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J Immunol 2010; 185:2563-2569; Prepublished online 14 July 2010; doi: 10.4049/jimmunol.0903664
http://www.jimmunol.org/content/185/4/2563

Supplementary Material
http://www.jimmunol.org/content/suppl/2010/07/14/jimmunol.0903664.DC1

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TLR4 Activation Is Required for IL-17–Induced Multiple Tissue Inflammation and Wasting in Mice

Haiqing Tang,* Shanshan Pang,* Min Wang,* Xiuying Xiao,† Yefei Rong,‡ Hui Wang,* and Ying Qin Zang*

IL-17 is a recently identified proinflammatory cytokine that plays pivotal roles in several chronic inflammatory disease models. Its expression was also found to be elevated in the serum of patients with chronic diseases. However, whether elevated systemic IL-17 expression can induce pathophysiological tissue inflammation is unknown. In this study, we demonstrated that systemic overexpression of IL-17 using an adenoviral vector could induce multiple tissue inflammation and wasting in mice. We also found that the expression of TLR4 was increased in tissues of IL-17–overexpressing mice. Moreover, TLR4 activation is required for IL-17–induced tissue inflammation and wasting, as evidenced by the absence of aggressive atrophy in gastrocnemius muscle, neutrophil accumulation, and expression of proinflammatory cytokines downstream of TLR4 in multiple tissues of TLR4-deficient mice. Further investigation revealed that TLR4 endogenous ligands high-mobility group box 1 and heat shock protein 22, were systemically upregulated and might be involved in the IL-17–induced TLR4 activation. Our results suggest that IL-17 may induce disease-associated tissue inflammation and wasting through TLR4 signaling. The study indicates a novel interaction between IL-17 and TLR4 activation and may have implications in the pathogenesis and treatment of chronic diseases. The Journal of Immunology, 2010, 185: 2563–2569.

Interleukin 17 is a recently identified proinflammatory cytokine that is produced predominantly by the newly discovered Th17 Th subset (1, 2) and other cell types, including γδ T cells, NKT cells, NK cells, neutrophils, and eosinophils (3). IL-17 induces massive cell and tissue reactions through its ubiquitously expressed receptor IL-17R (4, 5). Current evidence suggests that IL-17 plays pivotal roles in several chronic inflammatory diseases, such as multiple sclerosis, psoriasis, rheumatoid arthritis (RA), and inflammatory bowel disease (4, 6). Under these conditions, IL-17 acts as a potent inflammatory cytokine to coordinate tissue inflammation by inducing the expression of proinflammatory cytokines (TNF-α, IL-6, IL-1β), chemokines (CXCL1, CXCL8, CXCL10), G-CSF, and matrix metalloproteases (MMPs), which mediate neutrophil infiltration and tissue destruction (7, 8).

The inflammatory response often relates to the activation of innate immune system, during which TLRs play essential roles (9, 10). TLR4, the receptor for LPS, is an important receptor involved in the initiation of inflammatory responses. TLR4 can also recognize endogenous ligands released from stressed cells or damaged tissues, such as high-mobility group box 1 (HMGB1), heat shock proteins (HSPs), and breakdown products of heparin sulfate and hyaluronic acid (10–12). Once activated by endogenous ligands, TLR4 induces the production of proinflammatory cytokines and chemokines that alert the immune system and contribute to local inflammation (12, 13).

IL-17 has been found to be elevated in the serum of patients with chronic diseases (4, 14, 15). However, whether systemic overexpression of IL-17 can induce pathophysiological tissue inflammation is unknown. To gain insight into this, we systemically overexpressed IL-17 in mice through adenovirus-mediated gene transfer, because transgenic mice that ubiquitously overexpress IL-17 were infertile (16). Although a previous study showed that systemic overexpression of IL-17 by adenovirus delivery resulted in no obvious abnormalities other than granulopoiesis (16), in this paper we reported that mice receiving IL-17–expressing adenoviruses exhibited multiple tissue inflammation and wasting. Furthermore, we demonstrated that the expression of TLR4 and its endogenous ligands was elevated by IL-17 overexpression, and TLR4 activation is essential for IL-17–induced tissue inflammation and wasting.

Materials and Methods

Animals

Male wild-type (WT) (C57BL/6) mice (10–12 wk old) were purchased from the Shanghai Laboratory Animal Company (Shanghai, China). TLR4−/− mice were kindly provided by Prof. Vincent Deubel and Prof. Baoyue Ge (Institute Pasteur of the Chinese Academy of Sciences, Shanghai, China). The mice were individually housed in laboratory cages under a 12-h dark/light cycle in accredited animal facilities at the Shanghai Institutes for Biology Science (Shanghai, China), Chinese Academy of Sciences (Shanghai, China), with free access to standard chow and water. All experimental procedures and protocols were approved by the Institutional Animal Care and Use Committee of the Institute for Nutritional Sciences, Chinese Academy of Sciences. Fat and lean mass were measured...
by nuclear magnetic resonance with a Minispec Mq7.5 Analyzer (Bruker, Bremen, Germany).

**Construction and administration of recombinant adenovirus**

The recombinant adenoviruses used for mouse IL-17A overexpression were generated using the AdEasy Adenoviral Vector System (Stratagene, La Jolla, CA) in AD-293 cells according to manufacturer’s instructions. Briefly, DNA fragments encoding IL-17 were first cloned into pShuttle-CMV vector. Then, this vector and the empty pShuttle-CMV vector were transfected into B5183-AD-1 cells (pretransformed with pAdEasy-1 plasmid), individually, to get rIL-17-expressing adenovirus (Ad–IL-17) plasmid and control Ad plasmid. Viruses were prepared using AD-293 cells in endotoxin-free conditions and purified by CsCl ultracentrifugation. Adenoviruses were desalted and administrated through tail vein injection using ~5 x 10⁹ vp/mouse and 1 x 10⁹ vp/mouse for the lower dosage.

**Histology**

Tissues were fixed in 10% neutral buffered formalin, dehydrated in a series of ethanol dilutions, passed through xylene and xylene/paraffin, and finally embedded in paraffin. Sections were cut to a thickness of 5 µm and stained with H&E through standard procedures.

**Immunohistochemistry**

Formalin-fixed paraffin-embedded sections were deparaffinized and boiled for 10 min in 10 mM sodium citrate buffer (pH 6) for neutrophil detection and digested with 0.4% pepsin for TLR4 detection (12). Briefly, analysis of the TLR4-positive cellular infiltrate was performed by assessing 20 consecutive high-power fields (magnification ×40). The number of cells staining positively was counted and expressed as cells per 10 high-power fields.

**Western blot analysis**

Tissue was homogenized with RIPA lysis buffer and centrifuged for 20 min at 12,000 rpm to remove the debris. Serum proteins were analyzed directly without any further processing. Proteins were separated by SDS-PAGE, transferred to polyvinylidene difluoride membrane (Millipore, Bedford, MA) and blocked in 5% nonfat milk at room temperature for 1 h. The membrane was incubated overnight at 4°C with the following primary Abs: rabbit anti-mouse HSP22 (Cell Signaling Technology, Beverly, MA), and rabbit anti-mouse HMGB1 (ab18256, Abcam, Cambridge, U.K.). After washing, the blots were incubated with HRP-conjugated anti-rabbit IgG secondary Ab for 1 h at room temperature (Kangchen, Shanghai, China), and then developed with the SuperSignal West Pico Chemiluminescent Substrate (Thermo Fisher Scientific, Waltham, MA).

**Real-time PCR**

Total RNA was extracted from tissues using TRIzol reagent (Invitrogen) and treated with RQ1 RNase-Free DNase (Promega, Madison, WI). First-strand cDNA was synthesized with Moloney murine leukemia virus reverse transcriptase (Promega) and oligo(dT) primers (Takara Bio, Shiga, Japan). Specific primers were designed using Primer Express Software (Applied Biosystems, Foster City, CA) and sequences are as follows: actin (forward, 5’-ATCTGGCCACCAACCATCTTACA-3’; reverse, 5’-TTTTCACGTTG-GCCTTAGG-3’); CXCL1 (forward, 5’-CACCTCACCACCAACCAAGA- GT-3’; reverse, 5’-AGCATCTTGGGACAAATTTTCTGTA-3’); IL-6 (forward, 5’-TTCATCAGTGGCCTTCGG-3’; reverse, 5’-TGGGAGTT-GGTATCCTCTGTTGA-3’); TNF-α (forward, 5’-GAGCTGAAACGGC- AGAAG-3’; reverse, 5’-GCCCAAGCAGGAAIAGAGAAG-3’); MMP-9 (forward, 5’-GAGCTGAGCTGATATCCACC-3’; reverse, 5’-ATCACTGTC-TGCCGGAAGT-3’); BCL2 (forward, 5’-TTCGCTGGAGATGTACCA-3’; reverse, 5’-TCGCGGAGGTTGTGTGG-3’); muscle atrophy F-box (MAFbx) (forward, 5’-CTCTGGGCAAGTGTACCA-3’; reverse, 5’-GTCACCTGACCTGCTAGATG-3’); TL-R4 (forward, 5’-TGGTGCGTCTGGAGCACAA-3’; reverse, 5’-GGAAATGT-AATAATTTGAGGCAATCT-3’); HMB1 (forward, 5’-TGGCCGACT- TCAGTCCC-3’; reverse, 5’-GCCCTCTGGCTTTATAGGATCT-3’; and HSP22 (forward, 5’-GGAAGGTGGGATTTTGCTCACA-3’; reverse, 5’-GCGC- GCCTCTGGAGAAAG-3’). PCR amplifications were quantified using the SYBR Green PCR Master Mix (Applied Biosystems), and the results were normalized against actin gene expression.

**ELISA**

Tissue was homogenized in 50 mM Tris-HCl buffer (pH 7.5), containing 150 mM NaCl, 0.002% Tween-20, and complete protease inhibitor (Roche, Basel, Switzerland), and subsequently centrifuged at 12,000 x g for 10
min to remove debris. The concentration of IL-17 in the serum and TNF-α, IL-6, and CXCL1 in tissue homogenates was measured using ELISA kits (R&D Systems) according to the manufacturer’s instructions. Protein levels of cytokines and chemokine were corrected for the weight of tissue, and the results were expressed in picograms per milligram tissue. The detection limits were 5.1 pg/ml for TNF-α, 1.6 pg/ml for IL-6, 2 pg/ml for CXCL1, and 5 pg/ml for IL-17.

Statistics

Data are presented as the means ± SEMs. Statistical significance was determined using two-tailed unpaired Student t tests. A value of p < 0.05 was considered significant.

Results

Overexpression of IL-17 induces wasting in mice

To systemically overexpress IL-17, rAd–IL-17 or control empty adenoviruses (Ad-cons) were delivered into mice at day 0, and all experiments were conducted over the next 28 d. Ad–IL-17 infection significantly increased the serum levels of IL-17 throughout the period of 28 d with a peak at day 7 (Fig. 1A), whereas IL-17 levels in the serum of Ad-con–infected control mice were undetectable. Strikingly, Ad–IL-17–infected mice showed a gradual wasting phenotype, with much smaller body size than the control mice (Fig. 1B) and 10% loss of their initial weight at day 28 post-infection (Fig. 1C). Body composition analysis revealed that the weight loss was caused by depletion of both fat and lean mass (Fig. 1D). Taken together, our data demonstrate that overexpression of IL-17 caused wasting in mice characterized by severe loss of both fat and lean mass.

Because muscle wasting is a major feature of wasting, we sought to determine whether the lean mass loss in Ad–IL-17–infected mice was due to skeletal muscle wasting. H&E staining further revealed ongoing atrophy in the gastrocnemius muscle of Ad–IL-17–infected mice, with smaller muscle fibers but no inflammatory infiltrates or necrotic degeneration (Fig. 1E). Moreover, the mRNA levels of two critical ubiquitin ligases, MuRF-1 and MAFbx, for which the expression is elevated in various muscle atrophy models (17), were increased in the gastrocnemius muscle of Ad–IL-17–infected mice from days 4–28 (Fig. 1F). These data indicate that IL-17 induces skeletal muscle atrophy through activation of the ubiquitin-proteasome pathway.

Inflammatory phenotype in Ad–IL-17–infected mice

Next, we examined the tissue inflammation after Ad–IL-17 administration. In the liver, H&E staining showed that Ad–IL-17 but not Ad-con infection induced a moderate leukocyte infiltration at day 4, which became severe over time and eventually led to necrosis by day 28 (Fig. 2A). The pancreas seemed to be the most affected tissue, as Ad–IL-17–injected mice exhibited progressive fibrosis and atrophy in the pancreas, which totally disappeared by day 28. Histological analysis showed fibrosis and massive leukocyte infiltration in the pancreas at day 15 (Fig. 2B), further supporting the morphological observations. We also examined

![FIGURE 3. IL-17 induces the upregulation of inflammatory mediators in multiple tissues.](http://www.jimmunol.org/)

The mRNA levels of chemokine CXCL1 (A), MMP9 (B), and proinflammatory cytokines IL-6 (C) and TNF-α (D) were measured in the liver and gastrocnemius muscle by quantitative RT-PCR, as illustrated in Fig. 1. n = 5 per group. Data shown are means ± SEMs. *p < 0.5; **p < 0.01 versus the control mice.
other fetal organs and observed atrophy in the heart, but no morphological change was found in the lungs of Ad–IL–17–treated mice (Supplemental Fig. 1). In other inflammatory states, IL–17 acts as a potent inducer of neutrophil trafficking to sites of inflammation (18–20). Indeed, positive staining for the neutrophil marker Gr-1 by immunohistochemistry showed that most of the leukocytes infiltrating into the liver and pancreas were neutrophils (Fig. 2C). Consistent with the previous study, we also observed splenomegaly in Ad–IL–17–infected mice as determined by the spleen weight, with a peak at day 14 (Fig. 2D). In summary, these data suggest that IL–17 overexpression induces multiple tissue inflammation in mice. 

**IL–17 upregulates the inflammatory mediators in multiple tissues**

IL–17 induces inflammation through the upregulation of chemokines, MMPs, and proinflammatory cytokines. We therefore examined the expression of these mediators after adenovirus infection. The serum levels of TNF–α, IL–6, or CXCL1 were undetectable postinfection with either Ad–IL–17 or Ad-con, indicating that overexpression of IL–17 could not induce measurable expression of proinflammatory cytokines or chemokines in the circulation. We next tested whether IL–17 could induce the expression of inflammatory mediators in the related tissue microenvironments, including the liver and gastrocnemius muscle, representing the inflammatory and the wasting tissue, respectively. Postinjection of Ad–IL–17, mRNA levels of CXCL1, a direct target of IL–17 for neutrophil chemotraction, were dramatically increased in both liver and gastrocnemius muscle from days 4–28 (Fig. 3A). The mRNA levels of MMP9, another downstream target of IL–17, were increased in the liver through days 4–28, but no changes were detected in the gastrocnemius muscle (Fig. 3B), suggesting that it is not involved in the wasting. Next, we examined the mRNA levels of IL–6 and TNF–α, which are potent mediators of inflammation and wasting in many inflammatory states. IL–17 overexpression upregulated the mRNA expression of IL–6, with the most significant elevation in the gastrocnemius muscle from days 7–28, but only with a slight upregulation in the liver at day 14 (Fig. 3C). Unexpectedly, TNF–α was only slightly upregulated in the gastrocnemius muscle at day 14 (Fig. 3D). Therefore, these results provide evidence that IL–17 induces inflammatory genes expression in multiple tissues.

**IL–17 increases the expression of TLR4 and its endogenous ligands in vivo**

Given that the TLR4 signaling cascade plays an essential role in many inflammatory conditions, we sought to determine whether TLR4 contributes to the systemic inflammation and wasting induced by IL–17. To do this, we first examined the expression of TLR4 and found that IL–17 increased TLR4 mRNA levels in both the liver and gastrocnemius muscle (Fig. 4A). Immunostaining indicated that TLR4 protein was expressed by infiltrating leukocytes in the liver (Fig. 4B). The number of TLR4–positive leukocytes increased through days 4–28 (Fig. 4C). Muscle TLR4 expression increase was most evident on the myocytes (Fig. 4D).

Besides the upregulation of TLR4 expression, activation of TLR4 signaling often correlates with the release of endogenous ligands in other disease models (12, 13). We observed elevated serum levels of HMGB1 and HSP22 in Ad–IL–17–infected mice at day 4 and further elevations at days 14 and 28 when compared with the

![FIGURE 4](http://www.jimmunol.org/) 
**FIGURE 4.** TLR4 expression is elevated after Ad–IL–17 administration. A. The mRNA levels of TLR4 in the liver and gastrocnemius muscle measured by RT–PCR at indicated days. n = 5 per group. B. Immunohistochemistry staining for TLR4 in the liver of Ad–con–infected mice at day 28 and Ad–IL–17–infected mice at indicated days. Magnification ×40. C. Analysis of TLR4–positive leukocytes in the liver. n = 6 per group. D. Representative sections of the gastrocnemius muscle stained for TLR4 at day 28. Magnification ×40. Data shown are means ± SEMs. *p < 0.5; **p < 0.01; ***p < 0.001 versus the control mice.

![FIGURE 5](http://www.jimmunol.org/) 
**FIGURE 5.** Expression of endogenous ligands for TLR4 was elevated systemically after Ad–IL–17 administration. A. Levels of HMGB1 and HSP22 in the serum examined by immunoblotting at indicated days. B. The mRNA levels of HMGB1 and HSP22 in the liver measured by RT–PCR at indicated days. n = 5 per group. C. Protein expression of HMGB1 and HSP22 in the liver examined by immunoblotting at indicated days. D. The mRNA (D) and protein levels (E) of HMGB1 and HSP22 in the gastrocnemius muscle measured at day 14. n = 5 per group. Data shown are means ± SEMs. *p < 0.5; **p < 0.01 versus the control mice.
control mice (Fig. 5A). Next, we examined the expression of ligands in tissues. In the liver, IL-17 elevated the mRNA levels of HMGB1 and HSP22 from days 4–28 (Fig. 5B). Protein levels of both ligands displayed a similar pattern to the serum (Fig. 5C). In the gastrocnemius muscle, IL-17–induced ligand expression was confirmed at day 14 (Fig. 5D, 5E). Together, these data indicate that IL-17 elevates the expression of TLR4 and its endogenous ligands in vivo.

**TLR4**<sup>−/−</sup> mice are protected against IL-17–induced wasting

To investigate whether TLR4 is required for IL-17–induced wasting, we administrated Ad–IL-17 into TLR4<sup>−/−</sup> mice. Although TLR4<sup>−/−</sup> mice showed comparable serum levels of IL-17 to the WT mice after Ad–IL-17 infection (Fig. 6A), TLR4<sup>−/−</sup> mice were protected against IL-17–induced wasting. TLR4 deficiency restored the Ad–IL-17–induced body weight loss, which correlated with the reversal of both fat mass and lean mass (Fig. 6B). The atrophy in the gastrocnemius muscle was also absent in TLR4<sup>−/−</sup> mice after Ad–IL-17 infection, as the H&E staining showed that myofiber size was restored to the level of WT controls (Fig. 6C). Subsequent gene analysis revealed that mRNA expression of MAFbx and MuRF-1 induced by IL-17 was also inhibited in TLR4<sup>−/−</sup> mice, further supporting the morphological observations (Fig. 6D). Collectively, we conclude that IL-17–induced wasting and related gene expression are TLR4 dependent.

**Multiple tissue inflammation in TLR4**<sup>−/−</sup> mice is partially reversed

We next investigated whether TLR4 contributed to the multiple tissue inflammation induced by Ad–IL-17 infection. H&E staining showed that leukocyte infiltration and necrosis were totally absent in the liver of TLR4<sup>−/−</sup> mice after Ad–IL-17 infection (Fig. 7A). In addition, no leukocyte infiltration or fibrosis was observed in the pancreas of TLR4<sup>−/−</sup> mice (Fig. 7A). However, no differences in the spleen weights were observed between Ad–IL-17–injected TLR4<sup>−/−</sup> and WT mice at day 14 (Fig. 7B, upper panel). Unexpectedly, we found that the spleens of Ad–IL-17–infected TLR4<sup>−/−</sup> mice were still enlarged at day 28 (Fig. 7B, lower panel), when the spleens had recovered to control level in the WT mice. In summary, TLR4 deficiency protects mice from IL-17–induced neutrophil infiltration and damage in the liver and pancreas, whereas TLR4 is involved in the recovery but not the induction of splenomegaly.

Accordingly, we examined the protein levels of cytokines and chemokines in the liver and gastrocnemius muscle by ELISA. Consistent with the mRNA expression pattern, IL-17 increased the protein expression of TNF-α in the gastrocnemius muscle and CXCL1 and IL-6 in both the liver and gastrocnemius muscle of WT mice at day 14. In TLR4<sup>−/−</sup> mice, IL-6 and TNF-α expression induced by IL-17 was completely inhibited, whereas CXCL1 expression was not affected (Fig. 7C), indicating that TLR4 is not essential for IL-17–induced CXCL1 expression, and induction of CXCL1 alone is not enough for the mobilization of neutrophils. These data suggest that TLR4 contributes to the multiple tissue inflammation observed in IL-17–expressing mice.

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**FIGURE 6.** TLR4<sup>−/−</sup> mice are protected against IL-17–induced wasting. A, IL-17 expression in the serum was determined in both TLR4<sup>−/−</sup> and WT mice at day 7 after Ad–IL-17 infection. n = 5 mice per group. B, The body weight and lean mass weight at day 28 and the fat weight at day 7, n = 6 mice per group. Data shown are means ± SEMs. *p < 0.5; **p < 0.01. C, Representative histological images of gastrocnemius muscle at day 28. Magnification ×40; scale bar, 50 μm. D, The mRNA levels of MuRF-1 and MAFbx in gastrocnemius muscle determined by RT-PCR at day 14. n = 5 per group. Data shown are means ± SEMs. *p < 0.5; **p < 0.01 versus Ad-con–infected WT mice.

**FIGURE 7.** Multiple tissue inflammation in TLR4<sup>−/−</sup> mice is partially reversed. A, Representative H&E-stained sections of the liver (magnification ×20) at day 28 and the pancreas (magnification ×20) at day 14. B, Spleen weight measured at days 14 and 28. n = 5 per group. C, Protein levels of IL-6, TNF-α, and CXCL1 in the liver and gastrocnemius muscle measured by ELISA at day 14. n = 5 per group. Data shown are means ± SEMs. *p < 0.5; **p < 0.01.
Discussion
In this context, we demonstrated that the exposure of normal host to IL-17 through adenovirus-mediated gene transfer was capable of inducing multiple tissue inflammation and wasting. However, in the previous study (16), none of these abnormalities were observed in the mice infected with Ad–IL-17, which we propose to result from variation in the titration of virus in different laboratories. In fact, our experimental data showed that mice infected with one fifth of the present dosage of viruses (serum IL-17 levels range from 2.06–8.47 ng/ml) exhibited modest splenomegaly and leukocyte infiltration in both the liver and pancreas, together with elevated expression of cytokines and chemokine in the liver and muscle and increased endogenous ligand levels in the serum (Supplemental Fig. 2). The previous study may have possibly injected fewer viruses and missed the much less pronounced phenomena we observed.

Previous experimental data suggest that proinflammatory cytokines, including TNF-α, IL-1, IL-6, and IFN-γ, are a subset of cachectic factors (21–23). In our study, IL-17–expressing mice displayed significant body weight loss, caused by depletion of both lean mass and fat mass. Morphological analysis indicated an aggressive wasting phenomenon in the gastrocnemius muscle. Further study revealed that TLR4-deficient mice were protected against IL-17–induced cachexia. Collectively, these data demonstrated a previously undescribed cachectic effect of IL-17, which induces wasting through TLR4 signaling.

Next, we demonstrated that IL-17 induced multiple tissue inflammation characterized by massive neutrophil accumulation. The previous study (16) has reported that Ad–IL-17 infection-induced splenomegaly associates with neutrophilia and leukocytosis, which is a hallmark of chronic inflammation and in part represents the mobilization of leukocytes necessary to promote tissue-specific inflammation (22). Other studies indicate that IL-17 induces neutrophil chemotaxis through a CXC chemokine-dependent pathway (24, 25). In our study, we observed IL-17–induced splenomegally as well as the expression of CXCL1 in multiple tissues, irrespective of the existence of TLR4 or not. However, the neutrophil infiltration was absent in TLR4−/− mice, suggesting that mobilization of neutrophils in response to IL-17 may be interrupted by TLR4 deficiency. Indeed, TLR4 has been shown to play critical roles in regulating the migration, activation, and life span of neutrophils (26, 27), which provides further supporting evidence.

We also reported elevated expression of TLR4 in the liver and gastrocnemius muscle after Ad–IL-17 infection. In addition, we found that the expression of its endogenous ligands, HMGB1 and HSP22, was increased in the liver, gastrocnemius muscle, and circulation of Ad–IL-17-infected mice. Increasing experimental evidence indicates that engagement of the TLR4 by endogenous ligands is a major trigger of inflammation. For example, HMGB1 released from ischemic hepatocytes activates TLR4, which is critical in the development of lethal hepatic ischemia reperfusion injury (12). HSP22 is abundantly expressed in the synovial tissues of patients with RA and may be involved in the inflammatory process in RA through activation of TLR4 (28). Our data provided circumstantial evidence that endogenous ligands are the source of TLR4 activation in the present model. Studies will be necessary to determine the contributions of these ligands to IL-17–induced syndrome.

Previous studies have provided evidence that TLR4 is upstream of the production of IL-17 in several inflammatory models. For example, TLR4 is required for IL-23 induction and subsequent IL-17 production in both CD4+ and CD8+ T cells following Klebsiella pneumoniae infection (29). Furthermore, TLR4 gene deficiency protects IL-1Ra−/− mice from severe arthritis by reducing the number of pathogenic Th17 cells and the production of IL-17 (30). Although TLR4 expression has been reported to be upregulated by IL-17 in an IL-17–induced arthritis aggravation model (31), our study demonstrated that IL-17 could functionally modulate TLR4 signaling in vivo and provided evidence that TLR4 functioned downstream of IL-17 in our model system. Thus, it is conceivable that TLR4 may act downstream of IL-17 in those IL-17–mediated diseases, which needs further investigation.

Collectively, this study has suggested a new interaction between IL-17 and the TLR4 pathway. As the expression of IL-17 has been found to be elevated in several chronic diseases, our findings may also have general implications in the mechanism and treatment of chronic diseases.

Acknowledgments
We thank Prof. Vincent Deubel and Prof. Baoxue Ge (Institute Pasteur of the Chinese Academy of Sciences, Shanghai, China) for providing TLR4−/− mice.

Disclosures
The authors have no financial conflicts of interest.

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