2019

Fat-produced adipsin regulates inflammatory arthritis

Yongjia Li

Wei Zou

Jonathan R. Brestoff

Nidhi Rohatgi

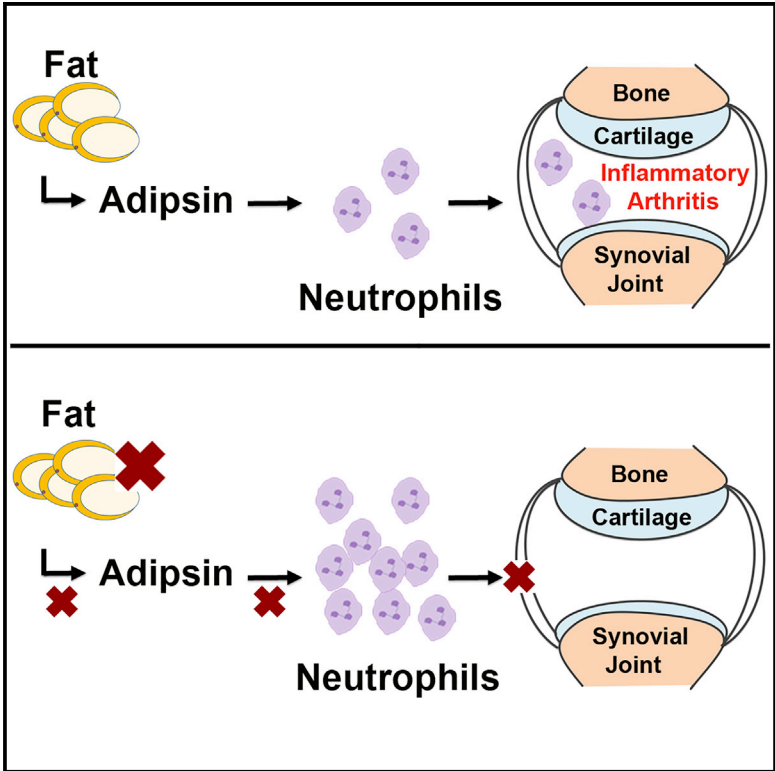Xiaobo Wu

See next page for additional authors

Authors

Yongjia Li, Wei Zou, Jonathan R. Brestoff, Nidhi Rohatgi, Xiaobo Wu, John P. Atkinson, Charles A. Harris, and Steven L. Teitelbaum

Cell Reports

Fat-Produced Adipsin Regulates Inflammatory Arthritis

Graphical Abstract

Authors

Yongjia Li, Wei Zou,
Jonathan R. Brestoff, ..., John P. Atkinson,
Charles A. Harris, Steven L. Teitelbaum

Correspondence

teitelbs@wustl.edu

In Brief

The relationship of fat and inflammatory arthritis (IA) is poorly defined. Li et al. generate fat-free (FF) mice and observe that they are completely resistant to IA because of a lack of adipsin. Their studies provide evidence that fat regulates IA development by adipsin activation of the complement pathway.

Highlights

- Depletion of fat completely protects against inflammatory arthritis

- Fat-derived adipsin is essential to development of inflammatory arthritis

- Adipsin deficiency prevents joint infiltration of neutrophils

CellPress

Report

Fat-Produced Adipsin
Regulates Inflammatory Arthritis

Yongjia Li,[1] Wei Zou,[1] Jonathan R. Brestoff,[2] Nidhi Rohatgi,[1] Xiaobo Wu,[3] John P. Atkinson,[3] Charles A. Harris,[4]
and Steven L. Teitelbaum[1,5,6,7,*]
[1]Division of Anatomic and Molecular Pathology, Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO 63110, USA
[2]Division of Laboratory and Genomic Medicine, Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO 63110, USA
[3]Division of Rheumatology, Department of Medicine, Washington University School of Medicine, St. Louis, MO 63110, USA
[4]Division of Endocrinology, Metabolism and Lipid Research, Department of Medicine, Washington University School of Medicine, St. Louis, MO 63110, USA
[5]Division of Bone and Mineral Diseases, Department of Medicine, Washington University School of Medicine, St. Louis, MO 63110, USA
[6]Shriners Hospitals for Children, St. Louis, MO 63110, USA
[7]Lead Contact
*Correspondence: teitelbs@wustl.edu
https://doi.org/10.1016/j.celrep.2019.05.032

SUMMARY

We explored the relationship of obesity and inflammatory arthritis (IA) by selectively expressing diphtheria toxin in adipose tissue yielding "fat-free" (FF) mice completely lacking white and brown fat. FF mice exhibit systemic neutrophilia and elevated serum acute phase proteins suggesting a predisposition to severe IA. Surprisingly, FF mice are resistant to K/BxN serum-induced IA and attendant bone destruction. Despite robust systemic basal neutrophilia, neutrophil infiltration into joints of FF mice does not occur when challenged with K/BxN serum. Absence of adiponectin, leptin, or both has no effect on joint disease, but deletion of the adipokine adipsin (complement factor D) completely prevents serum-induced IA. Confirming that fat-expressed adipsin modulates the disorder, transplantation of wild-type (WT) adipose tissue into FF mice restores susceptibility to IA, whereas recipients of adipsin-deficient fat remain resistant. Thus, adipose tissue regulates development of IA through a pathway in which adipocytes modify neutrophil responses in distant tissues by producing adipsin.

INTRODUCTION

Inflammatory arthritis (IA) is a family of chronic immune diseases characterized by production of proinflammatory cytokines, immune cell infiltration into joints, and pathologic remodeling of bone and cartilage (Deng and Lenardo, 2006). Neutrophils are abundant in synovial fluid of RA patients and participate in development of the disease (Cross et al., 2005; Dominical et al., 2011).

Adipose tissue, long viewed as exclusively responsible for energy storage and structural stability, is now considered an endocrine organ that stores and secretes hormones and cytokines (adipokines) (Kershaw and Flier, 2004). Although the relationship of adipose tissue to IA is poorly defined, circulating adipokines, including leptin, adiponectin, and visfatin, are increased in arthritic patients (Otero et al., 2006; Yoshino et al., 2011). Adipsin, also known as complement factor D (FD), is abundant and exclusively expressed in adipose tissue, but its relevance, and that of fat, to inflammatory joint disease is enigmatic (White et al., 1992).

To explore this issue, we generated "fat-free" (FF) mice that lack all white and brown adipose tissues, although marrow adipocytes are preserved (Wu et al., 2018). As such, circulating adipokines are virtually undetectable. We then used the serum transfer K/BxN model of IA, which mirrors the effector phase of rheumatoid arthritis (RA), to investigate the role of adipose tissue in joint disease (Christensen et al., 2016; Misharin et al., 2014).

Although FF mice have abundant neutrophils in blood, spleen, and peritoneal cavity, in steady state, they are completely protected from K/BxN serum transfer arthritis. Moreover, genetic deletion and fat transplantation experiments establish that adipsin, but neither adiponectin nor leptin, is required for development of IA. These data show that adipose tissue regulates immune diseases in the joints and identifies adipsin as a major link between adipose tissue and IA.

RESULTS

FF Mice Exhibit Systemic Neutrophilia

Given the essential role of neutrophils in the progress of murine (Wipke and Allen, 2001) and human (Cross et al., 2005; Dominical et al., 2011) IA, we first measured their abundance in three systemic compartments in FF mice. The percentage of neutrophils comprising CD45+ immune cells is significantly increased in FF mice, as are their numbers in blood, spleen, and peritoneal cavity (PEC) lavage (Figure 1). Serum acute phase proteins (APPs), including haptoglobin, C-reactive protein, α-1-acid glycoprotein, and α-macroglobulin, are indicative of acute phase inflammatory markers in IA patients and are enhanced (Table S1) (Cylwik et al., 2010).
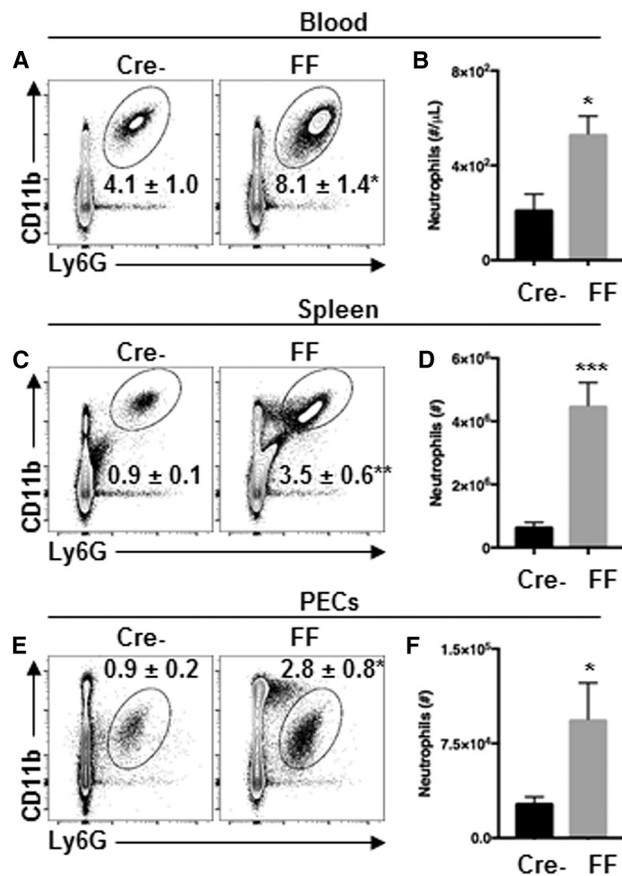
Figure 1. FF Mice Exhibit Systemic Neutrophilia
Frequencies and numbers of neutrophils in (A and B) blood, (C and D) spleen, and (E and F) peritoneal cavity (PEC) of FF mice. Two-way ANOVA with Tukey post hoc testing; mean ± SD; n = 5 mice; *p < 0.05 and ***p < 0.001.

FF Mice Are Refractory to IA

The hyperinflammatory state of FF mice suggests that they are prone to IA. To determine if this is so, we used the K/BxN serum transfer-induced model of the effector phase of RA (Kouskoff et al., 1996). Transfer of serum from arthritic K/BxN mice into Cre− mice induces profound paw and ankle swelling, appearing within 48 h and maximizing at day 4 (Figures 2A–2C). Contrary to our prediction of hypersensitivity, however, injection of K/BxN serum failed to increase joint thickness in FF mice (Figures 2A–2C). Micro-computed tomographic (μCT) imaging reveals extensive bone erosion in the ankles of Cre− serum-injected mice, while those lacking fat are essentially spared (Figure 2D). Histological analysis demonstrates massive infiltration of synovial space by inflammatory cells, bone and cartilage destruction, and an abundance of tartrate-resistant acid phosphatase (TRAP)-expressing osteoclasts in joints of Cre− but not FF mice (Figures 2E and 2F). Consistent with these observations, serum-injected FF mice exhibit no evidence of synovial immune cell infiltration (Figures 2G and 2H). The majority (80%–85%) of the infiltrating cells in Cre− mice are neutrophils (Figure 2I), and the relative magnitude of their increase following serum injection mirrors that of total immune cells (Figures 2I and 2J).

Synovial macrophages and monocytes are also dramatically induced only in Cre− mice when serum challenged (Figures 2K and 2L). Interleukin 1 β (IL1β), which is expressed by neutrophils and promotes their migration (Guma et al., 2009; Kay and Calabrese, 2004; Rider et al., 2011), is robustly increased in the joints of serum-injected Cre− but not FF mice (Figure 2M). Thus, complete lipoatrophy prevents IA despite substantial systemic innate inflammation.

### Adiponectin and/or Leptin Deficiency Does Not Affect IA

Although FF mice have virtually no circulating adipokines, adiponectin and leptin are increased in patients with RA (Otero et al., 2006; Yoshino et al., 2011). Adiponectin is a particularly relevant candidate to influence RA because it is the most abundant adipokine in synovial fluid (Schäffler et al., 2003). It induces matrix metalloproteinase and proinflammatory cytokine expression by joint-resident immune cells (Frommer et al., 2010).

To determine if IA is dependent on adiponectin, we injected K/BxN serum into wild-type (WT) or adiponectin-knockout (KO) mice. Surprisingly, WT and adiponectin-KO mice exhibit similarly severe limb swelling, joint bone erosion, joint inflammation, and osteoclast induction (Figures S1A–S1E). Thus, serum-induced IA is independent of adiponectin.

We performed similar experiments to determine if the absence of leptin mediates the joint-protective effect of fat depletion. Leptin-KO mice develop serum transfer-induced IA in a manner mirroring controls, as characterized by similar paw and ankle swelling, bone erosion, joint inflammation, and osteoclast induction (Figures S1F–S1J). These data indicate that neither isolated adiponectin nor leptin deficiency affects serum transfer-induced IA. To determine if their combined absence, as occurs in FF mice, may influence joint destruction, we administered serum to mice lacking both adiponectin and leptin (DKO). Like their single gene-deficient counterparts, double KO (DKO) mice develop IA in a manner identical to WT controls (Figures S1K–S1O). Thus, neither adiponectin nor leptin, alone or in combination, is involved in the pathogenesis of serum transfer IA.

Adipsin-KO Mice Are Resistant to IA

Adipsin, also known as complement factor D, is a secreted serine protease derived almost exclusively from adipose tissue and is diminished in obesity (Flier et al., 1987; Lowell et al., 1990; Wu et al., 2018). Adipsin is an essential component of the alternative complement pathway and therefore may also regulate innate immune responses in the joint. Mirroring FF mice and unlike those lacking adiponectin and/or leptin, injection of K/BxN serum into adipsin-deficient mice produces no paw and ankle swelling, periarticular bone erosion, osteoclast induction, or recruitment of innate immune cells such as neutrophils, macrophages, and monocytes into synovial fluid (Figures 3A–3L). In keeping with their resistance to IA, Il1β mRNA is reduced in the joints and synovial fluid of serum-injected, adipsin-deficient mice (Figure 3M). Expression of adipsin by fat therefore appears central to the development of IA. To confirm this hypothesis, we transplanted white adipose tissue, obtained from WT or adipsin-KO mice, into FF mice. Six weeks after fat
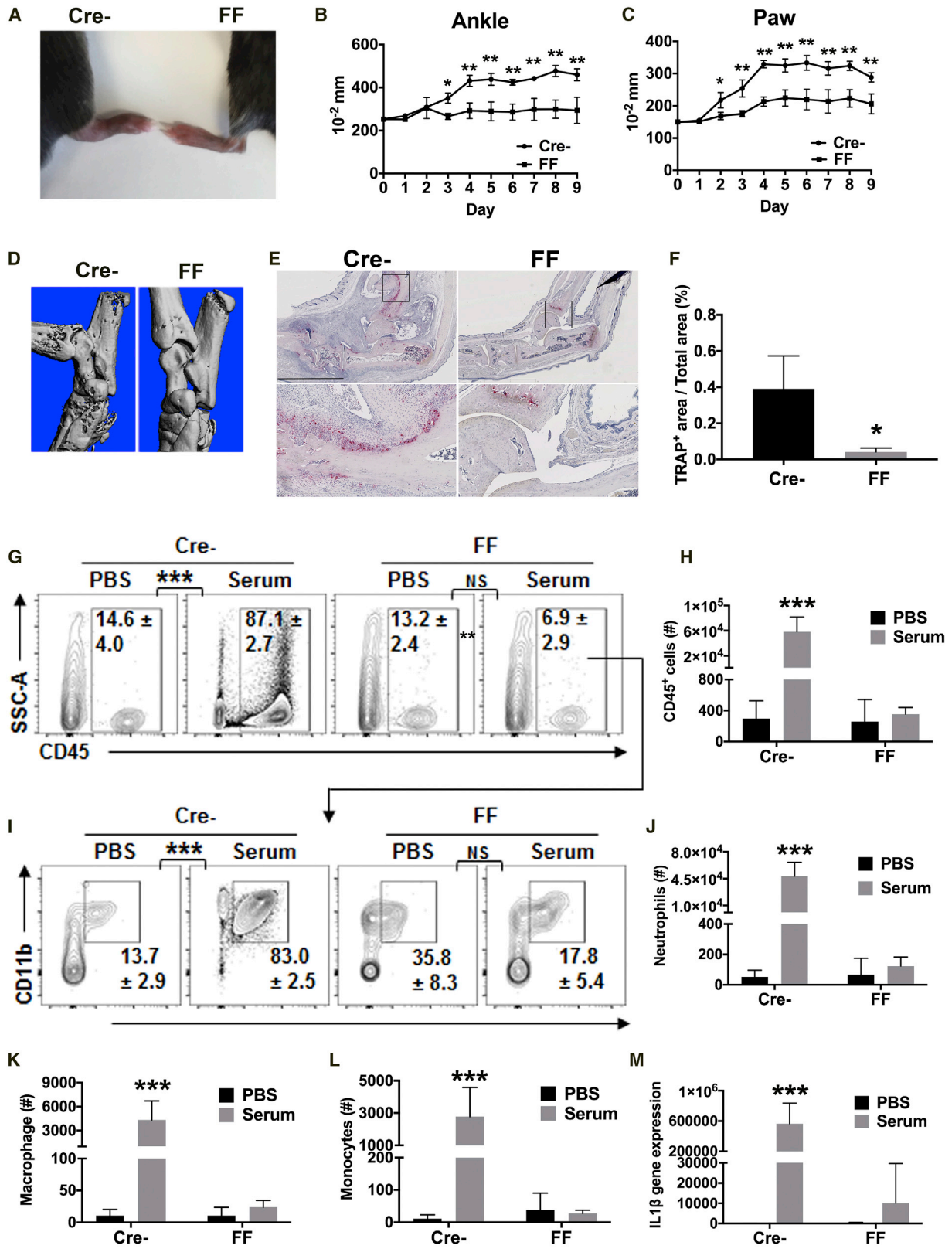
(legend on next page)

transplantation, we administered K/BxN serum to recipient mice. Nine days later, adipsin is evident in serum of FF mice transplanted with WT adipose tissue, although less than that of Cre− controls (Figure 4A). In contrast, the cytokine is undetectable in mice grafted with adipsin-KO fat. Confirming the essential role of adipose tissue in the pathogenesis of IA, WT fat transplantation substantially normalizes the susceptibility of FF mice to serum-induced ankle and paw swelling (Figures 4B and 4C). Alternatively, adipsin-deficient fat transplanted into FF mice induces only a slight increase of paw swelling and does not affect ankle thickness (Figures 4B and 4C). Further confirming that fat-derived adipsin is essential for the development of IA, WT but not adipsin-KO fat transplantation restores the ability of K/BxN serum to induce bone erosion and increase inflammation and osteoclast number in FF mice (Figures 4D–4F).

DISCUSSION

Obesity, originally considered exclusively a metabolic disease, is an inflammatory condition characterized by immune cell activation and increased expression of proinflammatory cytokines that drive both innate and adaptive immunity. In particular, obesity is associated with a type 1 inflammatory response as well as activation of the IL-17A/neutrophil immune axis, resulting in systemically increased neutrophils in obese mice and humans (Johnson et al., 2012). A reasonable hypothesis holds, therefore, that obesity likely predisposes to and accelerates progression of IA. Although it appears that obese patients are more susceptible to IA, it is perplexing that progression of the disease may be slower than in affected, lean individuals (Baker et al., 2014; van der Helm-van Mil et al., 2008).

Various adipokines have been linked to IA. For example, there is experimental evidence that targeting visfatin, which is modestly increased in obesity, promotes joint inflammation (Gómez et al., 2011a). However, the role of the two most abundant cytokines, leptin and adiponectin, is unclear (Otero et al., 2006; Yoshino et al., 2011). Leptin, which increases in the circulation with obesity, is postulated to aggravate IA (Toussirot et al., 2015). Complicating our understanding of leptin's role in IA, its circulating abundance correlates inversely with IA joint damage (Gómez et al., 2011b; Rho et al., 2009; Tong et al., 2008). Similarly, adiponectin, which is reduced in obesity, promotes IL-6 and MMP-1 expression by synovial fibroblasts (Ehling et al., 2006) and is predictive of radiographic RA progression (Klein-Wieringa et al., 2011). Yet administration of adiponectin reduces the severity of collagen-induced arthritis in mice (Ebina et al.,

2009). Thus, despite the endemic nature of obesity and the frequency of IA, the impact, if any, of adipose tissue and its products on the pathogenesis of IA is enigmatic.

Given this conundrum, we asked if the absence of fat, attended by a virtual lack of adipokines, modulates IA. We find that FF mice are completely protected from IA in face of systemic neutrophilia and increased circulating biochemical indicators of acute phase proteins. Thus, induction of IA may be independent of the systemic inflammation of obesity or lipodystrophy. The complete resistance of FF mice to serum-induced joint inflammation and destruction, and particularly its reversal by WT fat transplantation, indicates that adipose tissue, per se, modulates inflammatory disease in joints. The virtual absence of adipokines in these animals also provided the opportunity to determine the influence of depletion of specific adipocyte products on this family of disorders. Indicating that absence of leptin and/or adiponectin is likely not responsible for the resistance of fat-deficient animals to serum transfer arthritis, the joints of injected mice lacking either adipokine, alone or in combination, are inflamed and degraded to the same extent as control. On the other hand, our findings indicate that adipose tissue has a critical role in the pathogenesis of the effector phase of inflammatory joint destruction by producing adipsin, an adipokine that is decreased in obese mice and humans. This conclusion is consistent with the resistance of mice with genetically induced obesity and a paucity of adipsin, to IA (Busso et al., 2001; Flier et al., 1987)

Complement activation commonly occurs in arthritic joints (Struglics et al., 2016). Moreover, the alternative complement pathway is central to development of both collagen-induced and serum transfer arthritis and likely contributes to the progression of human RA through generation of C3 convertases (Banda et al., 2006; Ji et al., 2002). Although C5a, C3, and factor B are established mediators of this arthritic state, the role of adipsin has not been explored. Because adipsin is the only complement component almost exclusively produced by adipose tissue in mice, it represents a possible link between obesity, lipodystrophy, and IA, particularly as circulating levels of this adipokine fall dramatically with weight gain (Cook et al., 1987; Flier et al., 1987). Furthermore, like that lacking adipsin, a mouse devoid of fat is incapable of complement activation via the alternative pathway (Wu et al., 2018). Thus, the alternative pathway functions as a powerful feedback loop. It is involved in the pathogenesis of many diseases, making it an attractive therapeutic candidate. In keeping with our findings, induction of joint inflammation in serum transfer arthritis depends on C3 (Ji et al., 2002)

Figure 2. FF Mice Are Protected from Serum Transfer Arthritis
FF and Cre− mice were injected with K/BxN serum on days 0 and 3.
(A) Hind paws of Cre− and FF mice 9 days after initial serum injection.
(B and C) Ankle (B) and paw (C) thickness measured over time.
(D) μCT image of rear ankle of Cre− and FF mice 9 days after initial serum injection.
(E) Histological image of rear ankle of Cre− and FF mice 9 days after initial serum injection stained for TRAP activity (red reaction product).
(F) TRAP-stained area as a function of total area (scale bar, 2.5 mm).
(G and H) Frequency (G) and number (H) of total immune cells in synovial fluid of Cre− and FF mice 7 days after initial serum or PBS injection.
(I and J) Frequency (I) and number (J) of neutrophils in synovial fluid of Cre− or FF mice 7 days after initial serum or PBS injection.
(K and L) Numbers of (K) macrophages and (L) monocytes in synovial fluid of Cre− and FF mice 7 days after initial serum or PBS injection.
(M) IL-1β mRNA expression in joints and synovial fluid of Cre− and FF mice 7 days after initial serum or PBS injection.
In (A)–(F), Student's t test. In (I)–(M), two-way ANOVA with Tukey post hoc testing; n = 4 or 5 mice; *p < 0.05, **p < 0.01, and ***p < 0.001.
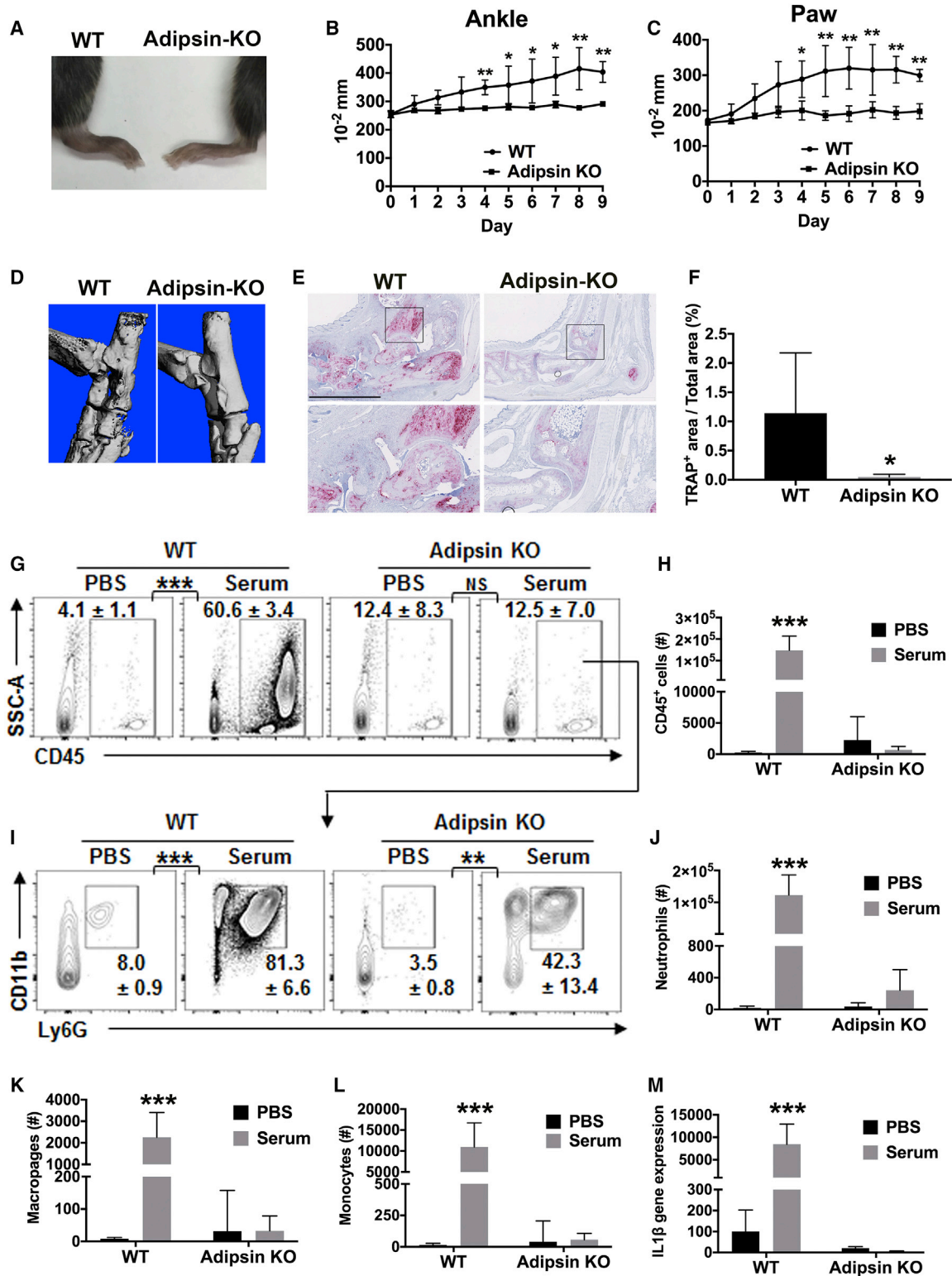
Figure 3. Adipsin Is Required for Development of Serum Transfer IA
Adipsin-KO and WT mice were injected with K/BxN serum on days 0 and 3.
(A) Hind paws of WT and adipsin-KO mice 9 days after initial serum injection.
(B and C) Ankle (B) and paw (C) thickness measured with time.
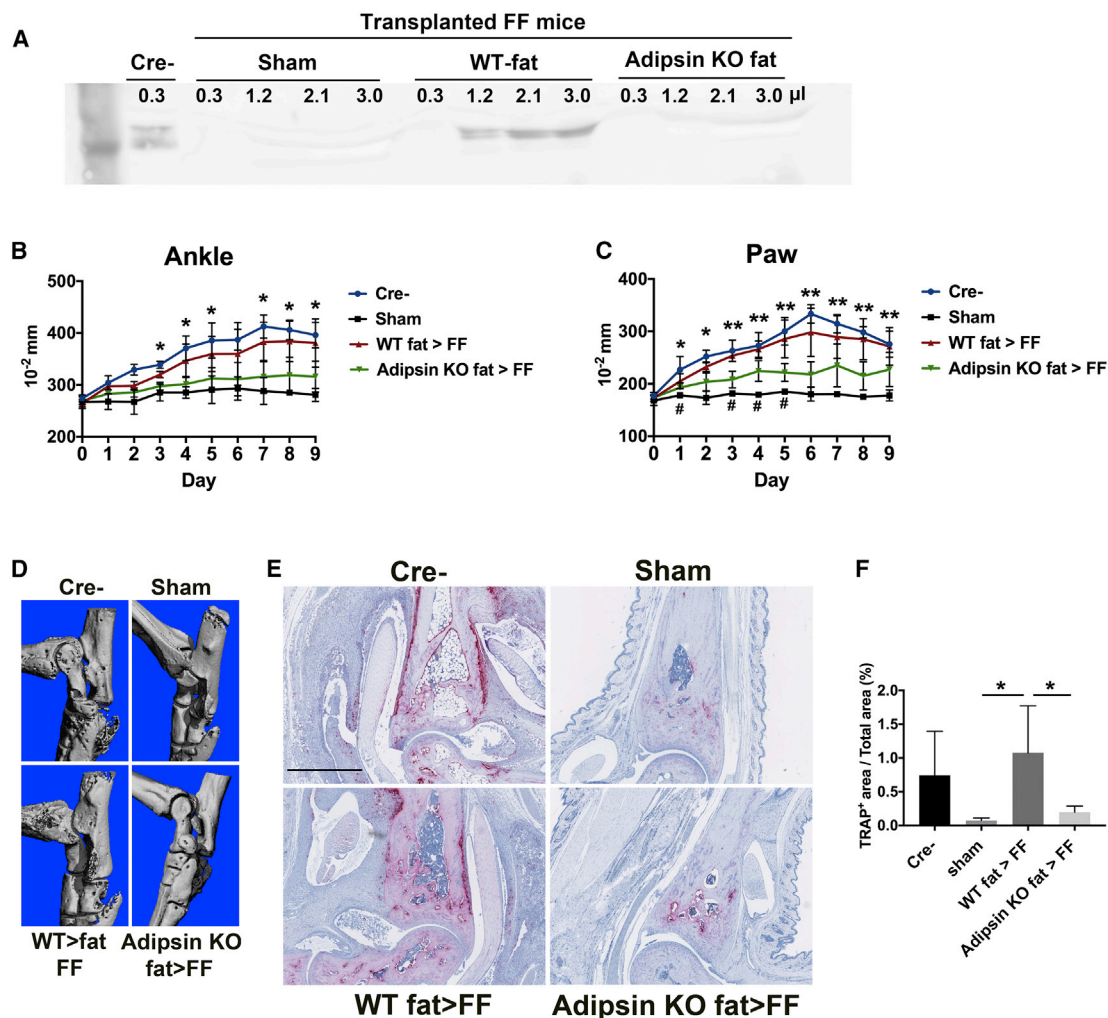
(legend continued on next page)

Figure 4. Fat-Residing Adipsin Promotes IA

FF mice were transplanted, subcutaneously, with white adipose tissue obtained from WT or adipsin-KO mice. FF mice subjected to sham surgery served as control. Six weeks after fat transplantation, mice were injected with serum on days 0 and 3. At day 9, mice were sacrificed and serum was collected.

(A) Adipsin, in increasing amounts of serum, detected by immunoblot in Cre− mice, sham-operated FF mice, FF mice transplanted with WT fat, and FF mouse transplanted with adipsin-KO fat.

(B and C) Ankle (B) and paw (C) thickness measured with time.

(D) μCT image of hind paws of control, sham-operated, and fat-transplanted mice 9 days after initial serum injection.

(E and F) Histological image of rear ankle joints of control, sham-operated, and fat-transplanted mice 9 days after initial serum injection stained for TRAP activity (red reaction product) (E) and TRAP-stained area/total area (F) of (E). Scale bar, 500 μm.

Two-way ANOVA with Tukey post hoc testing; n = 3–5 mice; *p < 0.05, **p < 0.01, ***p < 0.01, and ****p < 0.01 indicate WT-fat > FF compared with sham; #p < 0.05 indicates adipsin-fat > FF compared with sham.

and properdin (Kimura et al., 2010). Our results therefore add another alternative complement component, adipsin, to the list of IA-mediating molecules.

We used the K/BxN serum transfer arthritis model because of its convenience and speed of induction. This model and collagen antibody-induced arthritis (CAIA) are limited, however, by their

---

(D) μCT image of hind paws of WT and adipsin-KO mice 9 days after initial serum injection.

(E) Histological image of rear ankle joints of WT and adipsin-KO mice 9 days after initial serum injection stained for TRAP activity (red reaction product).

(F) TRAP-stained area/total area; scale bar, 2.5 mm.

(G and H) Frequencies (G) and numbers (H) of immune cells in synovial fluid of adipsin-KO mice 7 days after initial serum or PBS injection.

(I and J) Frequencies (I) and numbers (J) of neutrophils in synovial fluid of adipsin-KO mice 7 days after initial serum or PBS injection.

(K and L) Numbers of (K) macrophages and (L) monocytes in synovial fluid of adipsin-KO mice 7 days after initial serum or PBS injection.

(M) IL-1β mRNA expression in joints and synovial fluid of WT and adipsin-KO mice 7 days after initial serum or PBS injection.

In (A)–(F), Student's t test. In (I)–(M), two-way ANOVA with Tukey post hoc testing; n = 4 or 5 mice; *p < 0.05, **p < 0.01, and ***p < 0.001.

mirroring only the effector phase of RA. Thus, future studies warrant testing the effects of fat depletion on the collagen II-induced arthritis (CIA) model, which represents both the priming and effector phases of RA. Given that CIA is ameliorated by complement deficiency, it is likely to be dampened by fat depletion (Hietala et al., 2002).

Progression of IA is characterized by immune cell migration into joints. Neutrophils are abundant in synovial fluid of patients with active RA and mediate its progression (Cross et al., 2005; Dominical et al., 2011). Nevertheless, the mechanism of neutrophil infiltration into joints in IA is unclear. Complement activation promotes neutrophil adhesion to endothelial cells, while it is reduced by complement blockade (Riedl et al., 2016). Indeed, our data show that adipsin depletion prevents neutrophil accumulation into IA joints, indicating fat regulates neutrophils via an adipsin-dependent pathway. Activated neutrophils also trigger the alternative complement pathway (Camous et al., 2011), suggesting that crosstalk between neutrophils and adipsin-dependent complement activation.

Because adipsin is a relatively small protein (molecular weight ~25 kDa) and is required for efficient alternative pathway activation and amplification, it is an attractive target for pharmaceutical intervention. Many monoclonal anti-adipsin antibodies as well as small molecules have been developed to block the adipokine (Le et al., 2015). The goal is to treat human diseases such as age-related macular degeneration, paroxysmal nocturnal hemoglobinuria, atypical hemolytic uremic syndrome, and other diseases that may be mediated by alternative complement pathway activation. Of note, adipsin-KO mice grow and develop normally (Xu et al., 2001). Also, in standard animal husbandry conditions, they require challenge with certain infectious agents to bring out their immune deficit. However, the positive effect of adipsin on β cell function in diabetes challenges this conclusion and indicates that direct suppression of joint-residing adipsin may be necessary (Lo et al., 2014).

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- CONTACT FOR REAGENT AND RESOURCE SHARING
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
- METHOD DETAILS
  - Fat Transplantation
  - Isolation of immune cells and flow cytometry
  - Immunoblot
  - Serum Transfer Arthritis
  - Histology
  - Micro-computed tomography (μCT)
  - RNA extraction and qPCR
  - Acute phase proteins (APP) assay
- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.celrep.2019.05.032.

REFERENCES

Baker, J.F., Ostergaard, M., George, M., Shults, J., Emery, P., Baker, D.G., and Conaghan, P.G. (2014). Greater body mass independently predicts less radiographic progression on X-ray and MRI over 1-2 years. Ann. Rheum. Dis. 73, 1923–1928.

Banda, N.K., Thurman, J.M., Kraus, D., Wood, A., Carroll, M.C., Arend, W.P., and Holers, V.M. (2006). Alternative complement pathway activation is essential for inflammation and joint destruction in the passive transfer model of collagen-induced arthritis. J. Immunol. 177, 1904–1912.

Busso, N., So, V., Peclat, A., and Gabay, C. (2001). Mice deficient in leptin (ob/ob) or in liptin receptor (db/db) have a milder form of antigen-induced arthritis. Arthritis Res. 3, P023.

Camous, L., Roumenina, L., Bigot, S., Brachemi, S., Frémeaux-Bacchi, V., Lesavre, P., and Halbwachs-Mecarelli, L. (2011). Complement alternative pathway acts as a positive feedback amplification of neutrophil activation. Blood 117, 1340–1349.

Christensen, A.D., Haase, C., Cook, A.D., and Hamilton, J.A. (2016). K/BxN serum-transfer arthritis as a model for human inflammatory arthritis. Front. Immunol. 7, 213.

Cook, K.S., Min, H.Y., Johnson, D., Chaplinsky, R.J., Flier, J.S., Hunt, C.R., and Spiegelman, B.M. (1987). Adipsin: a circulating serine protease homolog secreted by adipose tissue and sciatic nerve. Science 237, 402–405.

Cross, A., Bakstad, D., Allen, J.C., Thomas, L., Moots, R.J., and Edwards, S.W. (2005). Neutrophil gene expression in rheumatoid arthritis. Pathophysiology 12, 191–202.

Cylwik, B., Chrostek, L., Gindzienska-Sieskiewicz, E., Sierakowski, S., and Szmitkowski, M. (2010). Relationship between serum acute-phase proteins and high disease activity in patients with rheumatoid arthritis. Adv. Med. Sci. 55, 80–85.

Deng, G.M., and Lenardo, M. (2006). The role of immune cells and cytokines in the pathogenesis of rheumatiod arthritis. Drug Discov. Today Dis. Mech. 3, 163–168.

Dominical, V.M., Bértolo, M.B., Almeida, C.B., Garrido, V.T., Miguel, L.I., Costa, F.F., and Conran, N. (2011). Neutrophils of rheumatoid arthritis patients on anti-TNF-α therapy and in disease remission present reduced adhesive functions in association with decreased circulating neutrophil-attractant chemokine levels. Scand. J. Immunol. 73, 309–318.

Ebina, K., Oshima, K., Matsuda, M., Fukuhara, A., Maeda, K., Kihara, S., Hashimoto, J., Ochi, T., Banda, N.K., Yoshikawa, H., and Shimomura, I. (2009).

Adenovirus-mediated gene transfer of adiponectin reduces the severity of collagen-induced arthritis in mice. Biochem. Biophys. Res. Commun. *378*, 186–191.

Ehling, A., Schäffler, A., Herfarth, H., Tarner, I.H., Anders, S., Distler, O., Paul, G., Distler, J., Gay, S., Schölmerich, J., et al. (2006). The potential of adiponectin in driving arthritis. J. Immunol. *176*, 4468–4478.

Flier, J.S., Cook, K.S., Usher, P., and Spiegelman, B.M. (1987). Severely impaired adipsin expression in genetic and acquired obesity. Science *237*, 405–408.

Frommer, K.W., Zimmermann, B., Meier, F.M., Schröder, D., Heil, M., Schäffler, A., Büchler, C., Steinmeyer, J., Brentano, F., Gay, S., et al. (2010). Adiponectin-mediated changes in effector cells involved in the pathophysiology of rheumatoid arthritis. Arthritis Rheum. *62*, 2886–2899.

Gómez, R., Conde, J., Scotece, M., Gómez-Reino, J.J., Lago, F., and Gualillo, O. (2011a). What's new in our understanding of the role of adipokines in rheumatic diseases? Nat. Rev. Rheumatol. *7*, 528–536.

Gómez, R., Scotece, M., Conde, J., Gómez-Reino, J.J., Lago, F., and Gualillo, O. (2011b). Adiponectin and leptin increase IL-8 production in human chondrocytes. Ann. Rheum. Dis. *70*, 2052–2054.

Guma, M., Ronacher, L., Liu-Bryan, R., Takai, S., Karin, M., and Corr, M. (2009). Caspase 1-independent activation of interleukin-1beta in neutrophil-predominant inflammation. Arthritis Rheum. *60*, 3642–3650.

Hietala, M.A., Jonsson, I.M., Tarkowski, A., Kleinau, S., and Pekna, M. (2002). Complement deficiency ameliorates collagen-induced arthritis in mice. J. Immunol. *169*, 454–459.

Ji, H., Ohmura, K., Mahmood, U., Lee, D.M., Hofhuis, F.M.A., Boackle, S.A., Takahashi, K., Holers, V.M., Walport, M., Gerard, C., et al. (2002). Arthritis critically dependent on innate immune system players. Immunity *16*, 157–168.

Johnson, A.R., Milner, J.J., and Makowski, L. (2012). The inflammation highway: metabolism accelerates inflammatory traffic in obesity. Immunol. Rev. *249*, 218–238.

Kay, J., and Calabrese, L. (2004). The role of interleukin-1 in the pathogenesis of rheumatoid arthritis. Rheumatology (Oxford) *43* (Suppl 3), iii2–iii9.

Kershaw, E.E., and Flier, J.S. (2004). Adipose tissue as an endocrine organ. J. Clin. Endocrinol. Metab. *89*, 2548–2556.

Kimura, Y., Zhou, L., Miwa, T., and Song, W.C. (2010). Genetic and therapeutic targeting of properdin in mice prevents complement-mediated tissue injury. J. Clin. Invest. *120*, 3545–3554.

Klein-Wieringa, I.R., van der Linden, M.P., Knevel, R., Kwekkeboom, J.C., van Beelen, E., Huizinga, T.W., van der Helm-van Mil, A., Kloppenburg, M., Toes, R.E., and Ioan-Facsinay, A. (2011). Baseline serum adipokine levels predict radiographic progression in early rheumatoid arthritis. Arthritis Rheum. *63*, 2567–2574.

Kouskoff, V., Korganow, A.S., Duchatelle, V., Degott, C., Benoist, C., and Mathis, D. (1996). Organ-specific disease provoked by systemic autoimmunity. Cell *87*, 811–822.

Le, K.N., Gibiansky, L., Good, J., Davancaze, T., van Lookeren Campagne, M., Loyet, K.M., Morimoto, A., Jin, J., Damico-Beyer, L.A., and Hanley, W.D. (2015). A mechanistic pharmacokinetic/pharmacodynamic model of factor D inhibition in cynomolgus monkeys by lampalizumab for the treatment of geographic atrophy. J. Pharmacol. Exp. Ther. *355*, 288–296.

Lo, J.C., Ljubicic, S., Leibiger, B., Kern, M., Leibiger, I.B., Moede, T., Kelly, M.E., Chatterjee Bhowmick, D., Murano, I., Cohen, P., et al. (2014). Adipsin is an adipokine that improves β cell function in diabetes. Cell *158*, 41–53.

Lowell, B.B., Napolitano, A., Usher, P., Dulloo, A.G., Rosen, B.S., Spiegelman, B.M., and Flier, J.S. (1990). Reduced adipsin expression in murine obesity: effect of age and treatment with the sympathomimetic-thermogenic drug mixture ephedrine and caffeine. Endocrinology *126*, 1514–1520.

Misharin, A.V., Cuda, C.M., Saber, R., Turner, J.D., Gierut, A.K., Haines, G.K., 3rd, Berdnikovs, S., Filer, A., Clark, A.R., Buckley, C.D., et al. (2014). Nonclassical Ly6C(-) monocytes drive the development of inflammatory arthritis in mice. Cell Rep. *9*, 591–604.

Otero, M., Lago, R., Gomez, R., Lago, F., Dieguez, C., Gómez-Reino, J.J., and Gualillo, O. (2006). Changes in plasma levels of fat-derived hormones adiponectin, leptin, resistin and visfatin in patients with rheumatoid arthritis. Ann. Rheum. Dis. *65*, 1198–1201.

Rho, Y.H., Solus, J., Sokka, T., Oeser, A., Chung, C.P., Gebretsadik, T., Shintani, A., Pincus, T., and Stein, C.M. (2009). Adipocytokines are associated with radiographic joint damage in rheumatoid arthritis. Arthritis Rheum. *60*, 1906–1914.

Rider, P., Carmi, Y., Guttman, O., Braiman, A., Cohen, I., Voronov, E., White, M.R., Dinarello, C.A., and Apte, R.N. (2011). IL-1α and IL-1β recruit different myeloid cells and promote different stages of sterile inflammation. J. Immunol. *187*, 4835–4843.

Riedl, M., Noone, D.G., Khan, M.A., Pluthero, F.G., Kahr, W.H.A., Palaniyar, N., and Licht, C. (2016). Complement activation induces neutrophil adhesion and neutrophil-platelet aggregate formation on vascular endothelial cells. Kidney Int. Rep. *2*, 66–75.

Rohatgi, N., Zou, W., Collins, P.L., Brestoff, J.R., Chen, T.H., Abu-Amer, Y., and Teitelbaum, S.L. (2018). ASXL1 impairs osteoclast formation by epigenetic regulation of NFATc1. Blood Adv. *2*, 2467–2477.

Schäffler, A., Ehling, A., Neumann, E., Herfarth, H., Tarner, I., Schölmerich, J., Müller-Ladner, U., and Gay, S. (2003). Adipocytokines in synovial fluid. JAMA *290*, 1709–1710.

Struglics, A., Okroj, M., Swärd, P., Frobell, R., Saxne, T., Lohmander, L.S., and Blom, A.M. (2016). The complement system is activated in synovial fluid from subjects with knee injury and from patients with osteoarthritis. Arthritis Res. Ther. *18*, 223.

Tong, K.M., Shieh, D.C., Chen, C.P., Tzeng, C.Y., Wang, S.P., Huang, K.C., Chiu, Y.C., Fong, Y.C., and Tang, C.H. (2008). Leptin induces IL-8 expression via leptin receptor, IRS-1, PI3K, Akt cascade and promotion of NF-kappaB/p300 binding in human synovial fibroblasts. Cell. Signal. *20*, 1478–1488.

Toussirot, É., Michel, F., Binda, D., and Dumoulin, G. (2015). The role of leptin in the pathophysiology of rheumatoid arthritis. Life Sci. *140*, 29–36.

van der Helm-van Mil, A.H., van der Kooij, S.M., Allaart, C.F., Toes, R.E., and Huizinga, T.W. (2008). A high body mass index has a protective effect on the amount of joint destruction in small joints in early rheumatoid arthritis. Ann. Rheum. Dis. *67*, 769–774.

White, R.T., Damm, D., Hancock, N., Rosen, B.S., Lowell, B.B., Usher, P., Flier, J.S., and Spiegelman, B.M. (1992). Human adipsin is identical to complement factor D and is expressed at high levels in adipose tissue. J. Biol. Chem. *267*, 9210–9213.

Wipke, B.T., and Allen, P.M. (2001). Essential role of neutrophils in the initiation and progression of a murine model of rheumatoid arthritis. J. Immunol. *167*, 1601–1608.

Wu, X., Hutson, I., Akk, A.M., Mascharak, S., Pham, C.T.N., Hourcade, D.E., Brown, R., Atkinson, J.P., and Harris, C.A. (2018). Contribution of adipose-derived factor D/adipsin to complement alternative pathway activation: lessons from lipodystrophy. J. Immunol. *200*, 2786–2797.

Xu, Y., Ma, M., Ippolito, G.C., Schroeder, H.W., Jr., Carroll, M.C., and Volanakis, J.E. (2001). Complement activation in factor D-deficient mice. Proc. Natl. Acad. Sci. U S A *98*, 14577–14582.

Yoshino, T., Kusunoki, N., Tanaka, N., Kaneko, K., Kusunoki, Y., Endo, H., Hasunuma, T., and Kawai, S. (2011). Elevated serum levels of resistin, leptin, and adiponectin are associated with C-reactive protein and also other clinical conditions in rheumatoid arthritis. Intern. Med. *50*, 269–275.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rat-anti-monoclonal adipsin antibody	R&D systems	Cat# MAB5430; RRID:AB_210837
FcBlock	BD Biosciences	clone 24G.2; RRID:AB_394656
Rat anti-mouse CD45-BUV395	BD Horizon	clone 30-F11; RRID:AB_2651134
Rat anti-mouse Ly6G-PE/Cy7	BioLegend	clone 1A8; RRID:AB_1877262
Rat anti-mouse/human CD11b-BV650	BioLegend	clone M1/70; RRID:AB_11125575
Rat anti-mouse Ly6C-FITC	BioLegend	clone HK1.4; RRID:AB_1186134
Mouse anti-mouse CD64-PE-Dazzle594	BioLegend	clone X54-5/7.1; RRID:AB_2566558
Rat anti-mouse F4/80-APC	BioLegend	clone BM8; RRID:AB_893493
IL1β Forward primer (GTGCAAGTGTCTGAAGCAGC)	Integrated DNA Technologies	N/A
IL1β Reverse primer (CAAAGGTTTGGAAGCAGCCC)	Integrated DNA Technologies	N/A
GAPDH Forward primer (TTCGACAGTCAGCCGCATCTTCTT)	Integrated DNA Technologies	N/A
GAPDH Reverse primer (CAGGCGCCCAATACGACCAAATC)	Integrated DNA Technologies	N/A
Chemicals, Peptides, and Recombinant Proteins		
Zombie UV	BioLegend	Cat #423108
Critical Commercial Assays		
iScript ^{TM} cDNA synthesis kit	Bio-RAD	Cat #170-8891
Powerup ^{TM} SYBR ^{TM} Green	Thermo Fisher Scientific	Cat #25742
Experimental Models: Organisms/Strains		
Fat free mice	Wu et al., 2018	N/A
Adipsin-KO mice	Wu et al., 2018	N/A
C57BL/6 mice	The Jackson Laboratory	Stock No: 000664
Adiponectin-KO mice	The Jackson Laboratory	Stock No: 008195
Leptin-KO	The Jackson Laboratory	Stock No: 000632
Adiponectin-leptin double KO mice	N/A	N/A
KRN mice	From Dr. Christophe Benoist	N/A
NOD mice	The Jackson Laboratory	Stock No: 001976
Software and Algorithms		
GraphPad Prism 7	GraphPad	https://www.graphpad.com/
Bioquant Osteo V7 10.10	Bioquant	https://bioquant.com/

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Steven L. Teitelbaum (teitelbs@wustl.edu).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

FF mice were generated by cross-mating animals expressing diphtheria toxin (DTA) containing a flox-STOP-flox cassette to those expressing Cre under control of the adiponectin promoter (Wu et al., 2018). Cre-littermates (Cre-) served as control in all experiments involving FF mice. Since FF mice die at birth at room temperature, they were housed at 30°C and maintained at 22°C in a 12-h light-dark cycle when studied. All other mice were kept at 22°C in a 12-h light-dark cycle. Adipsin-KO mice have been described (Xu et al., 2001). Adiponectin-KO mice and leptin-KO mice were obtained from Jackson laboratory and mated to generate adiponectin-leptin double-KO (DKO) mice. WT mice served as control for experiments involving adiponectin and leptin knockout mice. KRN mice were kindly provided by Dr. Christophe Benoist, Harvard Medical School. NOD mice were obtained from Jackson laboratory. The first generation of KRN mice X NOD mice are K/BxN mice and spontaneously develop arthritis. Their serum was collected and stored at −80°C. FF mice, adipsin-KO mice and adiponectin-KO mice are 6-9 weeks old. Leptin-KO mice and DKO mice are studied at

the age of 3 weeks because of joint swelling with obesity. Animal experiments were performed according to the policies of Animal Studies Committee (ASC) at Washington University School of Medicine in St. Louis. Mice were analyzed by approved protocols and received appropriate care in compliance with the standards in the Guide for the Use and Care of Laboratory Animals and the Animal Welfare Act.

METHOD DETAILS

Fat Transplantation
Six-week-old male C57BL/6 mice served as donors. Fat pads were removed from the inguinal or the intra-abdominal perigonadal (epididymal) region after donor mice were euthanized with CO2. Recipient mice were anesthetized by isoflurane. Fat pads of 1.5 donor mice were transplanted into the dorsal subcutaneous area of recipient mice. All recipient mice are on a C57BL/6 background.

Isolation of immune cells and flow cytometry
Synovial fluid was collected by washing the synovial cavity with 1 mL PBS. Collected immune cells were subjected to centrifugation at 500 x g at 4°C for 5 min and stained with the dead cell exclusion dye ZombieUV (1:600; BioLegend) in 50 mL PBS. Cells were incubated with 10 mg/mL FcBlock (clone 24G.2; BD Biosciences) in PBS containing 2.5% heat-inactivated fetal bovine serum (FBS) and 2 mM EDTA before being stained for flow cytometric analyses, as previously described (Rohatgi et al., 2018). The following antibodies were used: rat anti-mouse CD45-BUV395 (BD Horizon; clone 30-F11; 1:200), rat anti-mouse Ly6G-PE/Cy7 (BioLegend; clone 1A8; 1:300), rat anti-mouse/human CD11b-BV650 (BioLegend; clone M1/70; 1:400), rat anti-mouse Ly6C-FITC (BioLegend; clone HK1.4; 1:400), mouse anti-mouse CD64-PE-Dazzle594 (BioLegend; clone X54-5/7.1; 1:300), and rat anti-mouse F4/80-APC (BioLegend; clone BM8; 1:300). Flow cytometric analyses were performed with FlowJo (version 10, Ashland, OR), and cells were gated on singlets and live cells. Bulk immune cells were defined as $CD45^+$ cells. Neutrophils were defined as $CD45^+$ $CD11b^+$ $Ly6G^+$ cells. Monocytes were defined as $CD45^+$ $CD11b^+$ $Ly6G^-$ $Ly6C^{hi}$ cells. Macrophages were defined as $CD45^+$ $CD11b^+$ $Ly6G^-$ $Ly6C^-$ $F4/80^+$ cells.

Immunoblot
After denaturing in sodium dodecyl sulfate (SDS), serum was subjected to 10% SDS–polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride transfer membranes. The membranes were blocked with 0.1% casein in PBS and incubated with primary Rat-anti-monoclonal adipsin antibody (R&D systems, Minneapolis, MN) at 4°C overnight followed by probing with fluorescence-labeled secondary antibody. Proteins were visualized with the Odyssey infrared imaging system (LI-COR Biosciences).

Serum Transfer Arthritis
K/BxN serum (200 ml) were injected i.p. into recipient mice at days 0 and 3. The mice were monitored daily for the development of arthritis. PBS injection served as control. Paw and ankle thickness were measured with digital calipers. On day 9, blood was collected prior to mice being anesthetized for dissection.

Histology
Nine days after injection of K/BxN serum, animals were sacrificed and paws removed and fixed in 10% neutral buffered formalin for 24 h and rinsed in water before bones were decalcified in 14% EDTA (pH 7.2) for 20 days. Histological sections were stained with hematoxylin & eosin (H&E) and tartrate-resistant acid phosphatase (TRAP). The stained sections were scanned with Nanozoomer. To quantitate the TRAP positive area in the boxed region of interest (ROI), we used Bioquant Osteo V7 10.10 (Bioquant Image Analysis Corp., Nashville, TN).

Micro-computed tomography (μCT)
Bone structure of rear paws was assessed using a Scanco μCT40 scanner (Scanco Medical AG, Bassersdorf, Switzerland). Because of variation of bone mineral density with age, a threshold of 450 was used to evaluate scans of fat transplanted mice, and a threshold of 320 for all other mice.

RNA extraction and qPCR
Total RNA of soft tissue and fluid in synovial joints was extracted with TRIzol. Complementary (c)DNA was synthesized from RNA (1 μg) using the iScript cDNA Reverse Transcription kit (Bio-Rad) according to the manufacturer's instructions. Real-time PCR was performed using the SYBR Green Master Mix kit and gene-specific primers were listed in STAR METHOD. Quantitative PCR was performed on ABI PRISM 7500 sequence detection system (Applied Biosystems, Foster City, CA). Gene expression was calculated using the DDC_T method.

Acute phase proteins (APP) assay
The serum concentrations of APP in FF mice were detected by EVE Technologies. Briefly, haptoglobin, C-reactive protein, α-1-acid glycoprotein and α-macroglobulin in serum of FF mice were measured with Discovery Assay®.

QUANTIFICATION AND STATISTICAL ANALYSIS

Data are presented as means ± standard deviation (SD). Differences between groups were evaluated by unpaired Student's t test or two-way analysis of variance (ANOVA) with Tukey post hoc testing as recommended by Prism (GraphPad La Jolla, CA). The details were indicated in figure legends. P values are indicated when differences between two groups were statistically significant (< 0.05). Analyses were performed in Excel and Prism 7.
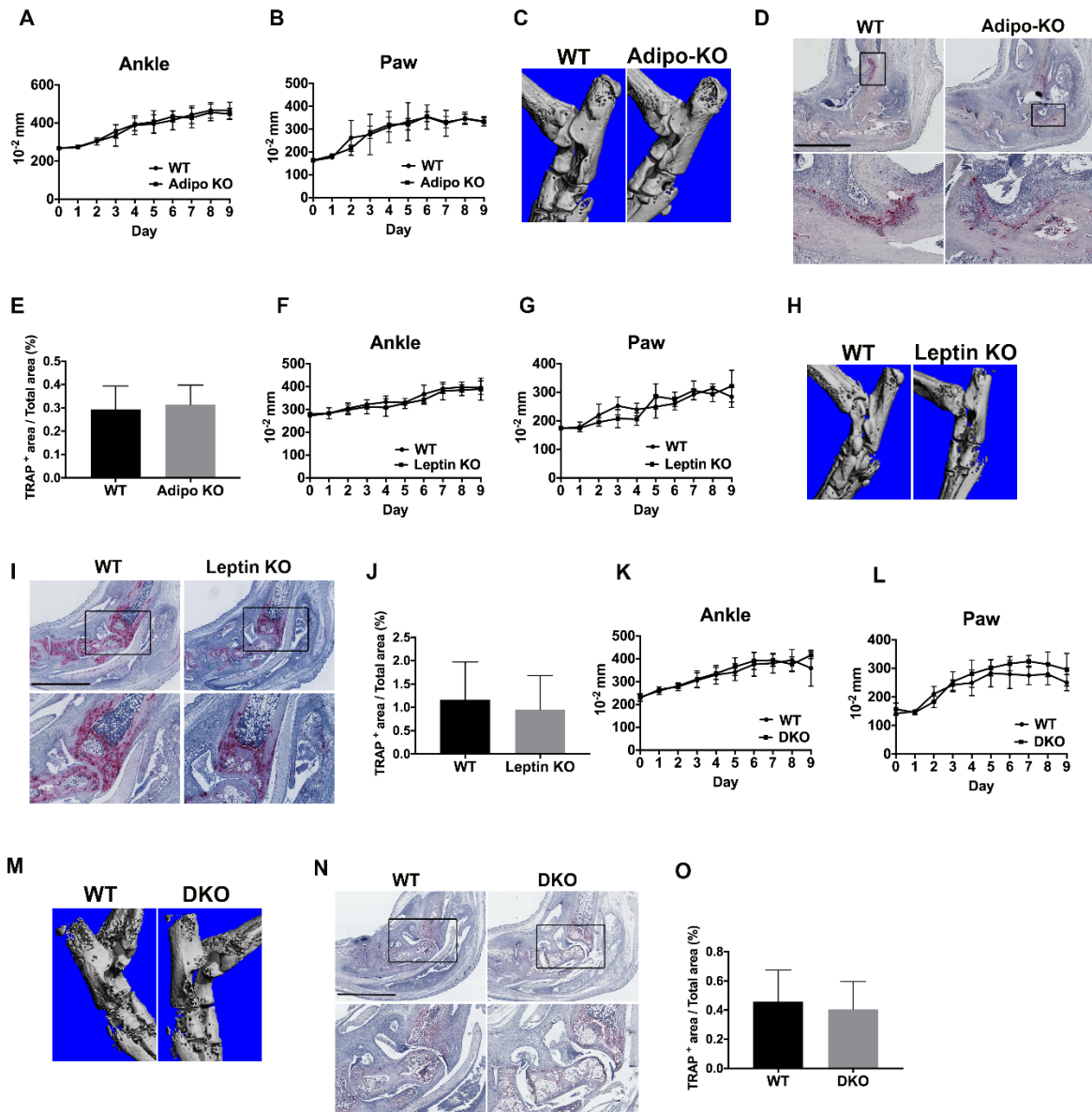
Supplemental Information

Fat-Produced Adipsin

Regulates Inflammatory Arthritis

Yongjia Li, Wei Zou, Jonathan R. Brestoff, Nidhi Rohatgi, Xiaobo Wu, John P. Atkinson, Charles A. Harris, and Steven L. Teitelbaum

Supplemental Figure S1, related to Figure 3. Serum transfer IA is independent of adiponectin and/or leptin. (A-E) Adipo-KO, (F-J) leptin-KO, (K-P) DKO and (A-PO) WT mice were injected with serum on day 0 and 3. (A,F,K) Ankle and (B,G,M) Paw thickness was measured with time. Nine days after initial serum injection (C,H,N) µCT image of hind paws was obtained, (D,I,O) histological image of rear ankle joints stained for TRAP activity and (E,J,P) TRAP stained area/total area (red reaction product) were determined, Scale bar=2.5 mm. Student's t-test, n=4-5 mice.

	Cre-	FF	P
Serum amyloid P	321±44 µg/ml	322±35 µg/ml	NS
Haptoglobin	24.1±12.6 µg/ml	508±247 µg/ml	0.028
C-reactive protein	9.51±0.16 µg/ml	28.3±5.0 µg/ml	0.003
Acid glycoprotein	196±22 µg/ml	295±25 µg/ml	0.007
Alpha-macrolobulin	2.76±0.35 mg/ml	4.36±0.306 mg/ml	0.004

Supplemental Table S1, related to Figure 1. Serum concentrations of acute phase proteins in FF mice. Student's t-test, n=3 mice, P-values are indicated in the table.