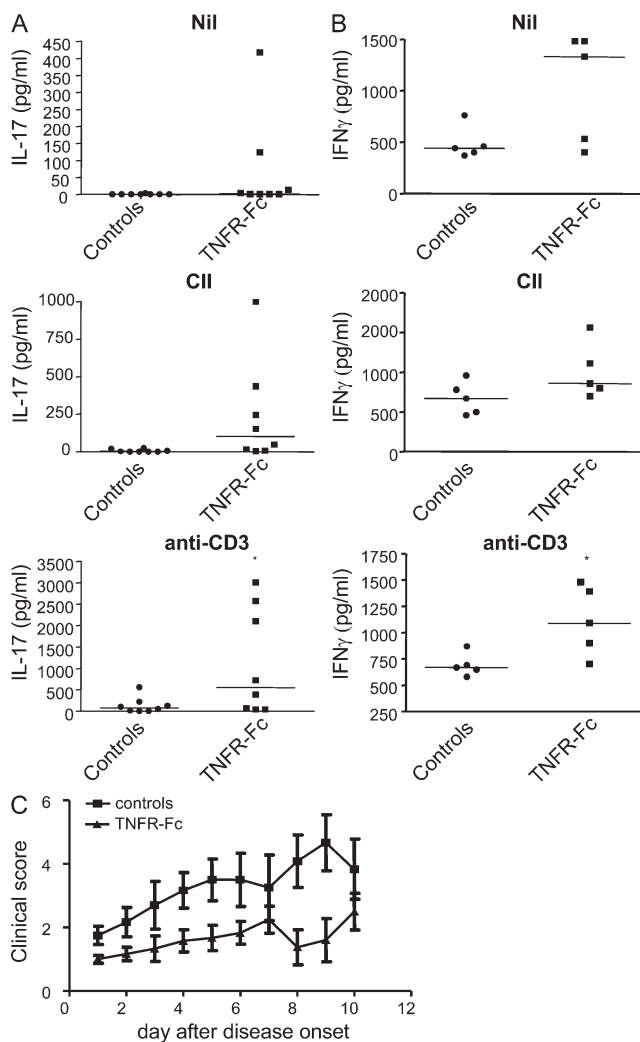


Figure 1. Increased IL-17 and IFN γ production in CIA after blockade of TNF α . DBA/1 mice with CIA were treated with TNFR-Fc or isotype control mAb (100 μ g/mouse on alternate days) from the time of disease onset. (A and B) LN cells were taken 10 d after disease onset and levels of IL-17 (A) and IFN γ (B) were determined by ELISA in the supernatants without further stimulation (Nil) or after stimulation with type II collagen (CII) or anti-CD3 mAb (CD3). Data show individual mice ($n = 8$; *, $P < 0.05$). (C) Clinical scores were assessed over the 10-d period in TNFR-Fc-treated and control mice. The data are representative of at least three experiments. Error bars show SEM.

that the proportion of CD4⁺ T cells producing IL-17 and IFN γ in the LN of p55^{-/-} mice was significantly greater than in those from WT or p75^{-/-} mice (Fig. 2 B). However, increased IFN γ and IL-17 responses were not observed in anti-CD3-stimulated LN cells from nonimmunized p55^{-/-} mice (Fig. 2, A and B), indicating that the T cells were not skewed toward Th1/Th17 responses before immunization. The percentage of CD4⁺ cells in immunized WT mice coexpressing IFN γ and IL-17 was low ($\sim 0.1\%$) and was not altered in p55^{-/-} or p75^{-/-} mice.

Despite the increase in Th1/Th17 responses observed in immunized p55^{-/-} mice, the proliferative responses of T cells from p55^{-/-}, p75^{-/-}, and WT mice did not vary significantly in response to collagen or anti-CD3 mAb stimulation (Fig. 2 C), and the percentages of CD4⁺ T cells in the LN of WT and p55^{-/-} mice were comparable, although slightly reduced in p75^{-/-} mice (Fig. 2 C). The percentage of regulatory CD4⁺Foxp3⁺ T cells remained unchanged in the p55^{-/-} and p75^{-/-} mice when compared with WT mice. IL-4 and IL-5 were undetectable in immunized WT, p55^{-/-}, and p75^{-/-} mice, which was attributed to the

strongly Th1/Th17-skewing properties of CFA. The fact that expanded populations of Th1 and/or Th17 cells were observed in inguinal LN of p55^{-/-} and anti-TNF-treated mice before the onset of arthritis indicates that there was not simply a redistribution of cells away from the joint to the LN.

We next sought to identify the mechanism by which TNF α reduces Th17 and Th1 cell activity. IL-12 and IL-23 share a common subunit, p40. Dimerization of p40 with p35 forms IL-12, which is involved in the differentiation of Th1 cells, whereas dimerization of p40 with p19 forms IL-23, which has an important role in the generation and/or survival of Th17 cells. Hence, one possible explanation for our findings is that TNF α conditions myeloid cells toward reduced p40 expression, and we set out to address this question using thioglycolate-elicited macrophages stimulated with LPS in vitro. Pretreatment of macrophages before LPS stimulation with 30 or 100 ng/ml of TNF α produced a dose-dependent reduction of p40 upon subsequent stimulation with LPS (Fig. 3). The maximum inhibition of p40 production by LPS-stimulated macrophages was $\sim 50\%$, and the failure to obtain greater suppression was attributed to the fact that LPS alone would inevitably produce significant quantities of TNF α . TNF α pretreatment also suppressed IL-6 production at 100 ng/ml, but not at 30 ng/ml, but had no effect on IL-1 β production at either dose (Fig. 3). This shows that the effect of TNF α on cytokine production was selective and did not cause global suppression of cellular activity.

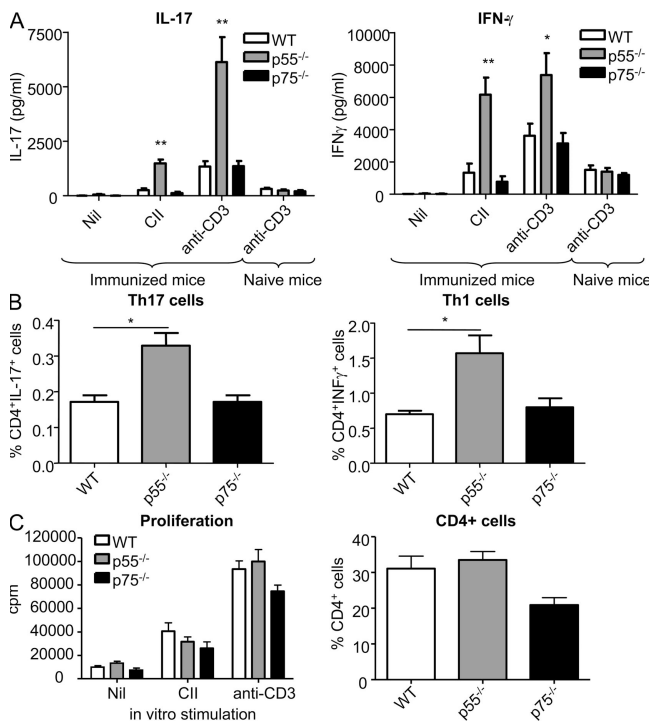


Figure 2. Amplification of Th17 and Th1 cell activity in p55 TNFR^{-/-} mice. LN cells from WT, p55 TNFR^{-/-}, and p75 TNFR^{-/-} mice were taken 14 d after immunization with type II collagen in CFA. (A) LN cells were either unstimulated or stimulated with collagen or with anti-CD3 mAb, and the level of proliferation was determined by [³H]thymidine incorporation. The percentage of CD4⁺ T cells in the LN was determined by flow cytometry on day 14 after immunization. (B) Levels of IL-17 and IFN γ were determined by ELISA. (C) The proportion of CD4⁺ cells in the LN producing IL-17 and IFN γ were determined by flow cytometry. Histograms show mean $\pm$ SEM ($n = 8$). *, $P < 0.05$; **, $P < 0.01$. Data are representative of two experiments.

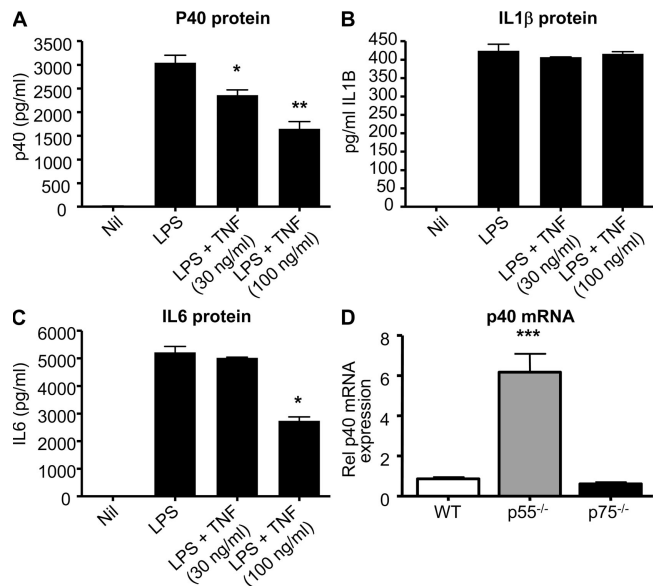


Figure 3. TNF α inhibits expression of IL-12/IL-23 p40. Thioglycolate-elicited macrophages were cultured in the presence or absence of 30 or 100 ng/ml TNF α for 8 h and then stimulated for a further 18 h with 1 ng/ml LPS. (A–C) Levels of p40, IL-1 β , and IL-6 protein were determined in the culture supernatants by ELISA. (D) Relative levels of p40 mRNA from WT, p55 TNFR^{-/-}, and p75 TNFR^{-/-} LN cells 14 d after immunization were determined by real time PCR. Histograms show mean $\pm$ SEM ($n = 4$). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Next, we addressed the question of whether p40 expression was elevated in vivo in the absence of signaling via the p55 TNFR. To quantify p40 expression without any form of manipulation in vitro, mRNA was extracted from LN cells immediately postmortem and analyzed by real-time PCR. LN cells from immunized p55^{-/-} mice were found to express sixfold greater levels of p40 mRNA compared with immunized WT and p75^{-/-} mice (Fig. 3). This confirms the ability of TNF α to suppress p40 expression in vivo.

We then questioned whether inhibition of IL-12/IL-23 p40 activity in p55^{-/-} mice would result in a reduction of IFN γ /IL-17 production and reduced numbers of Th1/Th17 cells. Treatment with a blocking anti-mouse p40 mAb in p55^{-/-} mice from the day of immunization to day 14 after immunization partially or completely abrogated both the increase in IL-17 and IFN γ production and the expansion of CD4⁺IL-17⁺ and CD4⁺IFN γ ⁺ cells (Fig. 4). These findings strongly suggest that at least one of the mechanisms by which TNF α influences the development and/or survival of Th1 and Th17 cells is by inhibition of IL-12/IL-23 p40 expression, although other mechanisms may also be involved.

These findings raise the important question of why TNF α blockade is effective in reducing disease activity despite increasing numbers of Th1 and Th17 cells. To address this question, we used an adoptive transfer system established previously (15) to confirm that the expanded Th1/Th17 cells were potentially pathogenic. Spleen and LN cells from arthritic mice treated for 10–14 d with anti-TNF α mAb or control Ab were pooled and injected into CB-17 SCID mice (5×10^7 cells/mouse or 10^7 cells/mouse). The SCID recipients were also injected i.p. with 100 μ g of type II collagen without adjuvant, which is required for the successful transfer of arthritis (15). The proportion of LN cells to spleen cells was $\sim 1:10$ and was identical in both anti-TNF α -treated and control groups.

Transfer of 5×10^7 cells from either anti-TNF α -treated or control mice led to efficient transfer of arthritis, although onset of arthritis was much earlier when donor cells were derived from anti-TNF α -treated mice (Fig. S2, available at <http://www.jem.org/cgi/content/full/jem.20072707/DC1>). However, when the numbers of donor cells were reduced to 10^7 cells, there was a dramatic difference between the two groups, with the cells from the anti-TNF α -treated donors showing vastly superior transfer of arthritis (Fig. S2). Remarkably, when the recipient SCID mice were treated with anti-TNF α mAb from the time of cell transfer, the development of arthritis was largely abrogated (Fig. S2). This experiment shows conclusively that anti-TNF α therapy expands the population of pathogenic T cells. However, the pathogenicity of these donor cells is largely blocked by anti-TNF α treatment of the recipients.

The enhanced capacity of lymphoid cells from anti-TNF α -treated mice to adoptively transfer arthritis to SCID mice demonstrates that the expansion of Th1/Th17 cells may have pathological consequences when the anti-TNF α “brake” is removed. However, in the presence of TNF α -neutralizing

antibodies, the pathogenic potential of these T cells is effectively neutralized.

One of the mechanisms by which anti-TNF α therapy is known to act in human RA is by preventing inflammatory cell infiltration into the joint (16). On this basis, we hypothesized that the mechanism by which TNF α blockade is able to reduce disease activity while expanding populations of Th1 and Th17 cells is by preventing the migration of T cells into the joint. To validate this hypothesis, arthritic DBA/1 mice were treated for 10–14 d with anti-TNF α mAb or control Ab. The mice were then killed and cells harvested from paws and inguinal LN for subsequent analysis by flow cytometry.

Anti-TNF α treatment of established arthritis reduced total numbers of CD4⁺IFN γ ⁺ and CD4⁺IL-17⁺ cells in joints

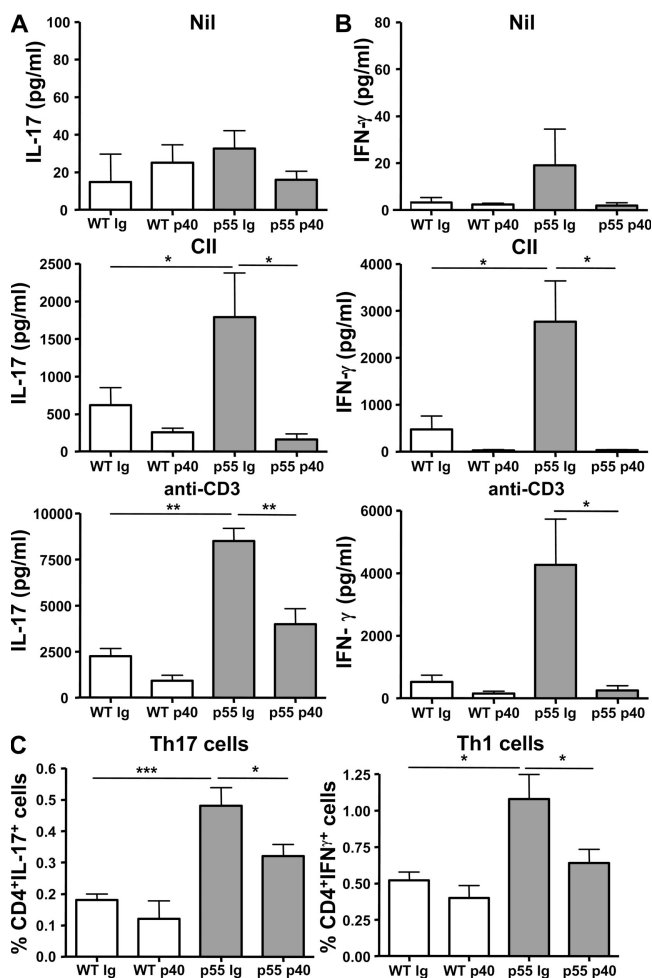


Figure 4. Blockade of IL-12/IL-23 blocks the expansion of Th1/Th17 cells. (A–C) LN cells from immunized WT (white bars) or p55 TNFR^{-/-} (gray bars) mice treated with control Ig (Ig) or rat anti-mouse p40 Ab (p40) were either unstimulated or stimulated with collagen or anti-CD3 mAb. Levels of IL-17 (A) and IFN γ (B) were determined by ELISA, and the proportion of CD4⁺ cells in the LN producing IL-17 and IFN γ were determined by flow cytometry (C). Histograms show mean $\pm$ SEM ($n = 5$). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Data are representative of two experiments.

by 63% and 67%, respectively, despite being present in increased numbers in inguinal LN (Fig. 5). It was concluded that one of the mechanisms of action of anti-TNF α therapy is to inhibit immigration of pathogenic T cells to the joint or prevent their emigration from LN. It was beyond the scope of the present study to investigate the mechanisms by which anti-TNF α prevents T cell accumulation in the joint, but they are likely to include reduced chemokine and adhesion molecule expression in the joint, as has been proposed for human RA (16, 17).

The key finding to emerge from this study is that, in addition to its proinflammatory role, TNF α is also responsible for dampening down Th1 and Th17 responses within the context of autoimmune arthritis. We propose that this represents a negative feedback mechanism that normally serves to limit the duration of T cell-driven inflammatory responses.

There are several published studies that support the findings reported here. For example, as observed in the present study, it was reported that TNF α selectively inhibits p40 expression in human and mouse myeloid cells in an IL-10-independent manner (18, 19). In CIA, it was shown that there was elevated IFN γ production (IL-17 was not measured) and increased numbers of CD4 $^{+}$ T cells in TNF $\alpha^{-/-}$ mice compared with WT mice (20). More recently, it was shown that i.p. administration of rTNF α reduced IFN γ production as well as the clinical severity of adjuvant-induced arthritis in rats (21), which is also consistent with our findings. In experimental autoimmune encephalomyelitis (EAE), it was reported that there was enhanced IL-12/IL-23 p40 expression in p55 $^{-/-}$ mice and increased Th1 (but not Th17) cells in the CNS compared with WT mice (22).

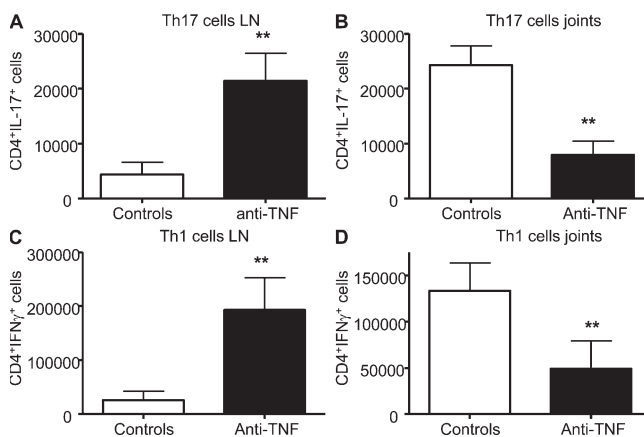


Figure 5. Anti-TNF α therapy prevents the accumulation of Th1/Th17 cells in the joint. Arthritic DBA/1 mice ($n = 6$) were treated once every 3 d for a total of 14 d with anti-TNF α mAb (TN3-19.12; 300 μ g/mouse) or control Ab. CD4 $^{+}$ cells from the inguinal LN and joints (obtained by enzymatic digestion of synovial tissue) were analyzed for intracellular cytokine expression by flow cytometry after stimulation with PMA/ionomycin. A, CD4 $^{+}$ IL-17 $^{+}$ cells in LN; B, CD4 $^{+}$ IL-17 $^{+}$ cells in joints; C, CD4 $^{+}$ IFN γ^{+} cells in LN; D, CD4 $^{+}$ IFN γ^{+} cells in joints. **, $P < 0.01$. Data are representative of two experiments. Error bars show SEM.

Anti-TNF α therapy is now undergoing evaluation in an increasing number of diseases and its effect is not universally beneficial. For example, TNF α blockade was shown to increase both the rate and frequency of relapse in patients with existing multiple sclerosis (23). In EAE, TNF $\alpha^{-/-}$ mice developed enhanced inflammation and demyelination, whereas treatment of susceptible mice with TNF α reduced the severity of disease (24). In another study, EAE failed to resolve in TNF $\alpha^{-/-}$ or TNFR $^{-/-}$ mice, suggesting that TNF α plays an important role in resolution of inflammation (25). Similarly, in murine lupus, administration of rTNF α was found to be protective (26), whereas TNF α deficiency was associated with increased production of antinuclear antibodies and accelerated onset of disease (27). Hence, an important question is whether the deleterious effects of TNF α blockade in these diseases are mediated via the expansion of Th1 and/or Th17 cells.

As discussed in a previous paragraph, TNF α has been reported to decrease p40 expression in human macrophages (18). Therefore, the findings presented in this paper may have implications for human RA. As in CIA, the therapeutic efficacy of TNF α blockade in human RA is indisputable (28), but it is possible that the rare occurrence of side effects, such as antinuclear autoantibodies and demyelination (29), could be explained by an amplification of Th17 and/or Th1 responses. However, it is also possible that the increased Th1/Th17 responses in the periphery versus the joint after anti-TNF α therapy may have beneficial consequences by increasing resistance to infection. This may help to explain the relatively low impact of TNF α blockade on susceptibility to infection (30).

In conclusion, the results of this study show that TNF α plays at least two distinct and opposing roles in CIA. First, it contributes to the accumulation of Th1 and Th17 cells in arthritic joints, and second, it plays an inhibitory role by limiting total numbers of these pathogenic T cell subsets in peripheral lymphoid organs.

MATERIALS AND METHODS

Mice. DBA/1, C57BL/6, and CB-17 SCID mice were purchased from Harlan. p55 $^{-/-}$ and p75 $^{-/-}$ mice were bred in house on a C57BL/6 background. All experimental procedures were approved by the Ethical Review Process Committee.

Immunization. DBA/1 and C57BL/6 mice were immunized with CFA plus bovine or chicken type II collagen, respectively (31). Arthritis severity was assessed as follows: 0, normal; 1, slight swelling and/or erythema; 2, pronounced edematous swelling; and 3, ankylosis. Each limb was graded, giving a maximum score of 12.

Anticytokine therapy. Blockade of TNF α was achieved using murine p75 TNFR-Fc (donated by GlaxoSmithKline) or hamster anti-mouse TNF α mAb (TN3-19.12; provided by R.D. Schreiber, Washington University School of Medicine, St. Louis, MO). Blockade of IL-12/IL-23 was achieved using rat anti-mouse p40 mAb (c17.8; donated by G. Trinchieri, then at Wistar Institute, Philadelphia, PA).

LN cell culture. LN cells were cultured at a density of 2×10^6 cells/ml in RPMI-1640 with L-glutamine plus 10% FCS, penicillin/streptomycin, sodium pyruvate, and β_2 -mercaptoethanol and stimulated with 50 μ g/ml

of type II collagen or 0.1 $\mu\text{g}/\text{ml}$ of anti-CD3 mAb (145-2C11). Supernatants were collected for cytokine analysis after 48 h. Cells were then incubated for a further 16 h in the presence of 1 $\mu\text{Ci}/\text{well}$ of [^3H]thymidine to quantify proliferation.

Thioglycolate-elicited macrophages. Mice were injected i.p. with 1 ml of 3% thioglycolate. After 3 d, mice were killed and peritoneal macrophages collected by PBS lavage. After overnight adherence, cells were incubated for 8 h in the presence or absence of 30 ng/ml rTNF α (PeproTech), followed by 18 h in the presence of 1 ng/ml LPS (Sigma-Aldrich).

Isolation of cells from joints. The skin was removed from arthritic hind paws, and synovial cells were liberated by digestion with 1.6 U/ml Liberase C (Roche) and 0.2 mg/ml DNase I (Roche) for 40–60 min at 37°C.

Cytokine measurement. Cytokines were measured using commercially available kits as follows: IFN γ (BD Biosciences); IL-17A (R&D Systems); and IL-12/IL-23 p40, IL-1 β , and IL-6 (eBioscience).

Flow cytometry. For intracellular cytokine staining, cells were stimulated for up to 10 h with PMA and ionomycin. Brefeldin A was added for the last 4 h. For surface staining, cells were incubated with anti-CD4 or anti-CD8 (BD Biosciences) for 30 min at 4°C, washed, and then fixed in Cytofix (BD Biosciences). Cells were permeabilized using PBS containing 1% FCS, 0.01% sodium azide, and 0.05% saponin and stained with anti-mouse IFN γ (BD Biosciences), anti-mouse IL-4 (BD Biosciences), and anti-mouse IL-17 (Cambridge BioScience) and analyzed on FACS Canto II using FACSDIVA software (BD Biosciences).

Real-time quantitative RT-PCR. RNA was isolated using the RNeasy protect mini kit (QIAGEN) and cDNA transcribed using the reverse transcription system (Promega). p40 gene expression was determined by real-time PCR using predesigned TaqMan primers and probe (Applied Biosystems) by the comparative method of relative quantitation. HPRT mRNA was used as an endogenous control to check for RNA and cDNA differences within samples. Differences in the mean threshold cycle (C_t) for the target gene p40 and the C_t for HPRT RNA, indicated by ΔC_t , were calculated to normalize differences in the mRNA extractions and the efficiency of the reverse transcription. The relative mRNA amount for each target gene was calculated as $\Delta\Delta C_t$ and expressed as fold change compared with a control sample.

Statistical analysis. The unpaired t test or one way ANOVA with Dunnett's multiple comparison test was used to test statistical significance. A p -value of <0.05 was considered significant.

Online supplemental material. Fig. S1 shows the expansion of Th1 and Th17 cells after treatment with anti-TNF α mAb. Fig. S2 uses an adoptive transfer model to demonstrate that the expanded population of Th1/Th17 cells is pathogenic. Online supplemental material is available at <http://www.jem.org/cgi/content/full/jem.20072707/DC1>.

This work was supported by the Arthritis Research Campaign and by the Kennedy Institute of Rheumatology Trustees.

The authors have no conflicting financial interests.

Submitted: 20 December 2007

Accepted: 22 September 2008

REFERENCES

- Kotake, S., N. Udagawa, N. Takahashi, K. Matsuzaki, K. Itoh, S. Ishiyama, S. Saito, K. Inoue, N. Kamatani, M.T. Gillespie, et al. 1999. IL-17 in synovial fluids from patients with rheumatoid arthritis is a potent stimulator of osteoclastogenesis. *J. Clin. Invest.* 103:1345–1352.
- Chabaud, M., J.M. Durand, N. Buchs, F. Fossiez, G. Page, L. Frappart, and P. Miossec. 1999. Human interleukin-17: A T cell-derived pro-inflammatory cytokine produced by the rheumatoid synovium. *Arthritis Rheum.* 42:963–970.
- Chabaud, M., P. Garnero, J.M. Dayer, P.A. Guerne, F. Fossiez, and P. Miossec. 2000. Contribution of interleukin 17 to synovium matrix destruction in rheumatoid arthritis. *Cytokine*. 12:1092–1099.
- Jovanovic, D.V., J.A. Di Battista, J. Martel-Pelletier, F.C. Jolicoeur, Y. He, M. Zhang, F. Mineau, and J.P. Pelletier. 1998. IL-17 stimulates the production and expression of proinflammatory cytokines, IL-beta and TNF-alpha, by human macrophages. *J. Immunol.* 160:3513–3521.
- Katz, Y., O. Nativ, and Y. Beer. 2001. Interleukin-17 enhances tumor necrosis factor alpha-induced synthesis of interleukins 1,6, and 8 in skin and synovial fibroblasts: a possible role as a “fine-tuning cytokine” in inflammation processes. *Arthritis Rheum.* 44:2176–2184.
- Van Bezooijen, R.L., L. Van Der Wee-Pals, S.E. Papapoulos, and C.W. Lowik. 2002. Interleukin 17 synergizes with tumour necrosis factor alpha to induce cartilage destruction in vitro. *Ann. Rheum. Dis.* 61:870–876.
- Hoeve, M.A., N.D. Savage, T. de Boer, D.M. Langenberg, R. de Waal Malefyt, T.H. Ottenhoff, and F.A. Verreck. 2006. Divergent effects of IL-12 and IL-23 on the production of IL-17 by human T cells. *Eur. J. Immunol.* 36:661–670.
- Oppmann, B., R. Lesley, B. Blom, J.C. Timans, Y. Xu, B. Hunte, F. Vega, N. Yu, J. Wang, K. Singh, et al. 2000. Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. *Immunity*. 13:715–725.
- Elliott, M.J., R.N. Maini, M. Feldmann, J.R. Kalden, C. Antoni, J.S. Smollen, B. Leeb, F.C. Breedfeld, J.D. Macfarlane, H. Bijl, and J.N. Woody. 1994. Treatment with a chimaeric monoclonal antibody to tumour necrosis factor α suppresses disease activity in rheumatoid arthritis: results of a multi-centre, randomised, double blind trial. *Lancet*. 344:1105–1110.
- Smolen, J.S., C. Han, M. Bala, R.N. Maini, J.R. Kalden, D. van der Heijde, F.C. Breedveld, D.E. Furst, and P.E. Lipsky. 2005. Evidence of radiographic benefit of treatment with infliximab plus methotrexate in rheumatoid arthritis patients who had no clinical improvement: a detailed subanalysis of data from the anti-tumor necrosis factor trial in rheumatoid arthritis with concomitant therapy study. *Arthritis Rheum.* 52:1020–1030.
- Iwamoto, S., S. Iwai, K. Tsujiyama, C. Kurahashi, K. Takeshita, M. Naoe, A. Masunaga, Y. Ogawa, K. Oguchi, and A. Miyazaki. 2007. TNF-alpha drives human CD14+ monocytes to differentiate into CD70+ dendritic cells evoking Th1 and Th17 responses. *J. Immunol.* 179:1449–1457.
- Williams, R.O., M. Feldmann, and R.N. Maini. 1992. Anti-tumor necrosis factor ameliorates joint disease in murine collagen-induced arthritis. *Proc. Natl. Acad. Sci. USA.* 89:9784–9788.
- Park, H., Z. Li, X.O. Yang, S.H. Chang, R. Nurieva, Y.H. Wang, Y. Wang, L. Hood, Z. Zhu, Q. Tian, and C. Dong. 2005. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nat. Immunol.* 6:1133–1141.
- Harrington, L.E., R.D. Hatton, P.R. Mangan, H. Turner, T.L. Murphy, K.M. Murphy, and C.T. Weaver. 2005. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat. Immunol.* 6:1123–1132.
- Williams, R.O., C. Plater-Zyberk, D.G. Williams, and R.N. Maini. 1992. Successful transfer of collagen-induced arthritis to severe combined immunodeficient (SCID) mice. *Clin. Exp. Immunol.* 88:455–460.
- Taylor, P.C., A.M. Peters, E. Paleolog, P.T. Chapman, M.J. Elliott, R. McCloskey, M. Feldmann, and R.N. Maini. 2000. Reduction of chemokine levels and leukocyte traffic to joints by tumor necrosis factor alpha blockade in patients with rheumatoid arthritis. *Arthritis Rheum.* 43:38–47.
- Tak, P.P., P.C. Taylor, F.C. Breedveld, T.J. Smeets, M.R. Daha, P.M. Kluin, A.E. Meinders, and R.N. Maini. 1996. Decrease in cellularity and expression of adhesion molecules by anti-tumor necrosis factor alpha monoclonal antibody treatment in patients with rheumatoid arthritis. *Arthritis Rheum.* 39:1077–1081.
- Ma, X., J. Sun, E. Papasavvas, H. Riemann, S. Robertson, J. Marshall, R.T. Bailer, A. Moore, R.P. Donnelly, G. Trinchieri, and L.J. Montaner. 2000. Inhibition of IL-12 production in human monocyte-derived macrophages by TNF. *J. Immunol.* 164:1722–1729.

19. Zakharova, M., and H.K. Ziegler. 2005. Paradoxical anti-inflammatory actions of TNF- α : inhibition of IL-12 and IL-23 via TNF receptor 1 in macrophages and dendritic cells. *J. Immunol.* 175:5024–5033.
20. Campbell, I.K., K. O'Donnell, K.E. Lawlor, and I.P. Wicks. 2001. Severe inflammatory arthritis and lymphadenopathy in the absence of TNF. *J. Clin. Invest.* 107:1519–1527.
21. Kim, E.Y., H.H. Chi, R. Rajaiah, and K.D. Moudgil. 2008. Exogenous tumor necrosis factor- α induces suppression of autoimmune arthritis. *Arthritis Res. Ther.* 10:R38.
22. Wheeler, R.D., S.P. Zehntner, L.M. Kelly, L. Bourbonniere, and T. Owens. 2006. Elevated interferon gamma expression in the central nervous system of tumour necrosis factor receptor 1-deficient mice with experimental autoimmune encephalomyelitis. *Immunology.* 118:527–538.
23. 1999. TNF neutralization in MS: results of a randomized, placebo-controlled multicenter study. The Lenercept Multiple Sclerosis Study Group and The University of British Columbia MS/MRI Analysis Group. *Neurology.* 53:457–465.
24. Liu, J., M.W. Marino, G. Wong, D. Grail, A. Dunn, J. Bettadapura, A.J. Slavin, L. Old, and C.C. Bernard. 1998. TNF is a potent anti-inflammatory cytokine in autoimmune-mediated demyelination. *Nat. Med.* 4:78–83.
25. Kassiotis, G., and G. Kollias. 2001. Uncoupling the proinflammatory from the immunosuppressive properties of tumor necrosis factor (TNF) at the p55 TNF receptor level: implications for pathogenesis and therapy of autoimmune demyelination. *J. Exp. Med.* 193:427–434.
26. Jacob, C.O., and H.O. McDevitt. 1988. Tumour necrosis factor- α in murine autoimmune 'lupus' nephritis. *Nature.* 331:356–358.
27. Kontoyiannis, D., and G. Kollias. 2000. Accelerated autoimmunity and lupus nephritis in NZB mice with an engineered heterozygous deficiency in tumor necrosis factor. *Eur. J. Immunol.* 30:2038–2047.
28. Feldmann, M., and R.N. Maini. 2003. Lasker Clinical Medical Research Award. TNF defined as a therapeutic target for rheumatoid arthritis and other autoimmune diseases. *Nat. Med.* 9:1245–1250.
29. Desai, S.B., and D.E. Furst. 2006. Problems encountered during anti-tumour necrosis factor therapy. *Best Pract. Res. Clin. Rheumatol.* 20:757–790.
30. Bongartz, T., A.J. Sutton, M.J. Sweeting, I. Buchan, E.L. Matteson, and V. Montori. 2006. Anti-TNF antibody therapy in rheumatoid arthritis and the risk of serious infections and malignancies: systematic review and meta-analysis of rare harmful effects in randomized controlled trials. *JAMA.* 295:2275–2285.
31. Inglis, J.J., G. Criado, M. Medghalchi, M. Andrews, A. Sandison, M. Feldmann, and R.O. Williams. 2007. Collagen-induced arthritis in C57BL/6 mice is associated with a robust and sustained T-cell response to type II collagen. *Arthritis Res. Ther.* 9:R113.