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*J Immunol* 2011; 187:2696-2701; Prepublished online 22 July 2011;
doi: 10.4049/jimmunol.1101149
http://www.jimmunol.org/content/187/5/2696

Supplementary Material [http://www.jimmunol.org/content/suppl/2011/08/02/jimmunol.1101149.DC1](http://www.jimmunol.org/content/suppl/2011/08/02/jimmunol.1101149.DC1)

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Zinc Finger Protein Tristetraprolin Interacts with CCL3 mRNA and Regulates Tissue Inflammation

Ju-Gyeong Kang,* Marcelo J. Amar,‡ Alan T. Remaley,† Jaeyul Kwon,‡ Perry J. Blackshear,§ Ping-yuan Wang,* and Paul M. Hwang*†

Zinc finger protein tristetraprolin (TTP) modulates macrophage inflammatory activity by destabilizing cytokine mRNAs. In this study, through a screen of TTP-bound mRNAs in activated human macrophages, we have identified CCL3 mRNA as the most abundantly bound TTP target mRNA and have characterized this interaction via conserved AU-rich elements. Compared to the wild-type cells, TTP<sup>−/−</sup> macrophages produced higher levels of LPS-induced CCL3. In addition, the plasma level of CCL3 in TTP<sup>−/−</sup> mice was markedly higher than that in wild-type mice. To determine the in vivo significance of TTP-regulated CCL3, we generated CCL3<sup>−/−</sup>/TTP<sup>−/−</sup> double-knockout mice. Along with decreased proinflammatory cytokines in their paw joints, there were significant functional and histologic improvements in the inflammatory arthritis of TTP<sup>−/−</sup> mice when CCL3 was absent, although cachexia, reflecting systemic inflammation, was notably unaffected. Furthermore, the marked exacerbation of aortic plaque formation caused by TTP deficiency in the APOE<sup>−/−</sup> mouse model of atherosclerosis was also rescued by disrupting CCL3. Taken together, our data indicate that the interaction between TTP and CCL3 mRNA plays an important role in modulating localized inflammatory processes in tissues that are dissociated from the systemic manifestations of chronic inflammation. The Journal of Immunology, 2011, 187: 2696–2701.

The temporal regulation of cytokine and chemokine mRNA levels, through posttranscriptional mechanisms, plays a critical role in regulating the cascades of inflammatory signaling events that when deranged can result in diseases such as autoimmune arthritis (1, 2). The identification of tristetraprolin (TTP), encoded by the zinc finger protein 36 gene, as an essential factor that binds to AU-rich elements (AREs) and destabilizes TNF mRNA provided a novel mechanism for modulating a central inflammatory cytokine (3). Mice with genetic disruption of TTP display an inflammatory syndrome comprised of cachexia, erosive arthritis, myeloid hyperplasia, and serologies consistent with autoimmune disorders (4). Since this seminal report, the expanding number of cytokines with ARE sequences that bind to TTP and other similar proteins have underscored the importance of this mechanism in modulating various immune responses (2, 5).

Identifying the molecular components involved in activating monocytes into effector macrophages may provide new insights into inflammatory diseases such as atherosclerosis (6, 7). Through gene expression analysis, we previously identified TTP as one of the most highly expressed transcriptional regulatory genes in macrophages purified from human atherosclerotic plaques compared with circulating monocytes (8). To further elucidate how TTP might regulate these inflammatory cells, we screened for TTP-interacting mRNA species in activated human macrophages by combining TTP immunoprecipitation with a sequencing-based gene expression technique. One mRNA species bound to TTP at high levels was CCL3 (MIP-1α), a cytokine belonging to the CC motif subfamily of chemokines that is involved in both acute and chronic inflammation (9).

CCL3 is secreted by activated macrophages and other inflammatory cells for diverse functions such as chemotaxis, phagocytosis, and mediator release (9, 10). Thus, as a chemokine present at high tissue concentrations, CCL3 promotes inflammation and has been proposed to be involved in a spectrum of diseases from asthma to multiple sclerosis (11–13). CCL3 is also known to be highly expressed both in the synovial fluid of patients with rheumatoid arthritis and in the plaque tissues of patients with atherosclerosis (14–17). As there is a well-established association between rheumatoid arthritis and atherosclerosis (18), CCL3 could serve as a common chemokine for the recruitment of inflammatory cells at disparate disease sites. Given our previous observation of high TTP expression in atherosclerotic plaque macrophages (8), we speculated that regulation of CCL3 mRNA by TTP may play an important role in atherosclerosis pathogenesis. Although CCL3 mRNA has been reported to have ARE sequences and can be inhibited by TTP (19, 20), the molecular mechanism and functional consequences of the interaction between CCL3 mRNA and TTP have not been well studied. In this study, we characterize the interaction between CCL3 mRNA and TTP. We provide genetic evidence that the loss of this interaction significantly contributes to the increase in inflammatory arthritis and atherosclerosis of TTP-deficient mice, highlighting CCL3 as a pathogenically significant target of TTP in these two common diseases.
Materials and Methods

Animals

All mice were maintained and handled in accordance with National Heart, Lung, and Blood Institute Animal Care and Use Committee standards. TTP^+/− mice were of the C57BL/6 background as described (4, 21). CCL3^−/− mice and APOE−/− mice were also of the C57BL/6 background and obtained from The Jackson Laboratory.

Abs

Abs used for the following proteins were: human TTP rabbit polyclonal (sc-14030; Santa Cruz Biotechnology); mouse TTP goat polyclonal (sc-8458; Santa Cruz Biotechnology); β-actin monoclonal (AC-15; Sigma-Aldrich); and negative control rabbit IgG (sc-2027; Santa Cruz Biotechnology).

Small interfering RNAs and plasmids

Non-specific and TTP-specific small interfering RNA (siRNA) duplexes were obtained from Dharmacon Research. TTP (open reading frame only), full-length CCL3, and GAPDH cDNA in pCMV plasmids were obtained from Genecopoeia. Point mutations in the ARE region of CCL3 were made using the QuikChange II kit (Stratagene) according to the manufacturer’s protocol.

Cell culture and transfection

THP1 human monocyctic cell line was obtained from the American Type Culture Collection and maintained as recommended. THP1 cells were transfected with siRNAs using Nucleofector (Amaxa) according to the manufacturer’s protocol and activated with 2 nM PMA as previously described (8). Bone marrow–derived macrophages (BMDM) were isolated from mouse femora and tibia and cultured for 6 d in DMEM (Invitrogen) supplemented with 10% FBS, penicillin/streptomycin (Invitrogen), and 1% 1,929 cell-conditioned medium. LPS (10 μg/ml, L2880; Sigma-Aldrich) was added to stimulate BMDM for the indicated times. For the mRNA decay assay, BMDM cultures were treated for 90 min with LPS prior to blocking transcription initiation with actinomycin D (5 μg/ml) and harvested at the indicated times. HEK293 cells were transiently transfected with the indicated plasmids using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s protocol.

TTP immunoprecipitation and serial analysis of gene expression

TTP–RNA complex immunoprecipitation was performed as previously described (8). Briefly, 10^6 THP1 cells were stimulated for 4 h with 2 nM PMA and used for cytoplasmic extract preparation, to which anti-TTP Ab (sc-14030; Santa Cruz Biotechnology) or control rabbit IgG (sc-2027; Santa Cruz Biotechnology) was added and incubated overnight at 4°C. RNA isolated from the immunoprecipitation was used to generate serial analysis of gene expression (SAGE) libraries as described (22). From each library, 40,000 tags were sequenced and analyzed using SAGE2000 software (8). Specific fold enrichment was determined by dividing the SAGE tag counts in the TTP versus nonspecific IgG libraries. The p values for SAGE tag counts were calculated as described (23).

mRNA quantification by real-time PCR

Candidate mRNAs identified by TTP immunoprecipitation–SAGE analysis were confirmed using RNA extracted for SAGE library synthesis. For joint tissue mRNA measurements, total RNA was isolated using the RNeasy Kit (Qiagen). mRNA from tissue total RNA or cultured cell lysates was purified by binding to polyethyleneimide acid magnetic beads (Invitrogen), reverse transcribed using Superscript II (Invitrogen), and quantified by real-time RT-PCR using SYBR Green fluorescence on the 7900HT Sequence Detection System (Applied Biosystems) as previously described (8). Cycle threshold values were normalized to the housekeeping gene eukaryotic translation initiation factor EIF3SS. Primer sequences are provided in Supplemental Table II.

Blood count and cytokine and lipid measurements

Differential cell counts from blood collected in EDTA tubes were determined using the Cell-Dyn 3700 hematology analyzer (Abbott). Plasma cytokine levels were measured by a Searchlight multiplex assay (Aushon Biosystems) or a mouse proinflammatory ultra-sensitive kit (Meso Scale Discovery) according to the manufacturer’s instructions. Plasma lipids were measured using an enzymatic assay (Wako Chemicals) on a ChemWell 2910 analyzer (Awareness Technology).

Body mass composition

Body composition (fat and muscle) was measured in nonanesthetized mice using the Bruker Minispec nuclear magnetic resonance analyzer (Bruker Optics) (24).

Grip strength measurement

For the functional quantification of arthritis, maximum four-limb grip strength was measured by using a digital grip strength meter (Columbus Instruments) as previously described (25, 26).

Joint histology and aortic plaque analysis

Front and rear paw joints were removed and fixed in 10% buffered formalin, decalcified in 30% formic acid, embedded in paraffin, sectioned, and stained with H&E. Aortas were fixed by perfusion (4% paraformaldehyde, 5% sucrose, and 20 mM EDTA [pH 7.4]), dissected from their origin in the heart to the iliac bifurcation, stained with Sudan IV solution, destained in 80% ethanol, and washed in water. Aortas were cut longitudinally, placed on glass slides, and quantified from their origin to the iliac bifurcation, excluding branch vessels. Quantification of the atherosclerotic lesions was performed using the Image-Pro Plus version 4.1 software (Media Cybernetics) with a ratio of sample identity and expressed as a fraction of total aortic surface.

Statistical analysis

A two-tailed Student t test was used for statistical analyses and considered to be significant if p < 0.05.

Results

Identification and characterization of CCL3 as a TTP target

To identify new TTP-interacting mRNAs in activated human monocytes, we combined the strategy of immunoprecipitating TTP protein followed by the sequencing of all bound mRNA species using the SAGE technique (Fig. 1A) (22). An advantage of the SAGE technique is that it can provide quantitative information about the abundance of a given mRNA species by the frequency
with which the SAGE tag, corresponding to its mRNA, is encountered during sequencing. After activating THP1 human monocytic cells for 4 h with phorbol ester (PMA), cytoplasmic extracts were immunoprecipitated with either anti-TTP or nonspecific Ab, and the communoprecipitated RNAs were extracted and used to synthesize SAGE libraries.

We performed a preliminary screen by looking for the presence of ARE motifs among the mRNA species that were 2-fold enriched in the anti-TTP Ab fraction ($p < 0.05$) (27). From this list of putative candidates, 15 were confirmed by RT-PCR to be increased in the anti-TTP Ab versus nonspecific (control IgG) Ab immunoprecipitated RNA (Supplemental Table I). Confirming the specificity of our technique, TNF was one of the more significantly bound mRNA species to TTP. Interestingly, another cytokine mRNA encoded by CCL3 was the most abundant TTP-bound species by SAGE tag number (Supplemental Table I). Our previous observation that CCL3 and TTP are highly coexpressed in human atherosclerotic plaque macrophages provided an in vivo correlation (8).

We first verified the SAGE data by performing RT-PCR on the same RNAs used to make the libraries. CCL3 mRNA was significantly enriched in the anti-TTP Ab fraction in parallel with TTP and TNF mRNAs, which served as positive controls (Fig. 1B). TTP destabilizes its bound mRNA, and therefore, cells in which TTP protein has been depleted should have elevated levels of its target mRNAs. Indeed, siRNA knockdown of TTP, which was confirmed by Western blotting, caused a significant increase in both CCL3 and TNF mRNA levels, whereas a non-TTP target mRNA GAPDH was not affected (Fig. 1C). These results provided additional evidence that TTP can negatively regulate CCL3 mRNA by direct interaction.

**TTP regulates CCL3 mRNA in vivo**

To examine the interaction between TTP protein and CCL3 mRNA in primary cells, BMDM were prepared from $TTP^{+/+}$ and $TTP^{-/-}$ mice. CCL3 mRNA expression levels peaked 3 to 4 h after LPS treatment in $TTP^{+/+}$ BMDM (Fig. 2A). In $TTP^{-/-}$ BMDM, CCL3 mRNA induction was significantly higher with a slightly delayed peak 4 h after LPS treatment. The dampening of CCL3 mRNA levels in $TTP^{+/+}$ BMDM correlated well with TTP protein expression (Fig. 2A). The effect of altered CCL3 mRNA levels was evident from parallel changes in CCL3 protein released into the medium (Fig. 2B).

To show that the increase in CCL3 mRNA in $TTP^{-/-}$ BMDM was due to posttranscriptional stabilization, we measured the steady-state level of CCL3 mRNA after blocking transcription with actinomycin D. With TNF and GAPDH mRNAs serving as positive and negative controls, respectively, the $t_{1/2}$ of CCL3 mRNA increased from 48 min in $TTP^{+/+}$ cells to 117 min in $TTP^{-/-}$ cells (Fig. 2C). The stabilization of CCL3 mRNA in $TTP^{-/-}$ cells provided in vivo genetic evidence that TTP negatively regulates it in macrophages.

**AREs mediate CCL3 mRNA binding to TTP protein**

The direct interaction between TTP protein and CCL3 mRNA was further assessed by in vitro cell transfection assays without the potential confounding effects of endogenous TFP (28). HEK293 cells were cotransfected with CCL3 and TTP cDNA in pCMV vectors (Fig. 3A). The transiently expressed TTP specifically reduced CCL3 mRNA, but not GAPDH mRNA, confirming their specific interaction in a heterologous cell system. Because important regulatory sequences are likely to be conserved, CCL3 sequences from five different species were queried, and their alignment revealed three conserved AREs (AUUUA) in the 3’ untranslated region (ARE 1, 2, and 3) (Fig. 3B). Point mutations were then introduced into each of these AREs to determine which mediated TTP regulation. The CCL3 mutants were cotransfected with either TTP cDNA or empty vector followed by CCL3 mRNA measurement (Fig. 3C). The 5’-ARE mutant (mutant 1) had the most significant effect on abolishing the inhibitory effect of TTP on CCL3 mRNA levels (Fig. 3C). The individual effect of the other two AREs (mutants 2 and 3) was weak, but the combined mutant (mutant 1-2-3) synergistically neutralized the effect of TTP on CCL3 mRNA stability.

**Deletion of CCL3 ameliorates the inflammatory arthritis of $TTP^{-/-}$ mice**

TTP-deficient mice develop an inflammatory syndrome consisting of cachexia, arthritis, systemic autoimmunity, myeloid hyperplasia, and extramedullary hematopoiesis (4, 29). One prominent feature of $TTP^{-/-}$ mice is the development of polyarticular arthritis characterized by paw swelling at 8–10 wk of age. This has been likened to human rheumatoid arthritis, although the serologies are more consistent with systemic lupus erythematosus.

To determine the effect of CCL3 mRNA regulation by TTP in vivo, we crossed $CCL3^{-/-}$ with $TTP^{-/-}$ mice to generate $CCL3^{-/-}TTP^{-/-}$ (double-knockout) mice. When paw grip strength was determined as a functional measure of arthritis, we observed a significant improvement in $CCL3^{-/-}TTP^{-/-}$ mice compared with $TTP^{-/-}$ mice (Fig. 4A). Notably, the improvement in female $CCL3^{-/-}TTP^{-/-}$ mice appeared more significant than that observed in the male mice, consistent with the influential role gender can play in autoimmune diseases ($p$ values for female versus male were 0.002 and 0.02, respectively) (Fig. 4A, Supplemental Fig. 1) (30). Based on this observation, we focused our study on female mice and found that, in parallel with the functional improvement, the severe synovial infiltration and bone

**FIGURE 2.** CCL3 expression is increased in $TTP^{-/-}$ mouse BMDM. A, CCL3 mRNA levels after LPS (10 ng/ml) stimulation are higher in $TTP^{-/-}$ compared with $TTP^{+/+}$ BMDM. The time course of TTP protein induction by LPS treatment is shown by Western blotting. B, CCL3 protein in the medium was quantified by ELISA. C, CCL3 and TNF mRNA $t_{1/2}$ are increased in $TTP^{-/-}$ versus $TTP^{+/+}$ BMDM. After stimulating with LPS for 90 min, actinomycin D (Act.D) was added to the cells. At the indicated times (Time after Act.D), mRNA was isolated from the cells and quantified by RT-PCR. Dashed line indicates 50% level. Data presented as mean ± SEM; $n = 3–6$. Downloaded from http://www.jimmunol.org/
CCL3 mediates the increased atherosclerosis of TTP−/− APOE−/− mice

Atherosclerosis is an inflammatory vascular disease that involves various cell types including plaque macrophages, and our previous study of human plaque macrophage transcriptome identified TTP as a potentially important regulator of gene expression (8, 32). To investigate whether TTP-regulated CCL3 is also involved in atherosclerosis, we crossed TTP−/− mice into an APOE−/− background, a well-established mouse model of hyperlipidemia and atherosclerosis (33). Again, we used female APOE−/− mice, as they have been shown to develop larger, less lipid-laden atherosclerotic lesions than male mice when fed a normal diet (34, 35). As predicted with the increase in inflammation caused by TTP deficiency, TTP−/−APOE−/− mice developed more severe atherosclerosis as measured by the total surface area of aortic plaques compared with APOE−/− mice (Fig. 6A).

Hyperlipidemia has been associated with systemic inflammation, but the plasma levels of triglycerides and total cholesterol were in fact reduced in TTP−/−APOE−/− mice, which is consistent with the cachexia caused by TTP deficiency (Fig. 6B). This suggested that inflammatory factors play a pivotal role in worsening the atherosclerosis of TTP-deficient mice. Therefore, to delineate the role of CCL3, we generated CCL3−/− TTP−/−APOE−/− (triple-knockout) mice. Although the plasma lipid level and body weight of triple-knockout mice remained decreased as with TTP−/− mice (Fig. 6B, 6C), the marked increase in atherosclerosis caused by TTP deficiency was prevented by the deletion of CCL3 (Fig. 6A). This observation provided additional in vivo genetic evidence that TTP-regulated CCL3 plays an important role in inflammatory diseases beyond those involving joint tissues.

Discussion

In summary, through a global screen for transcripts binding to TTP in activated human macrophages, we have identified CCL3 mRNA as the most abundantly bound chemokine species and have characterized the nature of its destabilization by TTP through ARE sequences. The posttranscriptional regulation of CCL3 mRNA by TTP is important, as the autoimmune arthritis and vascular inflammation observed in TTP−/− mice were both markedly reduced...
in the absence of CCL3. Furthermore, the disruption of CCL3 in the \( TTP^{-/ -} \) state dissociated localized tissue inflammation from systemic disease, a finding that is consistent with the function of CCL3 as an inflammatory chemokine and its previously observed role in a collagen Ab-induced model of arthritis (36). Together, these results provide in vivo genetic evidence underscoring the importance of both the spatial and temporal posttranscriptional regulation of chemokines in modulating the outcome of inflammation (37).

TNF plays a central role in coordinating complex inflammatory responses through associated mediator genes. A recent study has shown that the gene expression kinetics of both TNF and TNF-induced genes are largely influenced by their respective mRNA stabilities due to differences in ARE sequences (38). Although excessive TNF signaling, by loss of its mRNA destabilization in \( TTP^{+/ +} \) mice, has been shown to cause many of the inflammatory sequelae, it is possible that the expression level of CCL3 is regulated by TNF (42, 43). Regardless of the extent of the inflammatory response, there is a persistent reduction in the plasma protein levels of TNF in CCL3 \( TTP^{+/ +} \) mice compared with \( TTP^{+-} \) mice. This is consistent with a role for CCL3 as an inflammatory chemokine and its previously observed role in a collagen Ab-induced model of arthritis (36). Together, these findings demonstrate the importance of CCL3 in atherosclerosis via the regulation of chemokines in modulating the outcome of inflammation (37).

Our study also suggests a possible explanation for the increased prevalence of atherosclerotic disease in patients with rheumatoid arthritis by providing genetic evidence for a shared pathway in these two diseases whereby TTP regulates CCL3 mRNA stability. A growing body of work has implicated CCL3 and its receptor CCR5 in the pathogenesis of atherosclerosis (6, 7, 17). The marked increase in atherosclerotic plaque formation in \( TTP^{+/ +} \) APOE \( ^{-/-} \) mice is prevented by deleting CCL3. This suggests that CCL5 could be regulated by CCL3 (Supplemental Fig. 2D). To further understand the autoimmune arthritis of \( TTP^{+/ +} \) mice, more investigations are needed to determine the contributions of CCL3, CCL5, and possibly other chemokines, as the arthritis phenotype is not completely rescued by removing CCL3 alone.

It is possible that the expression level of CCL3 is regulated by both TTP and TNF. TNF has been shown to transcriptionally regulate CCL3 (40). Therefore, CCL3 could be a downstream messenger of TNF excess in \( TTP^{+/ +} \) mice, and our current study does not rule out the possibility that CCL3 mediates a subset of specific effects of TNF on the inflammation. Although it has been reported that the inflammatory phenotype of the \( TTP^{+/ +} \) mice can be prevented by treating with TNF Abs (4), \( TTP^{+/ +} \) mice continue to display myeloid hyperplasia even in the absence of TNFRs (29, 41). Thus, other TTP targets, which are independent of TNF signaling, appear to play a role in the pathogenesis of \( TTP^{+/ +} \) mice. In patients with rheumatoid arthritis, the blockade of TNF signaling using Abs does not affect CCL3 expression, whereas it reduces CCL2 and IL-8 levels (42, 43). Regardless of the extent of TNF involvement, our study reveals that CCL3 plays a critical role in localized tissue inflammation in \( TTP^{+/ +} \) mice, although its regulatory mechanism is likely complex and depends on various factors including cell type and species.

FIGURE 5. Deletion of CCL3 dissociates localized from systemic inflammation in \( TTP^{+/ +} \) mice. A, Growth curves of female \( TTP^{+/ +}, \ TTP^{-/-}, \) and \( CCL3^{-/-} \ TTP^{-/-} \) mice are shown. Arrows indicate the closely apposed growth curves of \( TTP^{-/-} \) mice and \( CCL3^{-/-} \ TTP^{-/-} \) mice \((n = 4–7)\). B, Body mass composition determined by nuclear magnetic resonance \((n = 3–6)\). C, The splenomegaly of \( TTP^{-/-} \) mice is unaltered in the absence of CCL3 \((n = 4)\). D, The plasma protein levels of TTP targets TNF and IL-1β continue to be elevated in the absence of CCL3 in \( TTP^{-/-} \) mice \((n = 3)\). E, The joint tissue mRNA levels of TTP targets TNF and IL-1β are reduced in the absence of CCL3 in \( TTP^{-/-} \) mice \((n = 3)\). Except as indicated in A, the mice used were in the age range of 17–22 wk. Data are presented as mean ± SEM. * \( p < 0.05 \) compared with \( TTP^{+/ +} \) mice.

FIGURE 6. Deletion of CCL3 prevents the increase in aortic atherosclerosis of \( TTP^{-/-} \) APOE \( ^{-/-} \) mice. A, The increased aortic atherosclerotic plaque area (Sudan IV stained) of female \( TTP^{-/-} \) APOE \( ^{-/-} \) mice (23–25 wk old) is prevented by deleting CCL3. Scale bar, 2 mm. B, Plasma lipids levels are reduced both in \( TTP^{-/-} \) APOE \( ^{-/-} \) and in \( CCL3^{-/-} \ TTP^{-/-} \) APOE \( ^{-/-} \) mice compared with APOE \( ^{-/-} \) mice \((n = 6–8)\). Data are presented as mean ± SEM. C, Body weights of 23–25-wk-old female mice of the indicated genotypes. Data shown as mean ± SEM; \( n = 4–8 \); * \( p < 0.05 \) compared with APOE \( ^{-/-} \) mice.
tion in a mouse model is applicable to human disease, as there is
abundant expression of both TTP and CCL3 in human atheroscle-
rotic lesions (8, 17). In addition, CCR5 is also present in arterial
smooth muscle cells, which are involved in plaque formation (17).
Although hyperlipidemia is thought to be a primary driver of ath-
erosclerosis, the unregulated expression of CCL3 appears to be piv-
otal in worsening atherosclerosis in TTP−/−APoE−/− mice despite
their significantly lower lipid levels compared with APoE−/− mice.

The CCL3 pathway may represent a potential target for site-
specific modulation of autoimmune arthritis and atherosclerotic
disease activity. Circulating cytokines have pleiotropic effects on
multiple systems, and their chronic blockade may lead to potential
complications such as the immune suppression seen with TNF an-
tagonist treatments. As chemokines spatially and selectively target
subsets of leukocytes, their blockade may reduce the likelihood of
systemic side effects (11). By disrupting CCL3 chemokine signal-
ing, the vicious cycle of recruiting more inflammatory cells to the
disease site may be prevented. Given our observations using two
different disease models, TTP regulation of CCL3 is likely to be
important for the pathogenesis of other common inflammatory
conditions and may merit therapeutic targeting.

Acknowledgments
We thank Milton Pryor, Rafaela Molina, Deborah Stumpo, Cory Lago, Ho
Joong Sung, Wenzhe Ma, and Toren Finkel for helpful advice and assistance.

Disclosures
The authors have no financial conflicts of interest.

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mRNAs interacting with TTP identified by immunoprecipitation-SAGE analysis and confirmed by RT-PCR. Table columns shown are gene description, tag sequence, Unigene identification number, tag count, tag count ratio in TTP versus IgG fraction, and P value comparing the two SAGE libraries are presented. A tag count of 0 was equated to 0.5 for fold-enrichment calculation.
### TABLE S2

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RT-PCR analysis primer sequences.
FIGURE S1. Four-paw grip strength of 9 wk-old male mice was measured by using a digital grip strength meter. TTP+/+ (square), TTP-/- (open circle), and CCL3-/- TTP-/- (filled circle).
Figure S2. Effect of deleting CCL3 on markers of systemic and joint tissue inflammation in TTP-/- mice. A, Absence of CCL3 does not affect the relative myeloid shift in the blood of TTP-/- mice. Fractions of myeloid- versus lymphoid-origin cells are increased in the blood of both TTP-/- mice and CCL3-/- TTP-/- mice (age range of 17 to 22 wk). Data presented as mean ± SEM, n = 5 to 9 samples. *P < 0.05 compared to TTP+/- mice. B, Absence of CCL3 does not affect the bone marrow hyperplasia of TTP-/- mice. Hypercellularity is observed in the bone marrow spaces (black arrowheads) of the first (right column) and third (left column) interphalangeal joints of 35 wk old TTP-/- mice and CCL3-/- TTP-/- mice by H&E staining. Scale bar: 500 µm. C, Absence of CCL3 prevents infiltration of macrophages into the joint tissue of TTP-/- mice. The increased mRNA levels of two macrophage markers CD11b and F4/80 in the joints of TTP-/- mice are markedly reduced by removing CCL3 (age range of 13 to 15 wk). Data presented as mean ± SEM, n = 3 samples. *P < 0.05 compared to TTP+/- mice. D, The increased mRNA level of another arthritis associated chemokine CCL5 in the joints of TTP-/- mice is also reduced by the absence of CCL3 (age range of 13 to 15 wk). Data presented as mean ± SEM, n = 3 samples. *P < 0.05 compared to TTP+/- mice.